Dental Age Estimates of Individuals Buried at Apollonia Pontica

by

Nathaniel Collin Moore

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

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FACULTY OF GRADUATE STUDIES
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MASTER OF ARTS

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Abstract

One primary challenge when attempting to ascertain adult age-at-death of unknown archaeological skeletal remains are the issues of subjective age estimation methods/techniques, age ranges, bias caused by the burial environment, and the use of multiple skeletal elements. Teeth, as opposed to bones, have several attributes that obviate these problems. This research uses a canine dental sample from the Apollonia Pontica site and addresses these issues using robust dental age indicators (transparency and pulp reduction), comparing the age estimates from two methods (digital and manual), applying multiple quantification techniques, and comparing these results to associated skeletal morphological age markers. The digital method of the transparency techniques corresponded closest to the skeletal morphological age estimates and was applicable on the largest sample portion. The dental age estimates have been established for the Apollonia Pontica sample and contributed by producing a single age estimate using objective techniques.

KEYWORDS: age-at-death estimation, adult, teeth, transparency, pulp reduction, Apollonia Pontica

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Table of Contents

Abstract.....ii

Acknowledgements.....iii

List of Tables.....ix

List of Figures.....x

Chapter 1: Introduction.....1

Chapter 2: Literature Review 4

Introduction.....4

Adult Age Estimation.....11

Age Estimation by a Single Dental Criterion 11

Secondary Dentin Apposition/Pulp Reduction.....11

Transparency.....23

Age Estimation by Multiple Dental Criteria.....36

Post-Depositional Influences.....51

Dentin Transparency.....53

Pulp Chamber/Root Canal and Secondary Dentin.....54

Summary..... 55

Chapter 3: Materials and Methods.....57

Apollonia Pontica: Biocultural and Bioarchaeological Context.....57

Biocultural Context.....57

Origin.....57

Trade and Dynamics.....58

Population.....59

Bioarchaeological Context.....59

Demographic Profile.....60

Skeletal and Dental Pathology.....62

Diet.....64

Sample Derivation.....65

Methods and Techniques: Age Estimation.....66

Manual Methods.....66

Apical Transparency.....66

Radiology and Radiography: Pulp Reduction.....69

Digital Methods.....71

Digital Images, Image Acquisition, Image Enhancement/Processing, Measurement, and Analysis.....71

Digital Images.....71

Image Acquisition.....72

Image Analysis.....75

Apical Transparency.....75

Pulp Reduction.....77

Age Calculation.....83

Chapter 4: Results 84

Digital and Manual Methods: Observer Error.....84

Digital Methods.....86

Pulp and Root Measurements.....86

Transparency and Root Measurements.....86

Manual Methods.....86

Pulp and Root Measurements.....86

Transparency and Root Measurements.....87

Comparison of Inter-Method Measurements and Ratios...87

Age Estimates and Distributions.....89

Comparison of Dental and Skeletal Age Estimates.....129

Chapter 5: Discussion and Conclusions.....131

Methodological Considerations.....131

Digital Methods: Intra-Observer Error.....131

Pulp Reduction: Linear and Area Measurements..131

Root Transparency: Transparency and Root Measurements.....132
Manual Methods: Intra-Observer Error.....133
Pulp Reduction: Linear Measurements.....133
Root Transparency: Transparency and Root Measurements.....133
Full Measurement Sets.....134
Possible Sources of Error.....134
Digital Methods.....134
Pulp and Root Measurements.....134
Manual Methods.....138
Pulp and Root Measurements.....138
Transparency and Root Measurements.....141
Comparison of Measurements.....143
Age Estimates and Distributions.....144
Comparison with Skeletal Age Estimates.....151
Other Factors to Consider.....153
Probable Age Estimates (AKA the 'Best Guess' Scenario).....156
Contributions to the Study of Apollonia Pontica.....158
Conclusions and Directions for Future Research.....165

References Cited.....170

Appendices.....181

Appendix 1: Total Dental and Skeletal Age Estimates.....181

List of Tables

2.1 Skeletal age indicators and variation in estimated age.....7

2.2 Pulp reduction publications illustrating correlation coefficients with known age separated by tooth type.....21

2.2 Transparency publications illustrating correlation coefficients with known age separated by tooth type.....35

3.1 Total adult age and sex distribution of the Apollonia Pontica sample.....61

3.2 Skeletal age estimates for the sub-sample of individuals from the Kalfata necropolis used in present research.....62

3.3 Apollonia Pontica sample dental inventory.....65

4.1 Paired samples *t*-tests for testing intra-observer error for repeated measures.....84

4.2 Full digital and manual measurement set comparison.....87

4.3 Digitally acquired dental age estimates.....90

4.4 Manually acquired dental age estimates.....91

4.5 Mean age estimates separated by individual.....121

4.6 Mean age estimates and standard deviation of techniques.....126

4.7 Digital and manual full age estimate set comparison.....127

4.8 Average of means and standard deviations of methods and techniques.....128

4.9 Dental and skeletal age estimates and correspondence.....129

5.1 Final age estimates of the Apollonia Pontica individuals.....158

5.2 Age distributions comparing % of age categories of Kalfata sample, usable thesis sample, and thesis dental sample.....163

List of Figures

2.1 Histological view of a tooth: A) secondary dentin/pulp reduction; B) transparency.....9

2.2 An illustration of the progressive shrinkage of the pulp chamber and increase in length of transparency over the lifetime.....11

3.1 Manual data acquisition of transparency height and root length.....67

3.2 Digital transparency image acquisition apparatus.....73

3.3 A specimen in image acquisition position.....74

3.4 The baseline digital transparency image.....74

3.5 Labial transparency height and root length digital measurement technique.....77

3.6 Minimum and maximum transparency digital measurement techniques.....77

3.7 Enhancement of a dental radiograph image.....78

3.8 Root and pulp width measurements at CEJ.....79

3.9 Root (A) and pulp (B) mesial length.....79

3.10 Pulp and root width measurements at CEJ (A), ¼ (B), and mid-root (C) levels...80

3.11 Cameriere et al.2004 labio-lingual tooth and pulp area measurements.....81

3.12 Cameriere et al.2004 pulp and root width measurements at mid-root.....81

3.13 Labio-lingual orientation tooth and pulp area measurements.....82

3.14 Mesio-distal orientation tooth and pulp area measurements.....82

4.1 Digital method age range, #301.....92

4.2 Manual method age range, #301.....92

4.3 Digital method age range, #19.....93

4.4 Manual method age range, #19.....93

4.5 Digital method age range, #253.....94

4.6 Manual method age range, #253.....94

4.7 Digital method age range, #289.....95

4.8 Manual method age range, #289.....95

4.9 Digital method age range, #352.....96

4.10 Manual method age range, #352.....96

4.11 Digital method age range, #336.....97

4.12 Manual method age range, #336.....97

4.13 Digital method age range, #244.....98

4.14 Manual method age range, #244.....98

4.15 Digital method age range, #215.....99

4.16 Manual method age range, #215.....99

4.17 Digital method age range, #192.....100

4.18 Manual method age range, #192.....100

4.19 Digital method age range, #307.....101

4.20 Manual method age range, #307.....101

4.21 Digital method age range, #249.....102

4.22 Manual method age range, #249.....102

4.23 Digital method age range, #207.....103
4.24 Manual method age range, #207.....103
4.25 Digital method age range, #284.....104
4.26 Manual method age range, #284.....104
4.27 Digital method age range, #242A.....105
4.28 Manual method age range, #242A.....105
4.29 Digital method age range, #305.....106
4.30 Manual method age range, #305.....106
4.31 Digital method age range, #250.....107
4.32 Manual method age range, #250.....107
4.33 Digital method age range, #321.....108
4.34 Manual method age range, #321.....108
4.35 Digital method age range, #304.....109
4.36 Manual method age range, #304.....109
4.37 Digital method age range, #359.....110
4.38 Manual method age range, #359.....110
4.39 Digital method age range, #374.....111
4.40 Manual method age range, #374.....111
4.41 Digital method age range, #242.....112
4.42 Manual method age range, #242.....112
4.43 Digital method age range, #309.....113
4.44 Manual method age range, #309.....113
4.45 Digital method age range, #67.....114

4.46 Manual method age range, #67.....114
4.47 Digital method age range, #254.....115
4.48 Manual method age range, #254.....115
4.49 Digital method age range, #338.....116
4.50 Manual method age range, #338.....116
4.51 Digital method age range, #354.....117
4.52 Manual method age range, #354.....117
4.53 Digital method age range, #302.....118
4.54 Manual method age range, #302.....118
4.55 Digital method age range, #330.....119
4.56 Manual method age range, #330.....119
4.57 Digital method age range, #331.....120
4.58 Manual method age range, #331.....120
4.59 Combined digital and manual method age distribution.....122
4.60 Combined digital method age distribution.....122
4.61 Combined digital method age distribution (w/o Cameriere techniques).....123
4.62 Combined manual method age distribution.....123
4.63 Combined digital method transparency age distribution.....124
4.64 Combined manual method age distribution.....124
4.65 Combined digital method pulp reduction age distribution.....125
4.66 Combined digital method age distribution (w/o Cameriere techniques).....125
4.67 Combined manual method pulp reduction age distribution.....126

Chapter 1: Introduction

The estimation of age-at-death of unknown individuals is routine practice in any osteological inquiry and is an inescapable feature in forensic, archaeological, paleoanthropological, and palaeodemographic contexts. It is also one of the most difficult tasks to execute properly as a multitude of forces are at play that introduce the potential for bias. The major conundrum surrounding the estimation of age-at-death is the inherent variation in the development and degeneration of age indicators across human populations separated by space and time. These deviations in expression of age markers can be attributed to cross-populational variances in biology, behavior, and genetics. In other words, each population is unique in the phenotypic and genotypic manifestation of age or the human body's reflection of the passage of time (biological and/or chronological).

The major and often singular source of age data for osteologists to analyze is the skeleton – bones and teeth. Over the linear lifetime, the skeleton transforms morphologically in a variety of ways. Despite the ever-present issues surrounding estimation of age from unknown persons, a wide array of methods and techniques have been developed and employed in order to determine age-at-death from various regions on the skeleton. However, not all locations on skeleton or dentition reflect chronological age or resist the post-mortem environment equally. Burial conditions have repeatedly been demonstrated to influence and bias age estimation for archaeological skeletal assemblages. The dentition has consistently proven to be one such robust and reliable data source comparable and possibly superior to bones, often surviving long after other hard parts have disintegrated. Another problematic area is how the age data is scored

(quantified) such as ordinal rankings that fit into phases or grades; these often give overlapping and or wide age estimate intervals. Subsequently, a more sophisticated means of quantification is metric measurement as it is expressed on a continuous scale and obviates observer subjectivity.

Two such adult dental age indicators that have proven most reflective of chronological age, are highly resistant to damage, and have parameters that are objectively measureable in both reference and archaeological samples are transparency of the tooth root and the reduction of the pulp chamber. Several quantification schemes have been developed for capturing transparency and pulp reduction, but not all of these techniques have correlated with age consistently. The Lamendin (1992) method and modifications (Drusini et al. 1989; Foti et al. 2005), Bang and Ramm (1970), and Miles (1963) have been well established as the most effective of transparency techniques. The techniques of Kvaal and Solheim (1994) and updates (Paewinsky et al 2005; Cameriere et al. 2004, 2007) have also been well received as applicable to archaeological samples. Transparency and pulp reduction have been applied to non-modern samples but in many instances multiple tooth types or large sample sizes are not available. In such cases, such, a strategy utilizing the two dental age parameters and applying multiple techniques and regression formulae is one such means of gathering maximum age data with minimal resources.

Although the dental age parameters have been assessed by traditional means, the advent of data acquisition from remote images and digital imaging and enhancement has more recently become utilized and will no doubt increase in use in the future to meet the needs of the global researcher.

The present research will apply the two techniques of dental age estimation using both manual and digital data acquisition methods on a sample of adult canines from the 5^{th}-3^{rd} centuries B.C. Greek colony of Apollonia Pontica, located on the Black Sea coast of Bulgaria. The dental age estimates will be assessed and compared with traditional morphological skeletal age markers. The current research objectives are:

1) using a single canine per individual, evaluate the use of two dental age indicators alone, for more effective age estimation over traditional/multiple skeletal age indicators,

2) test the utility and practicality of manual versus digital dental methods of measurements and techniques, and

3) create dental age estimates for a sample of individuals buried at the Apollonia Pontica site that produces one age estimate instead of an age range associated with skeletal age morphological indicators.

Chapter 2, Literature Review, establishes the context of the adult age estimation, and dental age markers and techniques. Chapter 3, Materials and Methods, illustrates the bioarchaeological and biocultural context of the Apollonia Pontica sample and describes the protocols used for the manual and digital data acquisition of the measurements, and the 17 techniques and regression equations used for age calculation. Chapter 4, Results, presents the findings of the manual and digital measurement techniques, intra-observer error tests, the age estimates and age ranges, age distributions, and correspondences with associated skeletal morphological age. Chapter 5, Discussion and Conclusions, elaborates on the age estimation results and implications for the study.

Chapter 2: Literature Review

Introduction

The ubiquitous phenomenon of aging or age-related change(s) as pertaining to practically all biological organisms may be defined as the "time-independent series of cumulative, progressive, intrinsic, and deleterious functional and structural changes that usually begin to manifest themselves at reproductive maturity and eventually culminate in death" (Arking 1998:12).While chronological time from a scientific perspective may be tracked or measured by the specific linear transgression of a calendrical sequence, biological 'time' is not so analogous and may be measured by "interlocking cascades of specific physical or chemical events" (Arking 1998:10). This is to say that there is a definitive complex difference between chronological age and biological age (Borkan and Norris 1980; Mitnitski et al. 2002). Chronological age remains unaltered or unchanging throughout time and space but biological age may increase or decrease in tempo and/or rate depending on somatometric, physiometric, and haematological variables (metabolism) as well as lifestyle, ancestry, and the environment (Dubina et al. 1984; Kemkes-Grottenthaler 2002).

Hard tissues, the source of age data, are often hardy and resist the burial environment and many cases are the chief source of information for physical anthropologists (Hoppa 2002; Larson 2002). Not all age indicators and techniques for age acquisition are identical in accuracy (how real or true is the final age estimate), precision (how refined is the age marker), reliability (probability that the age marker accurately yields the previous two criteria), and validity (how strong is the correlation between the

age indicator and chronological age) (White 2000; Kemkes-Grottenthaler 2002). Because of these unavoidable facts in sample and age indicator predispositions for inaccuracy on several levels, achieving realistic age-at-death and demographic reconstruction is difficult and not to be undertaken lightly (Woods et al. 1992).

When assessing any skeletal remains recovered in an archaeological or forensic context, the determination of sex, stature, ancestry, and age are all routine areas of inquiry (White 2000). The assessment of age is the focus of this research and the following will detail briefly methods of age acquisition and indicators of age located in the adult skeleton. In the case of reconstructing demographic profiles of unknown remains, the estimation of age is a fundamental and unavoidable practice (Hoppa and Vaupel 2002).

Age acquisition and, in turn, age determination of the unknown individual may be defined as the estimation of the age at the time of death through 1) the observation of morphological features on skeletal remains, 2) comparing this data to recent populations of known age (i.e. a reference population), 3) estimating the age of the individual, and then 4) determining sources of error or variability; variability is the discrepancy in the prediction of age between prehistoric and modern populations (Hoppa and Vaupel 2002). Step 2 is the most critical and is linked with the reference population for the developed method employed; it is at this phase that "morphological age criteria are established, given the known age in the reference sample" (Hoppa and Vaupel 2002:5). Skeletal age indicators or death structures are useful for age and demographic reconstruction because they are indicative of overall life expectancy, fertility, and population fluctuation (Kemkes-Grottenthaler 2002:48).

During physiological growth and maturation, the non-adult skeleton reflects chronological age with a high degree of predictability and accuracy; this is to say that there is a specific sequence to bodily changes (e.g. tooth eruption sequence, epiphyseal fusion, and bone growth rates) (Liversidge 2008; Schmitt et al. 2008). However, once maturity has been reached, accurately estimating adult chronological age becomes exponentially more challenging, especially with advancing age and most specifically in capturing older aged individuals - the right-most cohort or the 'oldest old' (Hoppa and Vaupel 2002; Miles 1958). The adult skeleton (bones and teeth) is constantly changing and altering with response to the passage of time, the environment, and variations in genetics. But, the "location, structure, and function" (Kemkes-Grottenthaler 2002:48) of these boney changes are reflected in different parts of the skeleton and change in tempo/rate at different times during life.

There are a variety of elements indicative of age located over the entire skeleton. Some markers include, but are not limited to: cranial suture closure, pubic symphysis surface morphology, the auricular surface of the ilium, sternal rib end morphology, clavicle epiphyseal closure, thyroid cartilage ossification, scapular ossification, vertebral lipping (Jakes 2000; Kemkes-Grottenthaler 2002; White 2000).

It should be noted that a certain degree of inter- and intra-element variance is always present – not all skeletal elements in one individual (or population) reflect the same degree of age related changes (either biological or chronological) or the same degree of correlation with true age (Bedford et al. 1993; Lovejoy et al. 1985; Wittwer-Backofen et al. 2008). These age markers each give different correlation coefficients as Table 2.1 demonstrates (adapted from Kemkes-Grottenthaler 2002:56). Each numeric

value in Table 2.1 illustrates the known age correlation coefficient with associated technique.

Table 2.1: Skeletal age indicators and variation in estimated age (years).

Indicator	♀	♂	Both sexes
Endocranial sutures (Acsadi and Nemeskeri 1970)	0.35	0.51	
Humerus (Acsadi and Nemeskeri 1970)	0.34	0.44	
Femur (Acsadi and Nemeskeri 1970)	0.58	0.56	
Pubic symphysis (Acsadi and Nemeskeri 1970, 5)	0.49	0.47	
Pubic symphysis (McKern and Stewart 1957, 1)		0.72	
Pubic symphysis (McKern and Stewart 1957, 2)	0.68	0.37	0.36
Pubic symphysis (Todd 1920, 1)		0.85	
Pubic symphysis (Todd 1921a, 2)	0.64	0.57	0.57
Auricular surface (Meindl et al. 1985, 3)			0.72
Multifactorial Summary Age (Lovejoy et al. 1985)	0.79	0.90	0.85
Dental wear (Lovejoy 1985)			0.71
Ectocranial sutures (Meindl and Lovejoy 1985)			0.57 lateral; 0.50 vault
Ectocranial sutures (Meindl and Lovejoy 1985)	0.34	0.59	0.56

(Adapted from Kemkes-Grottenthaler 2002:56. (1) Katz and Suchey 1986; (2) Meindl et al. 1985; (3) Meindle and Lovejoy 1985; (4) Kemkes-Grottenthaler 1996b); (5) Acsadi and Nemeskeri 1970)

The dentition has been demonstrated to match or even exceed traditional non-dental skeletal age estimation in terms of usefulness, accuracy, reliability, precision, and validity (when tested in reference populations) as relevant in paleodemography, and forensic or archeological contexts (Baccino et al. 1999; Ritz-Timme et al. 2000; Robbins 2000). As far as the analyses of ancient remains are concerned, teeth, because of their extreme hardness and high percentage of inorganic constituents, possess several useful and attributes that make them more prospective than bones. These include greater resistance to the burial environment (post-deposition), chemical alteration of their base physical (micro)structure (diagenesis , perimineralization, and fossilization), and are under the strictest genetic control and a slower metabolic rate (more constant/accurate reflection of inner biology/age related to chronological time), are more resistant to pathological insult, are more resistant to physical damage, and have a low incidence of remodeling and/or resorption (teeth do not remodel/resorb under normal circumstances). Teeth are, for all intents and purposes, biological stones (Lucas 2004). The following will elaborate on adult dental age changes.

For over a half a decade, the gradual time-metamorphic changes witnessed in the dentition have been utilized for ascertaining the age of the adult person (Gustafson 1947, 1950). There are at least 22 perceptible age-related changes in teeth which occur over the lifetime (adapted from Bang 1989:220; Rösing and Kvaal 1989:444):

- External changes:
    1) Attrition and abrasion
    2) Color changes
    3) Florescent changes

4) Etching characteristics

5) Microscopic changes in the surface

6) Gingival retraction/periodontal retraction

7) Addition of gross cementum thickness

8) Resorption of the root

9) Caries

10) Number of teeth

11) Cementum annulations

- Internal changes:

1) Vascular changes

2) Regressive changes to the pulp

3) Neural changes

4) Chemical composition

5) Loss of water from dentin

6) Increase in peritubular dentin

7) Dentin hardness

8) Secondary dentin deposition/pulp reduction (Figure 2.1, A)

9) Transparency of the root (Figure 2.1, B)

10) Racemization of amino acids

11) Stabilization of collagen

All of these changes progress with linear time, are interrelated, and affect one another to a certain degree, but not all reflect the passage of chronometric time equally. For example an individual or particular tooth may have similar transparency and/or pulp

reduction age estimates, but show no signs of caries or root resorption. In other words, not all of these regressive changes reflect chronological time and/or age identically, nor are all applicable to archaeological dental material. Figure 2.2 illustrates the progression of transparency and pulp reduction over the linear lifetime.

Figure 2.1: Histological view of a tooth: A) secondary dentin/pulp reduction; B) root transparency (thick-section prepared by Laurie Shead)

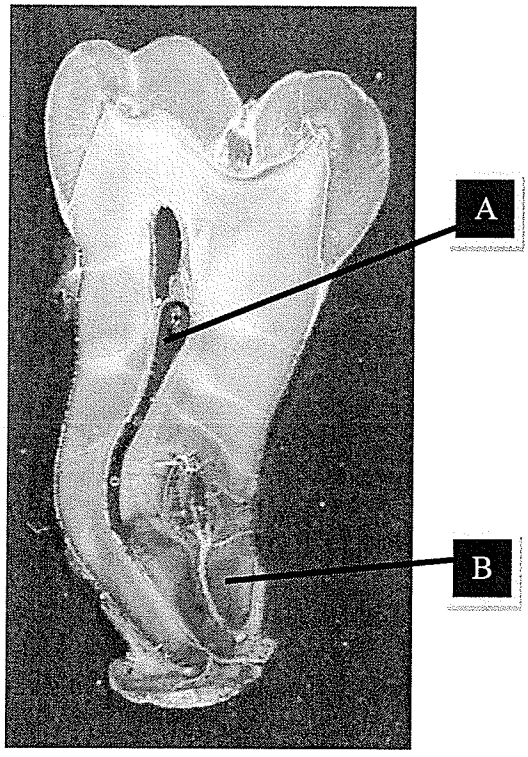
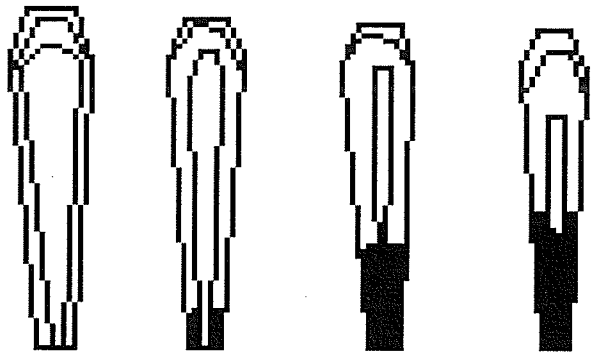


Figure 2.2: An illustration of the progressive shrinkage of the pulp chamber and increase in length of transparency over the lifetime.



Adult dental age estimation

Age estimation by a single dental criterion

Secondary dentin apposition/pulp reduction

Over the lifetime of the adult individual, the continuous and progressive formation of secondary dentin to the pulp chamber and root canal walls, causing a reduction in volumetric size, occurs (Morse 1991; Morse et al. 1991; Wakabayashi et al. 1993) (see Figure 2.1, A). This phenomenon is mainly related to age and appears to be a normal part of the process itself in a response to stimulus (masticatory, temperature, and osmotic changes) that the tooth is subjected to. This accumulation of tissue onto the pulp walls is not uniform and is most rapidly generated on the floor, although first beginning in the upper labial crown in anterior teeth because of occlusal forces (Philippas and Applebaum 1966, 1968). In contrast, generation of secondary dentin is much more stable

and/or even in tempo from the CEJ (cement enamel junction) to the central portion of the root, as these areas are protected from insults – it is these areas that reflect chronological age. It is not agreed upon precisely when secondary dentin first forms, but it is generally held that it does not begin prior to root completion and most likely after physiological maturational growth halts, generally around the age of 18 at the time of apex closure. The pattern and speed of formation is unique to each tooth type as different teeth are completed at different times and are placed under different kinds and amounts of pressure (Parkinson 2003). It appears that at younger ages (23 – 39 years) a reduction of the pulp occurs apically, coronally, and horizontally at a steady rate, but in advanced ages (60 – 80 years) an increase in this reduction occurs (Morse et al. 1991). Nevertheless, this morphological change has been determined to be a very accurate and reliable criterion for age. This is because of its protected location at the center of the tooth promoting stability and resistance to noxious (pathological and/or physical trauma) insults. Interestingly, surface caries on the lingual surface of roots and/or attrition do not affect the rate of secondary dentin formation patterning (Phillippas and Applebaum 1964; 1968). Nitzan and colleagues (1986) found a marked difference in the width of secondary dentin formation between functioning and impacted canines, where the latter had reduced additions to the pulpal walls indicating that mastication plays a causal role in gradual deposition (i.e. thickness).

Ito (1972, 1975) was the earliest to experiment with the metric assessment of the tooth-crown index alone and the relation to age of the individual. His technique made use of 32 teeth of all types from individuals aged 10 – 70. These were sectioned bucco-lingially to 1.00 mm and micro-radiographed (5 ma, 90 kv). Each tooth in the form of a

radiographic image was then measured by drawing a line at the CEJ, thereby indirectly examining the specimen by dividing it at the anatomical crown. Next, using a planimeter, the area of the crown, coronal pulp cavity, and coronal dentin was recorded and quantified as the following index: enamel area + area of coronal pulp cavity /area of coronal dentine x 100. In this way, crown attrition and the gradual addition of coronal secondary dentin was captured.

It was found that a negative correlation between age and the previously mentioned parameters occurs for all teeth. Furthermore, there is a age estimate relationship between tooth groupings (such as anterior, posterior, and premolar teeth), meaning that not all teeth reflect the measured phenomena equally. Specifically, molars are less accurate than anterior or premolar teeth; however, the canine proved to be the most unreliable tooth type in reflecting age. Because of this discrepancy in tooth groupings correlating to age, Ito (1972, 1975) created three different regression formulae for the respective tooth types. For the time, his method is unique in its use of indices instead of absolute scoring of criteria like Gustafson (1950) or Johanson (1971). In addition, a photorealistic record of the sample is produced as opposed to a hand sketched recreation of the tooth.

Using similar methodology to Ito (1972 and 1975), Ikeda and colleagues (1985) made improvements in acquisition techniques and used length measurements instead of area measurements to calculate age on a sample of 119 extracted teeth (56 incisors and 63 molars). Each whole tooth was radiographed and enlarged 4x. On these prints, the length of the coronal pulp cavity and crown was recorded in millimeters. To accomplish this, horizontal lines were placed at the CEJ, the highest coronal pulp horn, and the highest crown cusp. These values were then used to create a "tooth-coronal index" which he

adapted from Ito (1975:245). This study also found a negative correlation between the parameters and age, and acknowledged that the two tooth groups were required for individual regression formulae. This methodology has subsequently been very influential on using pulp and tooth dimensions for age determination, and has been cited by nearly every worker in this field.

Woods and associates (1990) undertook a cross-sectional study comparing age changes and pulp dimensions between sexes in two distinct populations. Their sample consisted of 234 randomly selected dental patients, aged 11 – 78 years, from 'black' (40 male/68 female) and 'white' (64 male/62 female) populations at the University of Tennessee, Memphis. Disease-free maxillary first incisors and mandibular canines and second molars were used. Full mouth radiographs with each tooth's labial/lingual aspect parallel to the film were collected, illuminated, and measured directly with a low-powered lens and sliding calipers. Maximum pulp widths and root lengths were recorded. A three-way factorial analysis of variance was used to test for black/white, sex, and age for each measurement. Regression analysis demonstrated that regardless of 'race', pulp diminution differs between sexes; in males, a relatively constant tempo in pulp shrinkage occurs but females display two distinct phases of secondary dentin apposition. During the first through fourth decades females exhibit regular deposition but between the ages of 30 – 50 the pulp rapidly occludes. In addition, 'white' males tend to undergo more rapid pulp shrinkage in older ages. Overall, age correlates were low regardless of tooth type, however, this research is valuable for the fact that population origin of the sample is an important consideration for accurate age estimation.

Solheim (1992) investigated secondary dentin in 1,000 half-sectioned anterior and premolar teeth and compared his quantification scheme to that of other researchers. His sample is identical to that used in Solheim (1989). Secondary dentin was measured by the half-tooth technique (labio-lingual orientation) under a stereomicroscope and assessed by the scores of Gustafson (1950), Dalitz (1962), and Johanson (1971). In addition, pen marks were made laterally across the ground root surface at four equal positions starting at the CEJ. The following measurements were then recorded: (CAP) area of crown pulp, (STC) total tooth width at CEJ, (SPC) pulp width at CEJ, (STCQ) total tooth width at ¼ root, (SPCQ) pulp width at ¼ root, (STM) total tooth width at mid-root area, (SPM) pulp width at mid-root area, (STA) total tooth width ¼ root, and (SPA) pulp width at ¼ root. Tooth/pulp ratios were assessed at each individual level and as the sum total of all four levels. The results showed that of the three scoring procedures, Johanson's (1971) method correlated strongest with age. The sum of the total root and pulp widths had stronger age correlation than any individual ratio; the coronal pulp area measurement also showed a strong negative correlation with age. Pulp widths are most strongly correlated with age at the CEJ and decrease as the apex is approached. Not all teeth are equal regarding age and secondary dentin formation; premolars showed a weaker correlation than other tooth types. In addition, canines may contain less pulp than other tooth types (Philippas and Applebaum 1968).

Kvaal and associates (1995) tested the indirect metric width measurements of secondary dentin and/or pulp size from periapical dental radiographs. Full mouth x-rays were collected from 100 persons ranging from 20 – 87 years of age. Mandibular second incisors, canines, and first premolars and maxillary first/second incisors and second

premolars were evaluated. This study differs from Kvaal and Solheim (1994) in that the measurements are slightly more complex; all other protocol being identical. Using Vernier calipers and a stereomicroscope, the following variables were recorded (labiolingual orientating in the mesial tooth surface): (T) maximum tooth length, (R) root length on mesial surface, (P) maximum pulp length, (A) root and pulp with at CEJ, (B) root and pulp width at mid-root, and (C) pulp and root width at ¾ towards the apex. Two different observers were used to assess for intra- and inter-observer error tests. The statistical tests were principal component and regression analyses, respectively (in said order). Age was the dependent variable and the principle component; likewise, sex was the independent variable was included in a stepwise procedure. Calculation was done separated by measurements for the upper/lower jaw and tooth type. Correlation coefficients between ratios and age showed that tooth/root length alone was not significant and all others were negative and significant. The strongest correlation with age was the mean value of all ratios when all six tooth types were included an approach which may best represent the overall size of the pulp. Width measurements at the CEJ were not significant in mandibular canines while maxillary canines demonstrated an overall low correlation with age. No differences were detectable between right or left sides of the jaw.

Murray and others (2002) directly measured secondary dentin and odontoblast density on labio-lingual thin-sections. Their sample consisted of 147 incisors, canines, and premolars taken from dental patients between the ages of 10 – 49 years. Specimen preparation included fixation and demineralization followed by encapsulation in paraffin wax, ground to 6 μm, and finally stained. At 50x magnification under light microscopy

the mean labio-lingual dentin thickness was ascertained by evaluating three locations on each specimen: crown dentin pulp border, CEJ, and at 75% the distance between the CEJ and apex. A comparison of all age groups (youngest cohort to oldest cohort) demonstrated that dentin thickness increases at a mean rate of 20 μm (10 μm per wall) per year in the root and 10 μm (6.5 μm per wall) in the crown, and odontoblast density deceases by 40.6% in the root and 15.6% in the crown. More precisely, the age reduction of odontoblast density was not readily observed in the 10-30 year old cohort while the inverse was found in the 31-50 year old cohort.

Bosmans and co-workers (2005) tested the measurements proposed by Kvaal et al. (1995) on a large sample measured on digital images. A total of 197 individuals (102 male/95 female) represented by six tooth types comprised their sample: maxillary first and second incisors, second premolars, and mandibular second incisors, canines, and first premolars. The sample was collected from a 'Caucasian' population, gathered from three Belgian dental practices, ranging in age from 19 – 75 years. The authors undertook a blind-study and kept prior patient knowledge unknown. The measurements were entered into Dental Age Estimation (ver. 2.0) software and automatically calculated for age.

Overall, the correlation coefficient for chronological and calculated age was very high (0.82 – 0.93). Other authors have also noted the chief source of discrepancies came from difficulty in defining reference points; vertical length measurements (root length), however, were not any less reproducible in the two methods. Their findings were different from the Kolltveit and colleagues' (1998) protocol, very high intra-observer agreement was found and suggests that digital imaging fares better in accuracy and precision than conventional means of measurement.

Paewinsky and colleagues (2005) published important research testing Kvaal et al.'s (1995) protocol on digital images with three different statistical analyses. Orthopantomograms (OPG), full mouth radiographs, were obtained from 168 individuals (91 male and 77 female), 14 – 81 years of age, from the Department of Dental Medicine, University of Munster. The sample consisted of mandibular second incisors (n=153), canines (n=158), first premolars (n=156) and maxillary first incisors (n=144), second incisors (n=138), and second premolars (n=101); teeth with caries or fillings were excluded. Each OPG was scanned so that the labial aspect of each tooth was visible and converted into a TIFF image file; the images were then exported onto a desktop computer. Digital image analysis was carried out with the program Hipax (ver. 3.01) following Kvaal and associate's (1995) linear measurements. Scoring was done by two independent observers. Intra-observer measurement variation was below 2%, inter-observer variation was larger although still small (<2%), and measurement values obtained by both examiners were larger (>10%) but the discrepancies were "systematical and monodirectional" (Paewinsky et al. 2005:29). Statistical analysis was carried out using nonparametric linear, cubic, and logistic regressions. No differences between sex or length measurement ratios were observed. Just as other researchers have found, width ratios were significantly correlated to age, and the strongest correlated ratio region was found at the CEJ. Combining all width values from all teeth, the linear coefficient was r=0.95 with a standard deviation of 5.6 years, which is moderately low. Mandibular canines alone were surprisingly quite high, r=0.81, which is significantly higher than what others researchers have found.

Cameriere and associates (2004) tested the Kvaal and coworkers' (1995) linear measurement technique as well as a new method of "pulp/tooth area ratio" or "RA" (Cameriere et al. 2004:1). The sample consisted of incisors, canines, and premolars taken from 92 Italian 'Caucasian' individuals (46 men and 46 woman) ranging in age from 18 to 72 years.

Using stepwise regression, the two variables found to match closely with chronological age (r^2=0.849) were 'AR' (pulp:tooth area ratio) and 'c' (pulp/root width ratio at mid-root). Controlling for tooth type and age parameter, maxillary canines measured by the AR ratio alone and not any other variable, demonstrated the highest correlation between estimated and knownage (r^2=0.842).

The variables that had the lowest correlation with age were tooth/root length. These variables are functionally dependant on criteria that appear to change little over time. This publication marks a positive trend in pulp research for age estimation that utilizes a digital scoring medium with fewer variables, tooth types, measurements and more robust statistical analyses in order to achieve higher and more accurate age correlations in older age groups (at least 72 years old) and young ones (compared to Kvaal et al. 1995 or Solheim 1992).

Cameriere and associates (2006) tested the correspondence of macroscopic skeletal age indicators and the pulp/tooth ratio (RA) age estimation method (Cameriere et al. 2004) on known-age archaeological/historical remains. Specifically, only maxillary canines were analyzed. The sample of 52 individuals came from three European Middle Age sites. Sample one included 33 adult fragmentary individuals; the second sample had ten individuals with complete skulls, and sample three consisted of nine known-age

mummies. The concordance of skeletal and estimated dental age of the first two samples was estimated to be 84% and the error between the mummies and the estimated ages was ±5.34 years at the maximum.

Building upon past research (Cameriere et al. 2004 and 2006), Cameriere and colleagues (2007) developed the most up-to-date, accurate, and simple method of using the pulp/tooth area ratio method (PTR). Examining both maxillary and mandibular canines, they tested a known-age comparative collection sample and achieved a S.E. of 5.4 years. PTR was assessed on periapical radiographs using a sample of 200 canines (114 teeth from 57 males; 86 teeth from 43 females) from a 'Caucasian' osteological collection at the University of Bolognia. The collection was comprised of individuals born between 1828–1916 and who died 1918–1932. Digital periapical x-rays were obtained on teeth without pathologies at an exposure of 10 minutes, 70 KV. In contrast to other age estimation methods using secondary dentin (e.g. Kvaal et al. 1995 and Solheim 1992), this study used area instead of linear measurements alone on both labiolingual and mesiodistal aspects of the canine. Dental maturity was defined by partitioning the pulp/tooth area ratio on both labiolingual mandibular (x_1) and mesial (x_2) teeth along with mandibular labiolingual (x_3) and mesial (x_4) teeth. Estimated age used the variables x_1, x_2, x_3, x_4 to create a multiple linear regression model with first order interactions between x1 and x3. In addition, separate prediction lines were estimated for upper and lower canines. Further, the regression model's accuracy was tested by comparing the true and estimated ages of the skeletons. Forty x-rays were re-examined for inter-observer error by concordance-correlation.

Cameriere et al.'s (2007) results indicated no inter-observer differences, and Pearson's coefficients showed that all morphological variables were significantly negatively correlated with age.

When both canines (x1 and x2) were included in the age prediction, the correlation was very strong results (r=0.925) and only slightly lower when either single canine was included (r=0.86). Sex differences in estimated age were not significantly different for either canine used. This research shows that age estimation is most accurate when both canines in both orientations are included. However, if only one canine is available it is acceptable to use the labio-lingual orientation for calculation alone. This method is universally applicable, accurate, rapid, non-destructive, and requires only one tooth.

Table 2.2: Pulp reduction publications illustrating correlation coefficients with known age separated by tooth type.

Publication	Tooth-Type(s) Used	Measurement Method	Measurement Technique (value inputted into equation)	Known-Age Correlation Coefficient
Kvaal and Solheim 1994	All incisors, canines, and premolars	Labio-lingual periapical x-rays. All measurements made with calipers	Max 1st I: pulp:root width CEJ ratio Max 2nd I: pulp:root width CEJ ratio Max C: pulp:root length ratio Max 1st PM: A) pulp:root width CEJ ratio, pulp:root length ratio, and transparency; B pulp:root width CEJ ratio, periodontal retraction, and pulp:root length ratio Max 2nd PM: A) periodontal retraction, pulp:root width CEJ ratio, and pulp:root length ratio; B) periodontal retraction and pulp:root CEJ ratio Mand 1st I: A) pulp:root CEJ ratio and transparency; B) pulp:root CEJ width ratio	Max 1st I: r=0.50 Max 2nd I: r=0.72 Max C: r=0.48 Max 1st PM: A) r=0.83; B) r=0.83 Max 2nd PM: A) r=0.90; B) 0.86 Mand 1st I: A) r=0.65; B) r=0.55 Mand 2nd I: r=0.71 Mand C: A) r=0.78; B) r=0.65 Mand 1st PM: r=0.78 Mand 2nd PM: A) r=0.82; B) r=0.77

			Mand 2nd I: pulp:root CEJ width ratio Mand C: A) pulp:root width CEJ ratio; B) pulp:root width CEJ ratio and transparency Mand 1st PM: pulp:root CEJ width ratio, pulp:root width mid-root ratio Mand 2nd PM: A) pulp:root width CEJ/mid-root ratio and transparency; B) pulp:root width CEJ/mid-root ratio	
Cameriere et al. 2004	Max canine	Labio-lingual full mouth dental x-rays, AutoCAD	A) Pulp:tooth area ratio and pulp:root mid-root ratio; B) pulp:tooth area ratio	A) r^2=0.849 ; B) r^2=0.8426
Paewinsky et al. 2005	Max 1st/2nd incisors and 2nd premolars; Mand 2nd incisors, canines, and 1st premolars	Labio-lingual full mouth dental x-rays	A) pulp:root CEJ width ratio; B) pulp:root ¼ root width ratio; C) pulp:root mid-root width ratio	Max 1st I:A) r=0.84; B) r=0.80; C) r=0.80 Max 2nd I: A) r=0.0.91; B) r=90; C); r=90 Max 2nd PM: A) r=0.91; B) r=0.89; C) r=88; Mand 2nd I: A) r=90; B) r=0. 86; C) r=88; Mand C: A) r=0.81; B) r=0.80; C) r=0.80; Mand 1st PM: A) r=0.89; B) r=0.90; C) r=0.88

Cameriere et al. 2007	Max and Mand canines	Labio-lingual LL) and mesio-distal (MD) periapical x-rays	Pulp:tooth area ratio. All Cs: P/T area ratio max C LL, P/T area ratio max C MD, P/T area ratio mand C LL, P/T area ratio mand C MD Max C: P/T area ratio LL and MD Mand C: P/T area ratio LL and MD	All Cs: r^2=0.94 Max C: r^2=0.89 Mand C: r^2=0.90

Transparency

Transparent (synonymous with: translucent, sclerotic, root dentin translucency, RDT, and apical translucency) dentin is the by-product of a progressive transition to a crystal-like appearance in the tooth roots with advancing age (see Figure 2.1, B). Coronal translucency also exists but this type is pathological or trauma related (Balooch et al. 2001). Like secondary dentin initiation, the exact age that translucency begins is also under contention with the ages of 18, 20, 25, and 30 years of age have been cited.(Olze et al. 2004; Prince and Konigsberg 2008). Most likely, this age discrepancy of researcher's estimation of initial formation is probably due in part to 1) the age range of samples, and/or 2) population-specific timing (though sex has been noted to have little effect on expression). This phenomenon begins at the root apex and gradually spreads towards the crown, eventually taking on a 'butterfly' appearance most marked in the mesial and distal portions of the tooth (see Figure 3.4; note the middle portion of transparency is longer

than the labial or lingual portions); in older aged persons the entire root may become translucent (Vasiliadis et al. 1983a, b).

The cause of this change from unaffected (non-transparent) or 'opaque' dentin to translucent or transparent dentin is caused by the gradual infilling of the dentinal tubules (but also the intratubular dentin, and/or peritubular dentin) with a mineral substance that closely resembles base hydroxyapatite or calcium salts (Senawongse et al. 2002, 2006; Traub et al. 1988; Weber 1967, 1974) resulting in 'needle-like' crystal growth in these areas (Porter et al. 2005). This substance is rhombus or cuboid in shape and once it has infiltrated the dentinal structure, it causes the refractive indices to equalize and transmit light instead of refracting it like 'opaque' dentin (Van Huysen 1960, 1961a, b). The phenomenon of root translucency is a natural part of the aging process and has been well documented as possibly a functional response of the tooth to gradually become more impermeable, thus more resistant to carious or physical insults with advancing age (Senawongse et al 2006). The amount of transparency is also similar in normally functioning and impacted, non-functional teeth, clearly indicating that age is probably the most important factor in progression (Azaz et al. 1977). Interestingly, Brinkman and Hartman (1980) found no differences in mineral content between translucent and normal dentin and proposed that it may be a loss of water as opposed to increased mineralization. In contrast to this, Kinney et al. (2005) did find a difference in mineral content between the two 'types' of dentin.

The first study to assess transparency as an age indicator alone was by Miles (1963). His research quantified the maximum length of transparency in millimeters on labio-lingual sectioned incisors of knownage. Although his publication is not clear on

methodology and sample size, it is the first metric analysis of this criterion for age estimation

Bang and Ramm (1970) offered the first validation study of age estimation by measurement of apical root translucency as a single age criterion. This measurement protocol has since become one of the most popular and extensively applied to date. Their sample consisted of 926 teeth of known age and sex with all tooth types represented. A unique aspect of this study is that both 400μm thick labio-lingual sections and intact roots were evaluated for comparison. Root measurements were taken in front of a constant light source with Vernier calipers (to the nearest 0.5 mm). The total root length "was measured bucally in the midline from the cemento-enamel junction to the apex" (Bang and Ramm 1970:4), and translucent dentin was measured from "the apex of the root in coronal direction to the borderline between transparent and opaque dentin" (Bang and Ramm 1970:4). If the junction of translucency was not a horizontal line then minimum (TL1) and maximum (TL2) measurements were taken and the mean was recorded. Also, some sections were enlarged for area measurement with a planimeter. As a cautionary note, if a large portion of apical cementum was present it was not included in the final measurement because of the error it may introduce by altering the pattern of visible transparency.

Bang and Ramm's (1970) results demonstrated that intra-observer error had no significant effect on differences in length measurements, but did impact area measurements. Incisors and canines were considerably easier to measure in both sections and intact root than any other tooth type. TL1 and TL2 had to be measured on 7.8% of intact specimens and increased to 21.7% on the sections. The sample was analyzed by

tooth type, age of root completion, sex, specimen collected at autopsy, and specimen collected in living subjects. Correlations ranged from 0.50 and 0.93 which means that not all tooth types reflect age accurately. It was found that whole, un-sectioned teeth, possessed higher correlation than sectioned ones. Interestingly, measurement of transparency length alone and expressed as a percentage of the entire root length were similar in age correlations. The small sample of 62 root filled teeth (dental restorations such as fillings) did exhibit variance in extent of transparency and 14 impacted teeth showed comparable results to normal ones. They conclude that translucency can be used alone for accurate age estimation.

Solheim (1989) investigated dentin translucency by evaluating length and area measurements related to age and sex. His sample consisted of 1,000 clinically extracted teeth from the states of Washington and Oregon, primarily from a cosmopolitan 'Caucasian' population. Only incisors, canines, and premolars were used; a total of 100 (50 per side) of each tooth type which had been stored in 4% formaldehyde. The source, reason for extraction, race, sex, and age of the patient was recorded. Linear measurements by Bang and Ramm (1970) were employed on both un-sectioned and half-teeth and on dry and wet specimens. Additionally, area^2 was measured off dry-sectioned roots superimposed by a photographic grid film; scoring procedures followed Gustafson (1950), Dalitz (1962), and Johanson (1971). Using Spearman rank and Pearson coefficients, their results demonstrated that measurement by light penetration through intact dry root correlated with age better than all other methods ($r=0.72$). No right or left size differences were found, contrary to the findings of Bang and Ramm (1970). Still other investigators have assumed identical dimensions of translucency in all tooth types

(Johanson 1971; Miles 1963). Separation by sex indicated that males have slightly longer zones of translucency than females, possibly due to the fact that males have larger and longer tooth dimensions. Interestingly, area of root/translucent zone measurements suggest that the area of translucency is influenced only to a small degree by the size of the root. No relationships were found between translucent zone size and periodontal destruction, cementum apposition, and root color.

Drusini and colleagues (1989, 1990) examined transparency in thin-sectioned specimens, ultimately favoring whole teeth as this allows for a more complete representation of the affected dentin. A thinner section, however, may not show the fullest extent of RDT. Therefore, sections from 500 – 600 μm appear to capture a larger portion of RDT. The sample consisted of 70 teeth from an Italian osteological collection (from individuals who died between 1890-1930), stored in ethylmetacrylate, and finally embedded and cut bucco-lingualy as close to the tooth's midline as possible; teeth with multiple or twisted roots posed more challenges than single-rooted teeth. The sections were examined under a microscope and enlarged 6x; metric measurement was done by a micrometer in the eyepiece. Scoring was made by two observers (based off Lamendin and Cambray (1989) technique), each recording the following: extension of root transparency (h) and total root height (H). These were then calculated as a percentage ratio: h x 100/H. Bang and Ramm's (1970) regression equations were then applied. This quantification is unique because it accounts for inter-tooth root variation in size compared to length of translucency instead of an absolute measurement. In contrast to previous studies, poor age correlation were obtained for the entire sample, with premolars holding the highest ($r=0.58$). Population differences and the use of an inappropriate regression

formula originally based on a Scandinavian sample may be sources of error. Another possible source of error may be that Bang and Ramm's (1970) regression formulae may not be compatible with Lamendin's measurements. They conclude that despite the use of an increased thickness of the sections (600 μm) compared to other authors (250 μm), the orientation of bucco-lingual sections may not capture the true extent of transparent dentin. Therefore, intact teeth should be used as they may be rotated over a light source which allows the examiner to observe the upper limit of transparent dentin more effectively than a section.

Similar in methodology, Drusini and associates (1991b) evaluated both modern and historical dental samples and compared conventional versus computer assisted measurements. The total sample consisted of 152 single-rooted teeth (incisors, canines, and premolars) from known-age individuals. The modern group consisted of 86 extracted teeth and the historical Italian sample of 66 teeth (see Drusini et al. 1990). For manual evaluation, each tooth was measured in front of a light source with the maximum transparency and root length recorded by Vernier calipers. Digital image conversion made use of black and white photographs of each tooth converted to 512 x 512 sized pixel images. Computer-assisted scoring made use of the IBAS 2000 software which permitted geometric measurements, adjustment of optical density, sharpness, and a grey value line profile feature; semi-automatic measurements were also programmable.

When the values from both methods were compared, the highest age correlation coefficients were for premolars measured by conventional means ($r=0.84$) and not for computer-assisted scoring. Moreover, the highest success with the lowest rate of error within ±5 years was for the historic premolars (48.49% for caliper and 45.46% with

image analysis software). Drusini and colleagues (1991b) conclude that a regression equation for a recent sample can be used for skeletal samples from at least (less than) 100 years ago. Additionally, despite traditional caliper measurements being slightly more accurate than computer assisted scores from images, digital evaluation does have some immediate advantages: semi-automatic illustration of the tooth, illustration of differences in transparency, and automatic generation of metric data.

In a similar study, Drusini (1991a) controlled for sex-related differences in the amount of transparency. Drusini (1991b) returned to the same sample as Drusini and associates (1991b), but also included molars (n=118) along with premolars (n=41) and anterior teeth (n=49). The regression results suggested that the highest correlations with age were for female anterior teeth; however, the slope lines were not significantly different. No significant differences were observed for the whole sample (male, female, and/or entire sample). In particular, the canine was said to have the greatest root thickness which may introduce measurement error. As a result, Drusini et al. (1991a) recommend partitioning this tooth type from others in regression analysis. The authors indicate that this slight increase in the amount of the translucent zone in males as opposed to females is probably due to males having larger tooth dimensions than females, with males often showing more attrition; this means that greater functional forces are generated on male teeth.

In a small but important publication, Bang (1993) examined three archaeological teeth from a single individual and evaluated both intact and sectioned specimens. The small dental sample was recovered from Norway and consisted of a mandibular canine and second premolar, and a maxillary canine. Radiocarbon dating placed the skeletal

remains to 7,950 ± 110 years old. Measurement protocol (and formulae) on both whole and sectioned teeth according to Bang and Ramm's (1970) instructions were carried out. The teeth were wax-embedded and sectioned labio-lingually to 400 μm thick.

The individual was estimated to be around 60 years of age when the mean of all calculations were examined. When the intact and sectioned measurements were compared, some slight discrepancies were found, specifically, differences in the limit of translucency caused differences in estimated age. For example, translucency length of the lower canine was 6 mm for the intact tooth and 5 mm for the section which gave calculated ages of 62 and 57 years of age, respectively. Furthermore, the extent of transparency was easily determined in all but the maxillary canine. The demarcation between opaque and translucent dentin was difficult to estimate on the canine because the boarder was indistinct. It is unknown if this difficulty in measurement is due to post-depositional alterations or physiological in nature and naturally occurring. Nevertheless, good age estimates were obtained in two out of the three teeth.

Ermenc (1997) experimented with thin-sectioned teeth and found a close connection between the metric increase in apical transparency height and age of the individual. The sample included 105 known-age single-rooted teeth from both upper and lower jaws taken from cadavers who ranged between 10 – 85 years of age. Each tooth was uniquely thinned in the mesio-distal longitudinal direction and polished; the sample was presumably not embedded and the exact thickness of each section was not given. The teeth were then illuminated with a halogen light by a Heliomat H2 (1,000 watt) with the height of transparency recorded in millimeters. Using linear bivariate regression, the author calculated the linear increase in height of transparency with advancing age of the

individual (with a 95% confidence interval). The author notes that transparency that is less than 2.5 mm in length is unreliable in age estimation. Further, the absence of transparency was only observed in individuals up to 18 years of age in most cases and no instances of transparency in individuals under 15 were found. This means that the lower age limit for initiation of transparency is realistically 15; however, 18 years of age is more probable because a higher incidence of these teeth with no transparency occurred in the sample. Moreover, teeth with a transparency height of 2.5 mm or more belong to individuals 23 years of age and realistically teeth from this same cohort should be considered the starting point for the age marker.

The effect of an individual's geographic population of origin and its expression on the variation of translucency formation related to age was discovered by Whittaker and Bakri (1996). A sample of 198 extracted single-rooted teeth of known age representing Malay, Chinese, Indian, and Welsh populations was evaluated. The teeth were stored in thymol (to prevent mold), bisected to slightly one side of the midline, imbibed with methylene dye, and longitudinally serial sectioned to 250 μm. Each section was then enlarged 25x and digitized (Apple 2 digitizer). This study utilized a unique index quantification of the parameters: area of translucent dentin (A1), area of non-transparent dentin (A2), the length of translucent dentin from apex to initial horizontal deviation from pulp (L1), root length from translucency deviation to highest point of translucency (L2), and the estimated length of translucency based on the previous measurements L3 = L2 x A1/A1+A2; all length measurement were made by Vernier calipers.

In all cases besides the Malay group, the highest age correlations were found when single sections through the mid-line of the tooth were included in the regression

formula instead of area measurements. The reason(s) for the Malay group being the exception is unknown. In addition, age correlation and length measurements were higher in the Welsh (r=0.79) sample than the others; within the non-Welsh groups the Malay (r=0.66) correlated highest, Chinese intermediate (r=0.60), and Indian lowest (r=0.62). Interestingly, when four sections were included the values for length and area were similar in correlation for all age groups besides the Chinese; length measurements were still found to be stronger in the Chinese despite the use of more sections. This publication strongly suggests that not all populations reflect age related changes in identical ways and that various factors may be at play within a single cohort to cause translucent dentin formation. Likewise, each researcher should consider making their own regression formulae for assessing each sample. The feather-like border that marks the interface between transparent and opaque dentin is not always easily identified, and imbibing with dye for examination with the unaided eye may be most appropriate in differentiating the junction. In these cases where the border between dentin is at an acute angle, the previously described quantification may more accurately capture the true measurement of translucency.

Sengupta and associates (1998) experimented with archaeological teeth and specifically addressed sampling and sectioning issues related to post-depositional problems. Conventional versus computer-assisted measurements were compared. No variable, such as sex, age, or specimen condition, were taken into account. Two skeletal assemblages of differing antiquity and burial environment were evaluated: 100 dentitions from coffin burials at St. Peter's Church (8^{th} – 19^{th} AD) and photographs of 220 prehistoric Danish dentitions. Non-carious extracted teeth were also examined to

compare with the archeological sample for insight into specimen preparation techniques. Bang and Ramm's (1970) measurements were performed on all samples using Vernier calipers. Teeth were embedded in methyl methacrylate which was found to stabilize the tooth structure better than epoxy resin. Each embedded tooth had three bucco-labio-lingual longitudinal sections cut from the midline, ground to 150 μm, and finally stained. Four measurements were recorded: length of RTD, % length RTD/length of root, area of RTD, and % area of RTD/area of root. If the transparent zone was not even then the average was recorded. The program MicroScale TM/TC was used for digital image analysis on monochrome macro photographs; this program allowed for contrast enhancement and semi-automatic tracing. Paired *t*-tests were used for statistical comparisons.

The authors favored single-rooted teeth due to ease of sectioning (longer and wider roots) and the fact that they are less prone to caries. They claim that methyl methacrylate is superior to epoxy in reinforcing friable ancient teeth; however, they make no mention of vacuum impregnation. Furthermore, methyl methacrylite may mimic the refractive index of RTD which would introduce artifactual error; despite this they found no such problems. Staining did not improve any contrast between opaque and translucent dentin. One problem that accompanies sectioned samples is that the entire complex 3D structure of RTD may not be captured. Furthermore, the angle of the cut will likewise affect the length and area measurements as well. Finally, they conclude that the choice tooth for ease of measurement and reproducibility is the mandibular canine when evaluating intact material by the same observer.

In a follow-up to this study, Sengupta and colleagues (1999) examined both modern and archaeological samples. They claim that dentin translucency as an age criterion is not appropriate to apply on non-modern samples because of burial alterations. The samples comprised only lower canines; the modern sample consisted of 56 extracted known-age, non-carious mandibular teeth from a 'Bristol' population and the archaeological sample consisted of 61 non-carious teeth from the Spitalfields collection. Each tooth was embedded in methyl methacrylate and three bucco-lingual longitudinal sections (150 μm) were prepared. Dentin was placed into three categories based on appearance: dentin translucency, post-depositionally altered 'chaulky' dentin, and non-altered dentin. Mean length measurements using Vernier calipers recorded the translucent zone alone and as a percentage of the entire root. Image analysis software (MicroScale) recorded mean area measurements of translucency alone and as percentage of the entire root. Statistical tests included reduced major axis regression, Pearson's correlation coefficient, and Bonferroni correction.

When the values of both samples were compared, they yielded polarizing results: length as a percentage of the root ($r=0.73$), instead of area as a percentage of the root ($r=0.46$) gave the best age correlations in the modern sample but in the archaeological sample no better measurement type (length or area) was identifiable. The reasons for this are due to destruction by diagenic influences. For example, when the three types of dentin were compared between the two samples, the modern sample had no occurrence of 'chalky' areas, but in the archaeological sample 48/61 teeth contained this feature. It is apparent that the burial environment may obliterate translucent dentin, rendering it useless as an age marker. This destruction appears random in nature with no way of

anticipating any kind of useable/unusable sample portion. Because of this variation in specimen usability, the authors do not recommend archaeological translucency as an appropriate age criterion.

Table 2.3: Transparency publications illustrating correlation coefficients with known age separated by tooth type.

Publication	Tooth-Type(s) Used	Measurement Method	Measurement Technique (value inputted into equation)	Known-Age Correlation Coefficient
Bang and Ramm 1970	All tooth types	Light penetration though intact root and caliper measurement	Mean transparency=maxi length/minimum length. Each tooth is given a different regression formulae partitioned by left/right and if transparency is < or > 9 mm in length	Max R 1st I: r=0.80 Max L 1st I: r=0.83 Max R 2nd I: r=0.84 Max L2nd I: r=0.83 Max R C: r=0.67 Max L C: r=0.64 Max PMs: r=0.78 Max R 2nd PM: r=0.82 Max L 2nd PM: r=0.80 Mand R 1st I: r=0.76 Mand L 1st I: r=0.61 Mand R 2nd I: r=0.65 Mand L 2nd I: r=0.79 Mand R C: r=0.73 Mand L C:0.73 Mand R1st PM: r=0.82 Mand L 1st PM: r=0.74 Mand R 2nd PM: r=0.73

				Mand L 2nd PM: r=0.82 Max 6 molars root no.1: r=0.83 Mand 6 molars root no. 1: r=0.82 Max 6 molars root no. 2: r=0.79 Mand 6 molars root no. 2: r=0.76 Upper 6 molars root no. 3: r=0.82
Drusini et al. 1989	All anterior teeth, premolars, and molars. The sample evaluated both modern (M) and known-age archaeological teeth (NM)	Light penetration through intact root and caliper measurement	Labial transparency height in MM (h) and labial root length (H) ratio = h x 100/H	Anterior M: r=0.57 Anterior NM: r=0.64 PM M: r=0.84 PM NM: r=0.84 All Ms M: r=0.82 All Ms NM: r=0.71 2nd M M: r=0.85 2nd M NM: r=0.85

Age estimation by multiple dental criteria

In 1947, the Swedish stomatologist Gösta Gustafson (Gustafson 1947, 1950) first published his pioneering method of age estimation of an individual from the adult dentition. He assessed six features that increase over the lifetime in dental tissues: attrition (A), periodontosis (P), secondary dentin (S), cementum apposition (C), root resorption (R), and transparency of the root (T) (Gustafson 1950:47). The method utilized

labio-lingual longitudinal ground sections .25 mm thick of 41 teeth spanning all tooth types, observed under a microscope; each specimen was evaluated and assigned an arbitrary 4-point score (0, 1, 2, 3) for each of the criteria. The author presumed that the sum of these points would correspond to chronological age and that an increase in points would equate to an older individual. After ranking each tooth, a linear regression equation for predicting age-at-death was calculated. The average prediction error was ±3.6 years, after which he decided that a double estimation method would achieve a lower error and evaluated each tooth twice. If the degree of age change was not clearly defined, a score of one point lower and higher would be assigned; the error then decreased to ±2.3 years. Finally, he stated that using more teeth from the same individual could greatly increase the accuracy of the estimate and/or decrease the average error in years as shown from one tooth to ±3.6, two teeth ±2.6, three teeth ±2.1, and four teeth ±1.8. He concluded that each individual reflects age at a different rate and that each researcher should make their own regression formula as Gustafson's own was based on a Scandinavian population.

The major weakness of this method lies in the fact that he assumed that all six variables combined equate accurately to age and that each variable alone does not (Johanson 1971:58, Kilian and Vlček 1989:259), when, in reality, this is far from the case. Other criticisms of his method are that it is destructive, somewhat subjective, and that a detailed knowledge of dental histology must be possessed by the observer for accurate interpretation (Prince 2004:20). Finally, his statistical analysis and age estimates have been found to be *quite* inaccurate (Lucy et al. 1995; Lucy and Pollard 1995; Maples and Rice 1979). Despite several flaws in his methodology, Gustafson did indeed take

notice of definite dental age changes and laid the foundation for future studies. Subsequent research has modified, verified, and supplemented this method *numerous* times in an attempt to improve various aspects.

Johnson (1968) attempted to improve upon acquisition techniques and quantification of translucent dentin and other dental age markers by employing the index of tooth dimensions instead of ordinal ranking systems as his predecessors did. Moreover, despite his research resulting in low correlation coefficients, his method was innovative and the first to experiment with area measurements and ratios. His sample consisted of 93 clinically extracted upper and lower anterior teeth (incisors and canines) of 27 male veteran patients (Veterans Administration Hospital, Washington, D.C.), ranging from 28 – 73 years of age; the patient's age was recorded but no other criteria such as health or reason of death were accounted for. Each tooth was thin-sectioned labio-lingually and ground to 250 μm. Following this, these sections were photographed in color (35 mm) behind a black background; the images were then projector-enlarged on graph paper (10 lines per inch) and pencil-outlined. Next, seven areas per tooth were counted: root transparent dentin (A), total tooth cross section (B), total tooth dentin (C), total tooth opaque dentin (D), root cross section (E), root dentin (F), and root opaque dentin (G). The area of these parameters was regressed against the actual age of the individual, and the highest age-correlated index was then compared to the sample when divided into tooth groups. When the area of transparency indices for all teeth was compared to the age of the individual, no significant relationship was found. He concluded that the area index is not a suitable method of age determination.

Johanson (1971), a pupil of Gustafson, undertook a validation study of all six age changes and implemented new data acquisition techniques and quantifications. The sample consisted of 46 known-age individuals (31 males and 15 females; 23-79 years old) and 62 teeth pulled at random (single-rooted premolars, canines, incisors) from 30 upper and 16 lower jaws. Prepared ground thin sections in a bucco-lingual longitudinal plane centered on the pulp cavity were made. He experimented with various section thicknesses to determine what allowed for the greatest visibility and concluded that 0.25 mm was best. The slides were then enlarged 3x in a magnifier and hand sketched on paper. He used point grading but further enlarged the phases into a finer 7-point system (i.e. 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0). Multiple regression analysis was determined for all six changes but each of the criteria were also determined alone. As such, the three variables that correlated most closely to age were: translucency r= 0.84, secondary dentin r= 0.63 and attrition r= 0.55. No difference between upper/lower jaw and sex was found.

Lorensten and Solheim (1989) published an important technical paper dealing with Gustafson's six dental age changes but focused on different quantifications of translucent dentin. Their sample consisted of 500 extracted teeth, 50 of each type excluding molars, from a 'Caucasion' population. Each tooth was prepared according to the half-tooth technique, photographed in color, and enlarged 50x and transferred to paper. A planigraph (in square mm) was used to acquire the following measurements: total area of tooth surface excluding enamel (TA), total root area apex from CEJ (TRA), area of translucent dentin (ATD), and the pulp area. In addition, the sample was also subjected to Johnson's (1971) 6 criteria and Bang and Ramm's (1970) dentin translucency measurement methods. Stepwise multiple regression analysis and Pearson's

coefficient was run for each tooth type, individual age, and tooth age. Surprisingly, the strongest coefficient was for maxillary canines while mandibular second incisors had the weakest. Of the canines, ATD was the major contributor to the regression. In some teeth rate of translucency decreases with age, and sex is also a negative factor for some teeth (maxillary second incisors and mandibular first incisors), meaning that females will tend to be estimated 8.23 – 9.73 years higher than males; this is because male teeth have increased deposition of minerals causing translucency.

Johanson's (1971) technique achieved higher scores than all other estimation methods (except for maxillary canines) because it includes 6 age changes. Bang and Ramm's (1970) technique proved weaker than Johanson's; the reason for this may be that it is less accurate than using a planigraphic measurement. The area measurement may largely be a result of age and only maxillary first incisors showed evidence that size of the translucent zone is influenced by the total tooth area. This study demonstrates that the spread of translucency is broadly independent of tooth size, and the rate is uniform for all individuals within a population; it would then be advantageous to select a tooth type with a long root so that the most age data could be obtained in an accurate way.

Lamendin and colleagues (1992) devised one of the most important, simple, and widely applied methods to date. This method has advantages over others because it requires little training and is non-destructive and based on the macroscopic caliper measurement of root transparency (T) and gingival recession or periodontitis (P). These two criteria are "expressed as an index by relating the measure to a fixed measurement on the tooth" or the height of the root (R). The index for T = (transparency height x 100)/root height and P = (periodontitis height x 100)/root height. T is measured in front

of a light source (16 watt negatoscope) from the apex to the maximum height on the labial surface; this aspect was chosen because it is less influenced by caries attack and where translucency is highest. Root height is assessed with the aid of a caliper square and ruler from the apex to the CEJ. The main aim was to compare their method with Gustafson's (1950) to test the age accuracy. The sample consisted of 306 extracted single rooted teeth from 208 persons aged 22 – 90 years, 135 males/73 females, and 198 'whites' and 10 'blacks' from both rural and urban areas. Tooth types used were as follows: 69 upper incisors, 66 lower incisors, 28 upper canines, 26 lower canines, and 45 premolars. Multiple regression analysis was employed to examine age-progressive changes.

It was found that the equation is not useful for individuals under 25.53 years of age because transparency does not begin until after this time. No differences between the sexes or inter-observer errors were observed. As for the whole sample was concerned, no tooth type fared better for age correlation, but, in the ages 40 – 49 years and 60 – 69 years, upper central incisors yielded the best age estimates. When comparing the Lamendin and Gustafson methods, the former gave a mean error much lower than the latter; 8.9 ± 2.2 versus 14.2 ± 3.4, respectively. In other words, the Lamendin method gave more but smaller errors (<10 years at 60%). As others have noted, this technique still has some limitations in that it has trouble correctly aging individuals under 40 and over 80 years of age. Nevertheless, it is one of the most accurate dental age methods to date and outperforms many other previous methods of measuring translucency, in that it is simple, rapid, non-destructive, and because of the use of indices, inter-observer error is eliminated.

Beyer-Olsen and colleagues (1994) investigated archaeological material from St. Olav's churchyard, Liberty Site, Trondheim, dating to 1100-1600 A.D. A total of 147 crania or 1,578/2,156 loose teeth (incisors, canines, and premolars) were available for age estimation, but from this set 111 teeth (of all types) randomly chosen were evaluated. The study compared age estimates between 'direct inspection' of cranial indicators/ attrition and Bang and Ramm's (1970) measurements/formulae of intact root translucency. For inter-observer error calculation, translucency measurements were recorded twice by two different observers. Their results showed that the mean age of the cranial method was 14 years lower than translucency estimates and that the correlations were low (r=0.61). The 'direct inspection' method gave age ranges between 15-63 years while translucency measurements gave age ranges of 30-72 years. The distribution between the two methods closely mirrors one another but the 'direct inspection' method produces ages 14 years younger. There were no significant inter- or intra-observer errors in translucency measurements between the two observers. No mention of post-depositional influence was noted. The authors conclude that translucency has considerable value as an age criterion because it is simple to score and findings are in accordance with the results of Bang (1993).

Kvaal and Solheim (1994) undertook an important study comparing three non-destructive dental age acquisition techniques. They macroscopically examined un-sectioned, extracted teeth and indirectly measured the pulp/secondary dentin from radiographs as well as macroscopically evaluating periodontal retraction and length of apical translucency. Their sample consisted of 452 incisors, canines, and premolars from a cosmopolitan 'Caucasian' population; any pathological or damaged teeth were

discarded. One tooth per individual was measured. For the radiographs each tooth was captured with the mesio-distal plane of the long axis against the film and the x-ray beam perpendicular. An Eggen holder was attached to the long cone of a Phillips Oralax 50 x-ray machine with the film at a distance of 22 cm and an exposure time of 0.32 seconds on Kodak Ultraspeed film. The following measurements were taken in millimeters with Vernier calipers: (T) apical translucency (Bang and Ramm 1970), (RL) mesial root length on radiographs, (P) periodontal retraction, (PWC) pulp width at CEJ on radiographs, (PL) pulp length on radiographs, (RWC) root width at CEJ on radiographs, (PWM) pulp width at midroot on radiographs, (FWC) PWC/RWC, (RWM) root width at midroot on radiographs, and (FWM) PWM/RWM. All root and pulp widths were measured with a stereomicroscope with measuring eyepiece. From these measurements they calculated the following ratios: (FL) pulp length/root length, (FWCE) PWC/RWC, and (FWMR) PWMR/RWMR. Ratios between tooth and pulp dimensions are used to reduce the possible effects of angulation and/or magnification that may occur with radiographic images.

There was a significant ($p<0.01$) correlation between: 1) the pulp/root width ratios at the CEJ for all tooth types, 2) the pulp/root width at the mid-root in all but three tooth types (mandibular canines/second incisors and maxillary canines), 3) and pulp/root length in all but three tooth types (mandibular first incisors and maxillary first/second incisors), 4) maxillary canine pulp/root length ratios are better than width ratios, 5) periodontal retraction was correlated with age in all but three tooth types, and 6) translucency was correlated with age in all but three tooth types (maxillary incisors and canine). Multiple regression formulae showed the strongest that age correlation was for maxillary second

premolars (r=0.90) while weakest for maxillary canines (r=0.48); mandibular canines correlated reasonably high (r=0.78). They caution that the sample was stored in formaldehyde for several years which may have an influence on root translucency.

The efficacy of the two-criterion Lamendin method was questioned by Foti and associates (2001) who demonstrated that periodontitis is not suitable for age determination in individuals less than 49 years of age because of naturally increasing periodontal disease. On the other hand, translucency proved to be the superior age marker and applicable to all age classes. Their sample consisted of 71 extracted single-rooted anterior teeth (incisors and canines) from 26 females and 45 males between the ages of 25 and 80. Two observers made double measurements for inter- and intra-observer error tests using the Wilcox non-parametric tests which yielded no significant differences. No significant age correlation between periodontitis for any tooth type was observed. In contrast, height of dentin translucency yielded much stronger results. One apparent problem is that the older the individual the greater the difference between calculated chronological and biological ages *or* that with increasing age there is a systematic underestimation in chronological age regardless of sex. Surprisingly, when individual tooth type was examined for mean errors the canine ranked lowest of all (7.31 years) as opposed to central incisors (9.23 years) and lateral incisors (9.16). This publication demonstrates that the Lamendin method of measurement on root translucency may be used alone to accurately assess chronological age and confirms the importance of this technique.

The two-criteria Lamendin technique was further evaluated by Prince and Ubelaker (2002) on a diverse known-age and sex skeletal sample in order to test if the

original regression equation is universally applicable. The sample consisted of 400 single-rooted teeth (incisors, canines, and premolars) from individuals ranging 25 – 99 years of age from the Terry Collection; this sample is specifically non-French and contains individuals of 'black' and 'white' ancestry. It should be noted that the authors used only Vernier calipers.

The authors demonstrated similar results to Lamendin's original study. Furthermore, the Terry Collection gave lower mean errors (8.23 years with a standard deviation of 6.87); however, it still gave values that over-aged younger cohorts and under aged the older ones. The ideal age cohorts that yield the most accurate age assessments are 30 – 69 years of age. Moreover, 'black' females showed the highest mean error (9.63) while 'black' males showed the lowest (7.17); 'white' females (8.46) and males (7.66) were similar in this regard. When ancestry and sex were examined individually, it demonstrated that the latter *does* have an effect on accuracy. They note that sex plays a hand in accurately estimating age and should be determined first before applying this technique. One important factor that was only briefly commented on is that the inclusion of periodontitis was the main source of error of the study, and it is known that in some populations a sex discrepancy in diet and dental health occurs.

In another test of the Lamendin et al. (1992) method, Megyesi and associates (2006) applied the technique to two archaeological samples in order to test if the technique and original French regression formula is applicable to a population removed in space and time. Their sample material came from Christ Church (Spitalfields, London) and St. Bride's (Fleet Street, London) from the 18th and 19th centuries whose ages-at-death are known (but undisclosed to the authors). The Spitalfields sample consisted of

176 individuals and 951 teeth, 44 individuals and 237 teeth came from St. Brides; both samples had about equal males/females. The individuals chosen were over the age of 25 and had one single-rooted tooth available. The condition of each tooth was scored qualitatively from 0 - 2 to assess if accuracy of the method is affected by burial condition alterations.

Overall, the sample contained a high number of unusable specimens (35%). ANOVA test of translucency and periodontitis suggested that the presence of both criteria may vary significantly between teeth in one sample. In other words, these parameters are influenced by burial conditions, and in turn, so too will the error of age estimation. Therefore, periodontitis and RTD readability and reliability are *closely* linked to the condition (or lack thereof) of the sample. For example, the mean error of age estimates of the sample using only those in good condition ***and*** exhibiting translucency is almost identical to the mean errors generated for translucency alone. Although post-mortem destruction is highly variable, this means that most of the errors surrounding the use of archaeological dentitions can be remedied by these exclusions. To reduce mean error in age estimation, it is recommended that all teeth not exhibiting RTD and in poor condition be removed from the regression line because 1) the individual has not yet reached the age at which translucency begins/appears, and/or 2) post-depositional forces have obliterated or altered translucency.

Lucy and colleagues (1996 and 2002) were some of the first to experiment with the alternative statistical approach of Bayes' theorem applied specifically to adult dental parameters. They propose that Bayes' theorem is superior to traditional linear or multiple regression in that it takes into account the prior probability (probability of an individual

belonging to a defined age group with no prior information other than the reference sample information), likelihood (probability that the observed data is true), and posterior probability (probability of the hypothesis being true assuming the observed value is correctly incorporated into the correct age category subsequently considering both the two previously described variables). Lucy and associates (2002) used data from 76 mandibular central incisors from the previous research of Solheim (1989), ascertaining the following variables: attrition, root roughness, secondary dentin, and translucency.

The nonparametric calibration approach was compared to multiple regression estimates for accuracy and precision of mean absolute deviation, regression slope, and the 95% confidence interval. Multiple regression analysis resulted in an underestimation of 15 years in the oldest individuals as opposed to the nonparametric approach which yielded bias a of 10 years in the same age category. Multiple regression gave a mean error of 9.5 years compared to nonparametric error of 11 years; however, this average error is less crucial than error at the left- and right-ends of the curve. In other words, bias, not mean error, is a more important consideration when assessing age reconstruction as nonparametric analysis tends to 'round' the ages rather than exaggerate them. Multiple regression analysis has reduced applicability to archaeological remains where as a Bayes' approach maintains accuracy and precision with reduced systematic bias at all ranges of the age distributions and especially at the extreme ends of the spectrum where confidence in age estimation is notoriously critical (Konigsberg and Frankenberg 1992).

Monzavi and coworkers (2003) tested the Gustafson (1947) method on unknown modern skeletal remains; it should be noted that they maintained the point system and did not use metric measurements of dental age parameters. Their sample consisted of 210

(185 males and 25 females) individuals ranging from 25 to 60 years of age. They included at least one mandibular single-rooted tooth (premolar, canine, and/or incisor). The dental sample was sectioned to approximately 0.5 to 1 mm in thickness. They found that the highest correlation coefficient ($p=0.641$) was produced when all six dental variables were combined instead of any single variable and that the best tooth type was the first premolar. Interestingly, the mean error (± 5 years) was not isolated to any single tooth type. They stress that the largest source of error may be assessing dental variables on teeth that have been desiccated instead of freshly extracted material.

Soomer and associates (2003) tested eight different dental age estimation techniques on a sample of known-age individuals. Their sample was small, consisting of only 20 teeth from 10 females and 10 males; two teeth were selected from each person regardless of type excluding molars. They tested the methods of: Johanson (1971), three formulae by Kvaal and Solheim (1994), Solheim (1993), Lamendin et al. (1992), and intact and sectioned formulae for Bang and Ramm (1970). Their results indicated that the methods using thin-sectioned teeth tend (although not significantly different) to be more accurate and precise than intact specimens. In addition, measurements on radiographs as opposed to thin-sectioned counterparts were not as accurate. When evaluating the age estimates, the two Kvaal and Solheim (1994) techniques drastically underestimated true age and had significantly lower precision and variability than another method. If secondary dentin is used they recommend Solheim's (1993) technique on a sectioned tooth as opposed to radiographic evaluation. The two transparency methods offered higher accuracy and precision than another technique without the need of specialized equipment like x-ray machines or specialized knowledge such as histology.

Prince and Konigsberg (2008) tested the Lamendin (1992) technique for intact root translucency and periodontal recession on a forensic sample. It has been demonstrated that periodontal recession adds inaccuracy to the Lamendin method; however, this publication's aim was to test statistical reliability between several formulae only. They applied Bayesian statistical analysis and compared their results to those of Lamendin's (1992) and Prince and Ubelaker (2002) (which were derived from different populations). The sample originated from Kosovo, Balkans and consisted of 401 known-age, single-rooted teeth from 359 males who ranged in age from 18 – 90; tooth types were either maxillary/mandibular incisors, canines, or premolars. They assessed mean error and correlation coefficients between these three formulae.

Bayes' theorem produced a mean error of 1.51 years, an absolute mean error for the whole sample of 9.01 years, and a correlation coefficient of 0.73 between predicted ages and estimated ages. Lamendin's and Prince and Ubelaker's formulae produced mean errors of 4.85 and 5.27 years respectively, and correlation coefficients of 0.67 and 0.70, respectively. The Bayesian approach also exhibited different predictions in older (60+) and younger (18-29) age groups compared to the other two statistical methods where the 'attraction to the middle' occurred (a common problem) (Prince 2004).

The two multiple regression methods produced overestimation in younger age groups and underestimation in older age groups, whereas Bayes' theorem did not completely remove this error but greatly reduced it. The authors note that the Bayesian approach is superior because it considers that a tooth may exhibit a 0 score for translucency and periodontal recession, whereas the other two tests would not. For example, Prince and Ubelaker's (2002) formula cannot predict the age for an individual

below the ages of 23.2 - 25.5. It is evident that Bayes' theorem takes into account age mimicry, age bias, and age range and reduces, but does not eliminate these and should be employed in age estimation especially in biased archaeological samples, over other statistical approaches.

Perhaps the most comprehensive test of several different age indicators on a single archaeological skeletal sample was carried out by Whittwer-Backofen and associates (2008). They tested the correspondence of several morphological skeletal, macroscopic dental, and chronometric dental age markers. The sample used for analysis was from Lauchheim, Baden-Wurttemberg, Germany (450-660/680 A.D) and consisted of 121 skeletons (54 males and 62 females). The age indicators applied to the sample included: common skeletal morphological markers, osteon density, root transparency (Lamendin et al. 1992), and cementum annulation counts. The observers consisted of 10 teams of 15 experienced individuals. When they examined the age estimates by mean age, the multifactorial skeletal and cementum annulation techniques failed to place age ranges that captured the upper age limit. In addition, no regular pattern of age estimates was found, but rather the estimates fell within the middle age categories. Interestingly, the transparency technique did succeed in placing individuals into the older age (70+) categories where other techniques did not. However, the transparency technique did exhibit the lowest consistency of any technique, meaning that the reference population and the applied sample are different (i.e. a population problem). When the age distributions were examined, different methods tended to place individuals into different age categories (lower, middle, upper). For example, the multifactorial skeletal age usually placed individuals in the high age range, the transparency technique placed individuals in

both lower and upper age extremes, and cementum annulation counts usually placed individuals in the low age categories.

Three factors at play that were responsible for the somewhat high variability of the techniques employed are: 1) post-mortem changes that influence observer scoring ability and age estimate quality, and 2) inter-individual biological variability that introduced problematic age estimation, and 3) the variability of age indicators correlated to chronological age have experienced changes over time (age markers can change morphologically over time). They caution that younger aged individuals are more accurately assessed than older aged individuals and that inter-observer measurement error can contribute to errors in age estimation. They conclude that when applying several different age indicators to one sample, a multitude of age estimates will usually be produced although they will usually compliment each other to a certain degree. The researcher should apply age marker techniques according to the skeletal elements available and *especially* the condition of the sample to avoid bias and error associated with taphonomically compromised material.

Post-depositional influences

Despite the advantage that teeth may hold over other mineralized tissues in resisting disintegration, teeth are undoubtedly chemically altered as opposed to their fresh counterparts (Kohn et al. 1999). Post-depositional modifications are random and variable in that predicting the amount of usable material is impossible. This variation in preservation stems from burial conditions and/or time since interment and ranges from skeletal sample to sample, within one sample, within one individual skeleton, within one individual set of jaws, and within one singular tooth. In reality, the time since burial has

little to do with the quality of preservation because a myriad of factors play a causal role such as heat, pressure, soil particle size, and pre-mortem pathological state, and depending on other geological activity these changes may be accelerated or decelerated (Bell et al. 1991 and 1996).

The chief factor in the destruction of the base physical form of dental tissues is the removal of the organic component (collagen) and infiltration by microbes, fungi, soil particulates, mineral replacement, and apatite alteration which all cause the architectural structure(s) to weaken; it is the extent and quality of this mineral replacement that dictates the outcome of integrity. Sometimes preservation is such that the collagen and cellular structures are maintained and intact with no trace of alteration (Doberenz and Wyckoff 1967) and other times diagenic changes may mimic and/or destroy that of the original (Falin 1961).

It should be stressed that not all types of depositional change should be viewed as detrimental in achieving accurate age ascertainment. This change may be divided into macroscopic and microscopic alterations, each with different consequences for age determination. For example, calcium concretions, staining, or sediment infiltration into the tubules may destroy or exaggerate root translucency but leave the pulp chamber unaltered. Conversely, geologic forces may crack and splay the root, making pulp chamber analysis difficult or impossible, but leaving areas suitable for transparency measurement. It should be doubly stressed that certain protocols and methodologies may not be cross-compatible or cross-applicable between fresh and archaeological dental material. The following will illustrate changes that the burial environment has on the

dentition and what implications and considerations should be taken into account before any analysis.

Dentin transparency

Archaeologically preserved dentin may be soft, brittle, and friable compared to that of fresh dentin which is tough and elastic. A commonly encountered pattern of damage is fracturing or splitting (splintering) that may cleave both the root and crown at the EDJ (Hillson 1996, 2005). In best cases, odontoblast processes have even been found to remain intact (Falin 1961) within the tubules. Tooth roots are most susceptible to contamination which usually encircles the dentin around the pulp chamber and stops at the CEJ with the root apex being most sensitive to this damage (Hillson 2005). Commonly cited microscopic changes are sediment infiltration into the tubules, staining that penetrates the surface, and microbial acid destruction causing white opaque patches (Robbins 2003, 2005; Sengupta et al. 1998; Wyckoff 1972).

It is the possibility of translucency mimicry and/or obliteration that is of concern. Several studies have stated that these types of alterations are frequent yet random in occurrence, lending testament to the variation in sample usability. It is transparency mimicry that is most detrimental because there is seemingly no way to discern what the true age-related area is from that of the altered portion; sometimes archaeological teeth show higher values than fresh counterparts (Mandojana et al. 2001).

Taphonomic changes to translucency have largely been unexplored and depending on the method of data collection, the consequences for age estimation are obvious. For example, if the outward appearance of the root appears opaque due to extraneous mineralization of the cementum, but root translucency remains underneath,

then this would require thin-sectioning as opposed to measurement by intact root. However, for non-destructive analysis, this in not a viable option and that particular specimen would be unusable, leaving a pulp reduction technique as one option.

Nevertheless, several of the previous studies have reported excellent correspondence with predicted age using root translucency on intact roots, and it should not be undermined as an important age criterion for archaeological samples. It is an unavoidable fact that some samples are so severe in taphonomic influence that transparency is useless and it is the danger of wasted time and effort that is of concern.

Pulp chamber/root canal and secondary dentin

The pulp chamber and/or root canal is the most resistant to post-depositional changes by virtue of the fact that it is protected within the innermost part of the tooth and after putrification of the soft tissue, it is essentially a void space. Although only limited literature regarding taphonomic changes is available, it appears that even under the most severe of alterations it still suffices as an accurate age criterion. This is not to say that if the pulp chamber was intact and the circumpulpal dentin was ablated that it would still be useful (it would be useless because the circupulpal dentin/pulp chamber ratio is required for age estimation), but that when examined alone, it is incredibly hardy. But, for example, if the zone of transparency was not present but otherwise the root was physically intact, the tooth would be useable.

The most commonly described change is infiltration of various crystalline ingrowths (Wyckoff 1972). These crystals may be composed of calcium, calcite, aragonite, or vivianite (Chandler and Fyfe 1997; McGowan and Prangnell 2006). The type of crystal is dependant on the geo-environment in proximity. In worst cases the

chamber is entirely occluded with mineral crystals that may radiate into the dentin, but in best cases the pulp tissue can be remarkably well preserved and allow for easy delineation of dental tissues. Despite the chamber being filled with extraneous material, it theoretically still is sufficient for use as an age indicator as the few publications utilizing archaeological material have obtained good results. Mandojana and associates (2001) comment that pulp dimensions can be larger and give higher age estimates than non-archaeological material.

Pulp reduction is the least utilized dental age criterion (Igbigbi and Nyirenda 2005), but, for obvious reasons it should not be disregarded as an effective age indicator, especially in archaeological situations.

Summary

Teeth begin the process of growth and development *in utero* which ceases around the age of 18 years with the closing of the root apex. Over the lifetime, the adult teeth continue to transform morphologically in numerous ways. Many of these changes, however, do not reflect chronological age and are inappropriate as criteria for age estimation and/or paleodemographic reconstruction. Of the 22 regressive adult dental age related changes, two parameters - apical root transparency and pulp reduction/secondary dentin apposition - have subsequently proven many times to correlate most strongly with chronological age and to have high utility for non-invasive analysis in archaeological contexts.

As will be outlined in the proceeding chapters, these two dental age parameters will be applied, using a variety of techniques, to a small sample of archaeological canines. The age-at-death will be assessed by both traditional manual and experimental

digital methods. These dental age estimates will then be compared to the respective macroscopic morphological skeletal age indicators in order to derive age estimates and age distributions for the Apollonia Pontica dental sample.

Chapter 3: Materials and Methods

Apollonia Pontica: Biocultural and Bioarchaeological Context

Biocultural context

Origin

Apollonia Pontica (5^{th} – 3^{rd} centuries B.C.E.) is the southernmost Black Sea Greek colony located on what is now Sozopol, Bulgaria (Isaac 1986; Panayotova 1998); Sozopol is literally built on top of the ancient city. Sometime near the end of the 7^{th} century B.C.E, the Ionian city of Miletus (western Turkey) experienced scarcity in food and resources and/or an increase in population which prompted small groups of settlers to establish independent colonies around the Black Sea; Apollonia was established in approximately 610-609 B.C.E. (Isaac 1986; Hoddinot 1975, 1981; Nedev and Panayotova 2003; Tsetskhladze 2004). The region adjacent to the colony was inhabited by the kingdom of Thrace. Evidence suggests that relations were peaceful between the Greek colonists and native Thracians, based on mutual trust and common interests (Nedev and Panayotova 2003). Despite the friendly terms of the two distinct ethnic groups, they remained largely spatially distinct (Nedev and Panayotova 2003). Apollonia was indeed a very desirable location and was chosen because the surrounding land and sea possessed several potentially advantageous attributes including excellent harbors, fertile agricultural land, abundant marine resources, and metal ore (Danov 1990; Hoddinot 1975; Isaac 1986). It should be stressed that the reason for establishing the colony was driven not by

trade, but because the land offered such high potential for prosperity. In other words, trade was a product of the abundant and diverse resources and not the reverse (Tsetskhladze 1998).

Another major advantage of the colony's location was that it was very strategically defensible, surrounded by the large Strandja Mountain range to the west and the Bay of Burgas to the east (Isaac 1986). As allies, Apollonia and Thrace undoubtedly protected one another on military and political levels as they shared common interests with one another.

Trade and dynamics

Due to the abundance of resources available, trade, craft-production, and metallurgy developed and transformed the colony into a thriving, wealthy, and politically important urban center. Specifically, Apollonia served as a large producer of goods as well as a central trade hub for relations and mediation between the Aegean and Thracian worlds. Other demonstrations of the city's wealth were the minting of coins, the construction of monumental public architecture, large protective walls, and several cemeteries (Hoddinott 1975; Nedev and Panayotova 2003). Apollonia also established its own settlement called Anchialos for salt production (Isaac 1986; Nedev and Panayotova 2003). In short, each Ionian Black Sea colony was an independently governing entity or *polis* (the city, the city state, and the citizens). Shortly after Apollonia was established, the settlement transformed into a thriving city lasting until the end of the 3rd century B.C.E (Nedev and Panayotova 2003).

Population

Despite Apollonia being one of the most successful Greek Black Sea colonies, there is a scarcity of literary evidence that exists regarding its history and/or population (Danov 1948). From the few literary sources that have been recovered, the population size was estimated to have been approximately 3,000 individuals (Danov 1948). Since Sozopol covers much of ancient Apollonia Pontica, much of the present and future knowledge about the population structure and dynamics comes from the skeletal remains recovered from the necropolises, as outlined below.

Bioarchaeological context

The dental material analyzed in the present research was excavated from the Kalfata necropolis, located on a 5 km strip situated parallel to the Bay of Burgas coast, just south of the Skaminey Peninsula in a sandy and littoral zone. Although not the only Apollonian cemetery, Kalfata has been the most intensely studied and has produced a substantial amount of skeletal remains. The cemetery was deliberately constructed outside the city limit walls in order to preserve the rich agricultural land (Nedev and Panayotova 2003). It is possible that this particular spot may have been used for the inhumation of both the non-elite and ruling class (Greaves 2002).

In 1938, a chance discovery of a grave prompted excavations of the necropolis. Following this, large-scale investigations took place from 1946-1949 by Bulgarian archaeologists who uncovered 801 inhumation graves (Nedev and Panayotova 2003). Since 1992, Kristina Panayotova of the Institute of Archaeology in Sofia has uncovered more than 1000 additional burials which date to the mid $5^{th} - 2^{nd}$ centuries B.C.E. The skeletal remains were recovered from several different types of graves that contained a

wide array of associated grave goods. Most recently, Dr. Anne Keenleyside of Trent University has examined these remains with a focus on the health and diet of the Apollonian population.

Bioarchaeological research on Black Sea Greek colonial sites is lacking. Therefore, little is known about these ancient Greek colonists and their biological, behavioral, and environmental adaptations to the region. To date, the only bioarchaeological centered studies of skeletal remains from this area have been accomplished by Keenleyside and colleagues (2005a, b, 2006, 2008). These studies have greatly contributed to our understanding of the health status and life-ways of these ancient people. This section presents what is currently known about ancient Apollonian skeletal biology, demography, and diet based on the skeletal remains recovered from the Kalfata necropolis.

Keenleyside (n.d.) presents the largest, most current, and detailed bioarchaeological study of these remains to date. Sex determination was accomplished by analysis of the crania and pelvic bones (Bass 1987; Ubelaker 1989) and adult age estimation was based on morphological examination of the pubic symphysis (Brooks and Suchey 1990), auricular surface (Lovejoy et al. 1985), and ectocranial suture closure (Meindle and Lovejoy 1985). Each of the adult individuals was categorized into one of three age cohorts: 18 – 35 years, 36 – 50 years, and 50+ years. Health and nutritional status of the remains was assessed by examination of multiple skeletal and dental markers including cribra orbitalia, porotic hyperostosis, trauma, infection, and dental pathology.

Demographic profile

A total of 240 nearly complete and intact skeletons were examined. Of these, 158 belonged to adults (69 males, 87 females, and 84 of indeterminate sex). Table 3.1 shows

the adult age distribution separated by sex and Table 3.2 illustrates the skeletal morphological age estimates of those individuals used in the current research (collected by Dr. Anne Keenleyside). Of the 158 skeletons available for age estimation analysis, 60 individuals (38%) were young adults (18-35 years), 60 individuals (38%) were middle aged adults (36-50 years), and 38 individuals (24%) were old adults (50+ years).

Table 3.1: Adult age distribution of the Apollonia Pontica sample (data provided by Dr. Anne Keenleyside, n.d.).

Adults	Males	Females	Indeterminate Sex	Total
18-35	21	35	4	60
36-50	33	26	1	60
50+	14	24	0	38
Total adults	68	85	5	158

Table 1.2: Skeletal age estimates for the sub-sample of individuals from the Kalfata necropolis used in present research.

Cat #	Skeletal Age Estimate
301	18-35
19	18-35
253	18-35
289	18-35
352	50+
336	36-50
244	18-35
215	36-50
192	36-50
307	18-35
249	50+
207	50+
284	18-35
242a	36-50
305	18-35
250	36-50
321	18-35
304	36-50
359	36-50
374	36-50
242	36-50
309	18-35

67	36-50
254	36-50
338	50+
354	36-50
302	50+
330	50+
331	50+

Skeletal and dental pathology

A total of 51/156 crania exhibited cribra orbitalia (24 of these individuals had healed lesions) with no statistically significant differences between males and females. Most of these lesions were mild to moderate in severity. A small number of the individuals (8/158) exhibited evidence of porotic hyperostosis on the frontal, parietal, and/or occipital bones; six of these individuals also showed evidence of cribra orbitalia.

A small number of individuals exhibited evidence of cranial trauma, 7/159 crania; no significant differences between sexes were observed. 28/205 individuals (13.7%) showed signs of infracranial trauma, with slightly more males (21.9%) than females (17.3%) exhibiting lesions. Nevertheless, all lesions were well healed. A small number (6/159 or 3.8%) of individuals showed possible evidence of infection. Interestingly, 19/150 individuals displayed evidence of infracranial infection, and in all but one case the lesions were well healed.

Dental calculus was a commonly observed, affecting 128/201 (63.7%) individuals or 1146/2506 (45.8%) of the permanent teeth. No sex differences were observed when comparisons were made by number of individuals. However, when compared by the number of teeth affected, males had a higher prevalence of calculus than females. Dental caries was the second most prevalent dental disease when recorded by individual, observed in 92/202 (45.5%) of the individuals. In contrast, however, only 215/2543 (8.4%) teeth had lesions, indicating that caries may not have been common. Antemortem tooth loss affected more individuals, 72/205 (35.1%) with no indication of a sex differences. However, when the number of teeth/alveoli was examined a higher proportion of young males than young females had lost teeth antemortem. Abscesses were the least prevalent dental pathological condition, affecting 32/195 (16.4%) of the individuals and only 55/3161 (1.7%) of the permanent teeth. No differences between males and females were observed, however, abscesses generally increased with age of the individual. Enamel hypoplasia on the canines and/or incisors was observed in 83/827 (10%) of these teeth. No sex differences were detected, yet interestingly, the middle age

male cohort showed a higher frequency than the female cohort with both sexes exhibiting higher incidence of defects in older age.

Diet

Keenleyside and Panayotova (2005b) and Keenleyside, Schwarcz, and Panayotova (2006) were the first to critically examine evidence of diet in the Apollonian skeletal sample. Isotopic evidence from rib fragments indicates that the colonists' diet relied heavily on terrestrial resources (C3 plants and animals) but also consisted of a significant portion of marine foods. Interestingly, few sex or age differences in diet were found; however, males may have consumed more protein/marine foods and females may have consumed more carbohydrates. Cribra orbitalia/porotic hyperostosis showed no significant sex differences but a significant number of sub-adults were afflicted. Despite historical evidence of malaria and thalassemia in this region of Bulgaria, the skeletal evidence suggested that iron deficiency related to diet was most likely the cause. This may have been due to the reliance on cereal crops that contain phytates which inhibit iron absorption. Prolonged breastfeeding, weanling diarrhea, and the use of cereal as weaning food may also have reduced the amount of iron in the body. Another likely contributing factor was infectious disease. Apollonia was an urban centre characterized by high-density occupation, which may have promoted the spread of pathogenic microorganisms including parasites.

The prevalence of carious lesions in adult teeth was markedly low for agricultural populations (Keenleyside 2008). In the Kalfata sample, middle aged females exhibited only a slightly higher incidence of caries than males, generally suggesting the population as a whole were consuming similar food types.

Sample derivation

The sample of teeth used for the current study originates from adult individuals buried in the Kalfata necropolis and is comprised of n=37 mandibular and maxillary canines (cuspids); n=31 were whole, n=6 were friable and/or a crown. For age estimation n=29 were usable and n=8 were not suitable for age estimation. The teeth were chosen at random by Dr. Anne Keenleyside regardless of element (mandibular or maxillary), pathological state, condition, and/or sex of the individual. Table 3.2 displays the dental sample; the 'Catalog #' indicates the grave number of the individual, the 'Original Description' indicates the tooth element/type as written on the associated specimen bag, the 'Condition' illustrates the tooth was whole, friable, or a crown, and the FDI designation categorizes the canine according to left/right and/or mandibular/maxillary.

Table 3.3: Apollonia Pontica sample dental inventory (separated by individual catalog #, original tooth description, specimen condition, and FDI tooth # designation).

Catalog #	Original Tooth Description	Condition	FDI
267	Cs (max, mand?)	Whole	43
501	R. Mand C	Whole	43
19	R. Mand C	Whole	43
253	R. Mand C	Whole	43
289	Mand C	Whole	43
352	Mand C	Whole	43
336	Mand C	Whole	43
215	C (max, mand?)	Whole	43
244	Mand C	Whole	43
192	mand C	Whole	33
307	Mand C	Whole	33
249	Mand C	Whole	33
207	Mand C	Whole	33
284	Mand C?	Whole	33
242a	Mand C	Whole	33
305	Mand C	Whole	33
250	Mand C	Whole	33
321	Mand C	Whole	33
304	Max C	Whole	23
359	Max C	Whole	23

374	Max C	Whole	23
242	Max C	Whole	23
309	Max C	Whole	23
67	L. Max C	Whole	23
254	Max C	Whole	23
338	Max C	Whole	13
354	Max C	Whole	13
302	R. Max C	Whole	13
330	Max C	Whole	13
331	Max C	Whole	13
267(2)	Cs (max, mand?)	Whole	13
320	Max C	Crown	?
358	Max C	Crown	?
291	Mand (?) C	Friable	?
233	C	Crown	?
219/104 (1)	Cs (max, mand?)	Friable	?
219/104 (2)	Cs (max, mand?)	Friable	?

Methods and techniques: age estimation

This study employs two dental age markers on the Apollonia Pontica sample of canines – tooth root transparency and pulp reduction. The published quantifications (Bang and Ramm 1970, Cameriere et al. 2007, etc.) are described below. Both conventional manual caliper and digital measurement values were recorded. The specifics of how these measurements were acquired are presented in the following sections.

Manual methods

Apical transparency

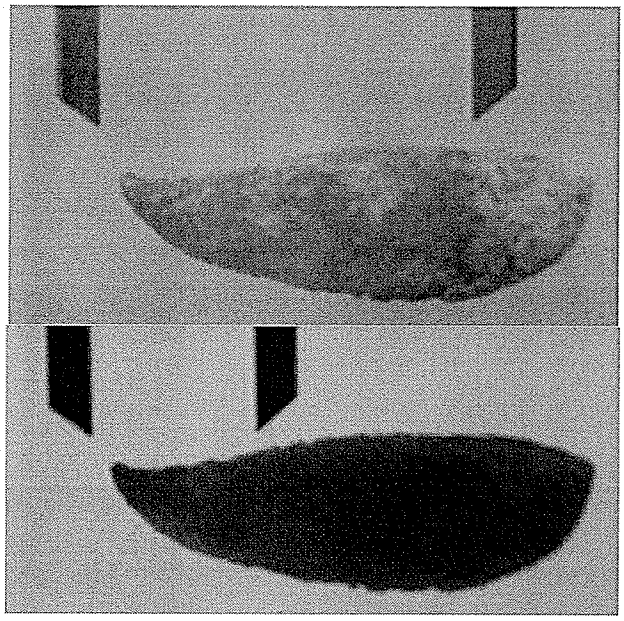
For manual/non-digital data acquisition of translucency by light penetration through intact root, the following equipment is required for all methods of quantification: a constant transmitted light source (GE Medical Systems x-ray light table), digital Vernier calipers (Mitutoyo® Absolute Digimatic digital Vernier calipers), and a magnification instrument (Spi® 10x scale lupe). This setup is the classic manual technique:

- each tooth is placed on the light table and transilluminated
- calipers assess the labial, maximum, and minimum length of apical translucency (root apex – translucent:unaffected dentin junction) along the central mesial or distal long axis of the root as well as the labial root height (root apex – labial CEJ); this is illustrated in Figure 3.1.

Each measurement was accomplished under magnification. All values were recorded to the nearest hundredth of a millimeter and entered into a database (Microsoft® Excel). It was noticed that the bright light and meticulous measurements caused eye strain and fatigue. To remedy this and avoid possible errors, several minutes (5 to 10) were taken between each tooth to allow the eyes to rest. All specimens (excluding crowns only) were scored over a three-day period and 1/4 of the sample was re-assessed at least seven days later for intra-observer error testing.

Canine roots have an elliptical cross-section (thinner mesio-distally and thicker labio-lingually) and it was decided that the best aspect to view the translucent zone most accurately was laying the tooth flat on either the mesial or distal side. This position allowed maximum light to pass through the root for delineation of the translucent/unaffected dentin junction. The techniques for quantifying translucent dentin for age estimation on the present dental material are the Bang and Ramm (1970), Drusini et al. (1989), Foti et al. (2001), and Miles (1963). The following will describe these methods of measurement (i.e. what variable is inputted into the regression equation). Below illustrates the typical conventional caliper manual measurement technique.

Figure 3.1: Manual data acquisition of transparency height and root length. The top image illustrates the root length measurement and the bottom image illustrates the measurement of transparency height.



The **Bang and Ramm (1970)** technique as applied to intact teeth is the average between the maximum (T2) and minimum (T1) heights of translucency (apex – transparency). This may be expressed as the following equation: (T1 + T2)/2. The maximum and minimum height of transparency on the labio-distal/labio-mesial and/or linguo-distal/linguo/mesial aspects was found to usually offer the clearest position for observation (see Figure 3.6).

The **Foti et al. (2001)** technique measures the maximum labial height of transparency (based on Lamendin et al. 1992). The maximum labial height on either the labio-distal or labio-mesial aspect was found to usually offer the clearest position for observation (see Figures 3.1 and 3.5).

The **Drusini et al. (1989)** technique (based off Lamendin et al 1992) is the index or ratio between the maximum heights of labial translucency (apex - translucency) and labial root (apex – labial CEJ). The maximum labial height on either the labio-distal or

labio-mesial aspect was found to usually offer the clearest position for observation (see Figure 5.1 and 5.5.

The **Miles (1963)** technique records the maximum height of transparency on either the labio-distal/labio-mesial and/or linguo-distal/linguo/mesial aspects (see Figure 3.6).

Radiography and radiology: pulp reduction

Radiographs offer a more precise and accurate view of the internal dimensions of teeth (pulp and dentin are measured indirectly) as opposed to thin-sections (direct measurement), as the former captures the entire shape and accounts for irregular root morphology whereas the latter may be limited in this regard. Furthermore, radiographs are non-destructive and also produce a permanent image record of the specimen. Radiolucent objects/materials easily allow the passage of x-rays and often appear darker. Conversely, radiopaque objects/materials are not easily penetrated by x-rays and appear lighter. For example, enamel appears lightest (nearly white), dentin as a shade of gray, and the pulp cavity (a void) as black. In a proper radiograph all three dental tissues should be easily differentiated by these color contrasts.

When creating x-rays of dental material several specifications must be maintained to ensure accuracy in measurement, as follows:

- the long axis of the tooth root must be parallel to the film to reduce magnification and angulation
- the x-ray film must remain parallel with the specimen sheet,
- the tooth must be placed as close as possible to the center of the x-ray beam,

- if metric measurements are to be cross-comparable, a calibration object should be inserted with each x-ray sheet.

The following protocol was used to produce radiographs of the total dental sample: 1) the x-ray film (Kodak X-OmatV 10" x 12") with specimen Catalog # for each tooth was adhered with tape to the center of the specimen sheet, 2) a thin piece of glass with a grid on it was used to easily move the teeth, 3) the teeth were adhered to the glass with wax attached to the crown tips and oriented as described above, 4) a calibration bar was placed on the film, and 5) the specimens were radiographed at 10 minutes, 80 kilovolts (Faxitron cabinet x-ray machine, model # 43855A). The Faxitron used for this research produces a micro-focus cone and not a conventional wide-beam cone. This reduces the level of magnification and ensures that the level of distortion is reduced. In any event of distortion, angulation, and/or magnification, the combination of the calibration bar and the measurement ratio (pulp:total dentin width) negates these errors (i.e. the use of ratios and not absolute measurements). To ensure that the errors did not occur, the actual tooth was compared to the image on the developed x-ray film. All teeth were x-rayed at the labio-lingual and mesio-distal orientations so that a quasi-3D view could be obtained. Each film was developed in a dark room using an x-ray film processing machine.

Manual caliper measurements were as follows:

- each film was placed in front of a light table and illuminated,
- reference points were marked with an ultra-fine marker to aid measurement,
- digital calipers were used for measurement recorded in millimeters.

The **Kvaal and Solheim (1994)** technique applied to mandibular canines measures the width ratio of pulp:total root width at the CEJ, the root length, and pulp length (all from a labio-lingual aspect) along the mesial portion of the root. This technique also has a separate equation that incorporates the Bang and Ramm (1970) transparency value (Figures 3.6, 3.8, and 3.9 illustrate the measurements that are used for this technique).

The **Paewinsky et al. (2005)** technique applied to mandibular canines records the width ratio of pulp:total root width at three levels (CEJ, ¼ root, and mid-root) measured along the mesial portion of the root. Three separate regression formulae are used for each ratio level (Figure 3.10).

The **Cameriere et al. (2004)** method was originally designed on digital images, however, for comparison with digital analog measurement, caliper measurement are also recorded. The root and pulp width of the maxillary canine is measured at the mid-root. This single manual measurement is then averaged with the digital scores for the final age estimates. The digital method presented below fully explains the measurement technique.

Digital methods: digital images, image acquisition, image enhancement/processing, measurement, and analysis

Digital images

In recent years the use and application of digital images in multiple areas of science has greatly increased (Russ 2006; Watkins et al. 1993), with the two now being inseparable at some level. Generally, osteologists often rely on the observation of skeletal material to answer a myriad of questions; however, these specimens may be difficult to measure and usually cannot be transported for obvious reasons, which may make

comparative analysis difficult. Fortunately, digital images provide a permanent copy of the original material which is universally transferable and may be archived. Although there is no substitute for hands-on/physical/direct observation of the specimen under scrutiny, digital images do provide a much simpler means to facilitate the global researcher (Sitchon and Hoppa 2005).

The program used for all digital analyses is Image-Pro Plus version 5.1 (Media Cybernetics) image analysis and processing software. The software allows for the importation and examination of digital images by a graphical user interface (GUI) with a variety of tools and features.

Image acquisition

For transparency and pulp/dentin measurements from digital images, the following protocol for each age marker is described.

For transparency acquisition, the set-up was as follows:

- a copy stand with a digital camera (Olympus Camedia C-720 Ultra Zoom 8x) and a light table was mounted to the base (see Figure 3.2)
- on the light board, a piece of matte black card stock with a 2 centimeter x 1 centimeter isosceles triangle cut-out for hyper-illuminating each tooth root was fashioned,
- a centimeter bar was positioned below the triangle cut-out for digital calibration
- a label with catalog # was placed above each tooth
- a digital camera with the lens was positioned parallel to the base of the copy stand. A level was used to ensure that the apparatus did not record off-center images. Using wax for stability, each tooth was placed over the triangle cut-out

and illuminated. The camera was set to 'macro' mode to record images in close proximity and the flash was turned off; some of the lights were dimmed in the room to reduce image glare. All images were saved in a color TIFF (Tagged Image File Format) format in 1984 x 1488 pixels, as this file format does not compress, distort, or degrade any information (see Figure 3.3).

Figure 3.2: Digital transparency image acquisition apparatus.

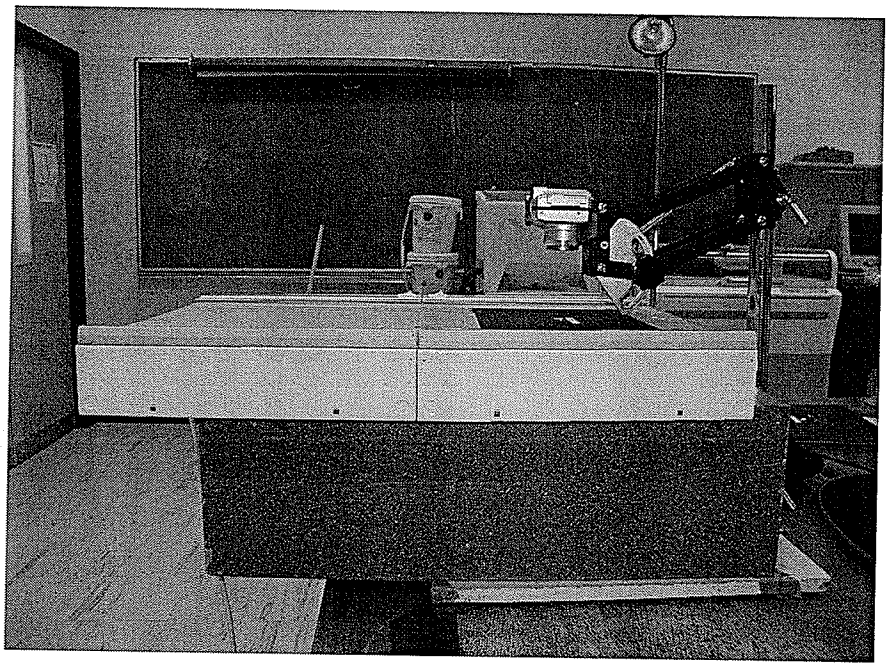
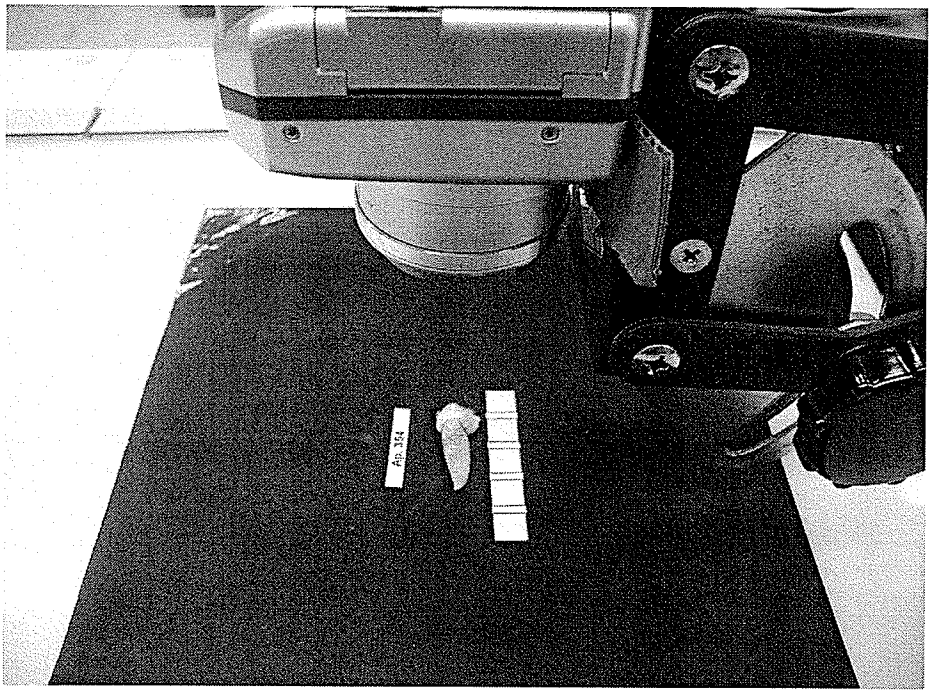


Figure 3.3: A specimen in image acquisition position.



The image below illustrates the unaltered base image acquired from the digital setup.

Figure 3.4: The baseline digital transparency image.



For the digital analysis of radiographs, all films were scanned on a high resolution x-ray scanner, and saved onto a CD-ROM in a DICOM (Digital Imaging and Communications in Medicine) format. These DICOMs were converted to a TIFF file format for digital measurement.

Image analysis

Apical transparency

Digitally assessing the extent of root translucency was analogous to the manual techniques, however, the software allowed for magnification, plotting of graphical linear lines, and reference points, to be placed directly on each image of the tooth root. It was noticed that the visual observation and measurement of translucency in a digital medium does not entirely prevent, but reduces the problems of morphological variation and idiosyncrasy that manual scoring has in some cases (i.e. some samples have a 'blurry' junction).

The following sequence was carried out on each image for assessment:

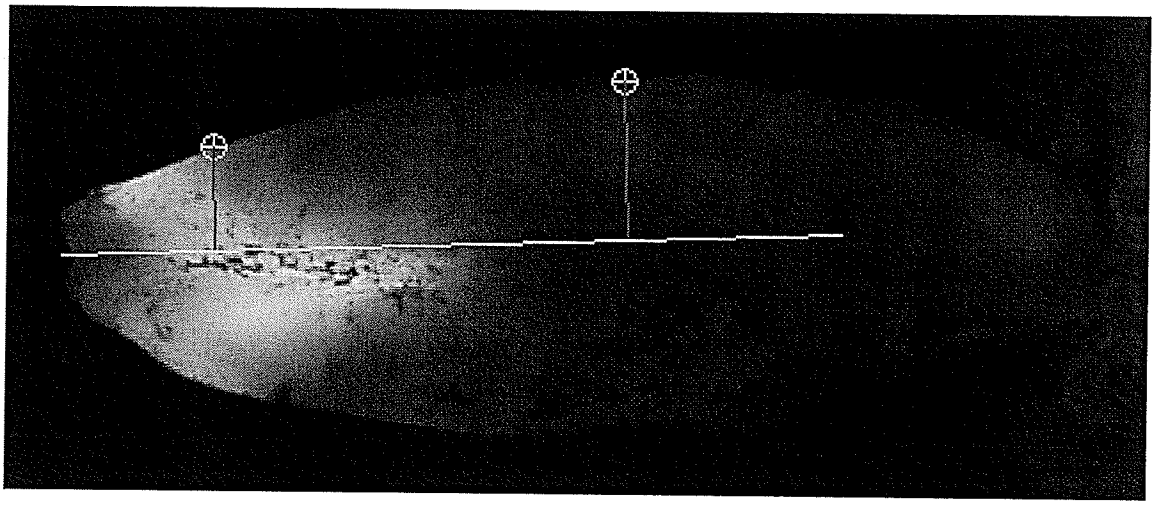
- the TIFF image was imported
- the Spatial Calibration feature was employed to calibrate each image (millimeters) using the calibration bar placed below each tooth as reference
- the image was enhanced with two Spatial Filters (De-speckle to remove black specks and Sharpen to bring out edge borders)
- to bring out distinctions in dark/light shade values, the gamma was reduced from 1.0 to 0.5 to make the border of translucency more vibrant,

- a point feature (small crosshairs) was placed for reference at the labial, maximum, and minimum limits of translucency, the central and labial CEJ and root apex
- using the Manual Measurement toolbar, the Perpendicular Line feature was used to mark the longitudinal midline of the root (apex to mid-CEJ)
- from this, perpendicular lines marked the labial root length (apex – CEJ), labial minimum, and maximum transparency height
- the image was magnified and de-magnified to get the best perspective on plotted measurements; adjustments were made accordingly
- the measurements were saved for later observation (.mrs file)
- measurement values were either available for manual collection or exported directly into a database. This technique allows for the digital assessment of translucent dentin without the limitations of eye strain and fatigue associated with the manual method of measurement.

Foti and Drusini techniques

The digital measurements were taken following the criteria outlined in Figures 3.5 and 3.6. The image below illustrates the measurements required for the Foti and Drusini techniques.

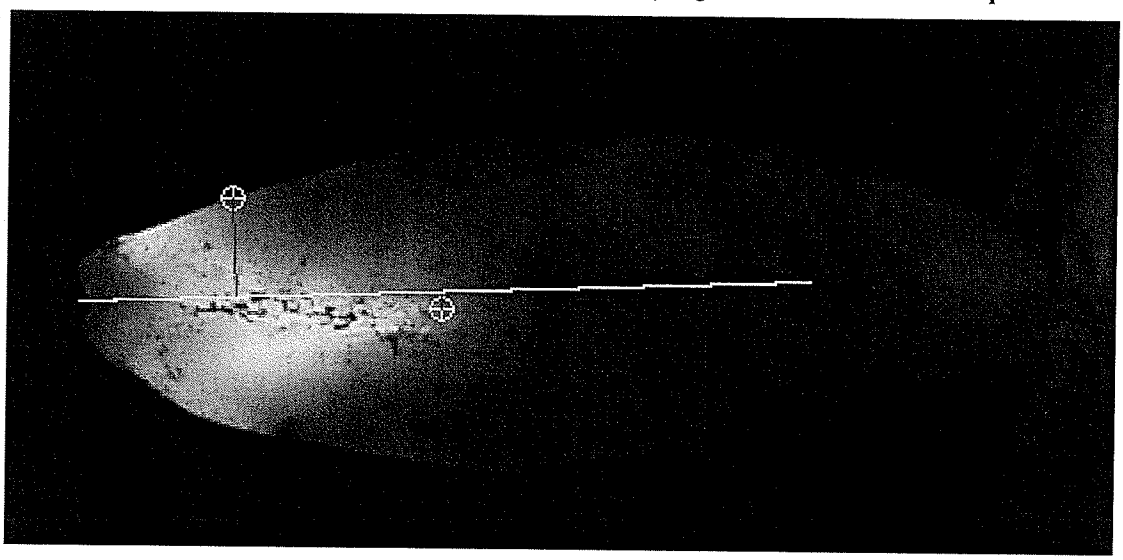
Figure 3.5: Labial transparency height and root length digital measurement techniques.



Miles and Bang and Ramm technique

The image below (Figure 3.6) illustrates the digital analog of the Bang and Ramm measurement technique.

Figure 3.6: Minimum and maximum transparency digital measurement techniques.



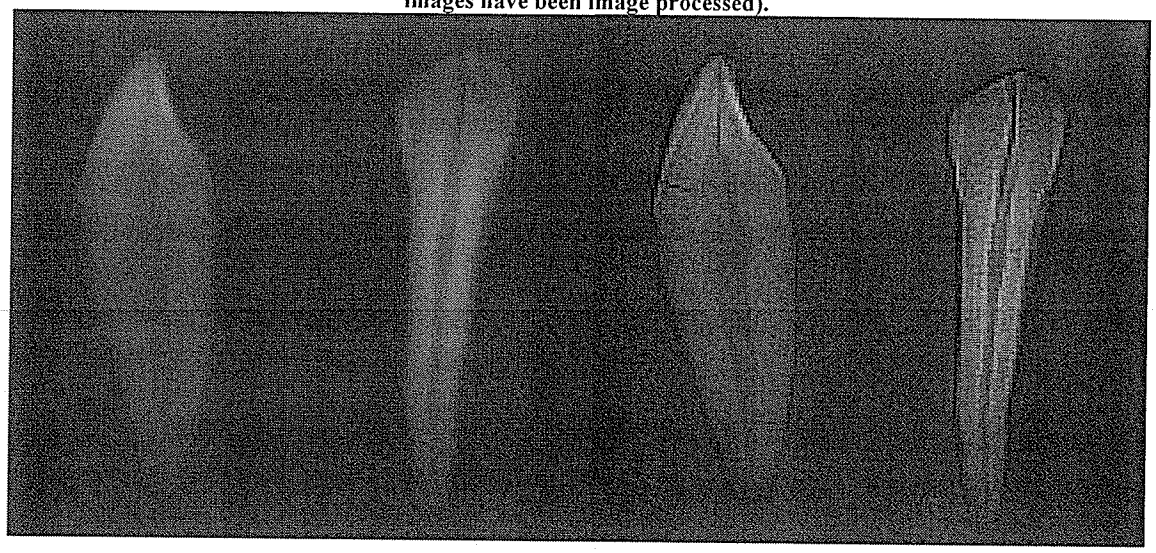
Pulp reduction

The digital scoring of tooth dimensions (pulp, root, and tooth) was accomplished in a similar manner as the manual linear measurement. The following describes the digital analog measurements of Kvaal and Solheim (1994), Paewinsky et al. (2005), and

Cameriere et al. (2004 and 2007) protocol. The following protocol was carried out on each image for scoring:

- each DICOM was imported and saved as a TIFF file
- each image was calibrated (millimeters) with the Spatial Calibration feature using the calibration bar in each image
- the rectangular Area of Interest (AOI) was placed around each tooth for isolated image enhancement
- each tooth was enhanced using the Spatial Filters feature, Sharpen filter (this makes the image more pixilated in order to make borders more distinct); the image was magnified (toggled between x2 or x3)
- using the Manual Measurements feature, several lines were drawn with the Linear Lines tool and the Create Polygon feature was used to create area measurements. Below is an example of a tooth in the labio-lingial and mesio-distal plains before image processing and after (Figure 3.7). Note how enhancement emphasizes sharpness in fine lines and shades where the original image is lacking

Figure 3.7: Enhancement of a dental radiograph image (the left two images are unaltered while the right two images have been image processed).



The images below demonstrate the **Kvaal and Solheim (1994)** digital technique (Figures 3.8 and 3.9).

Figure 3.8: Root and pulp width measurements at CEJ.

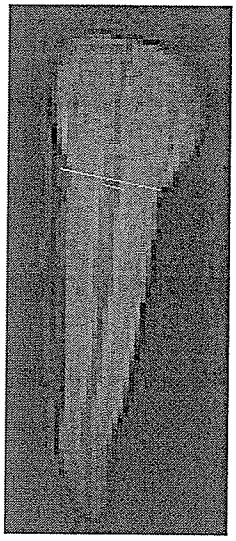
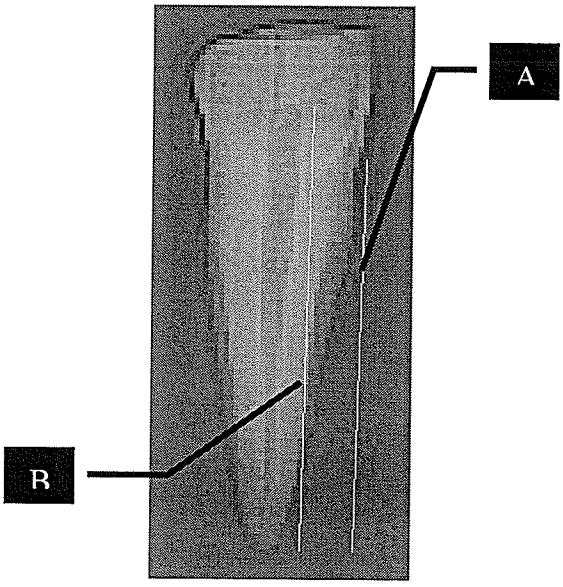
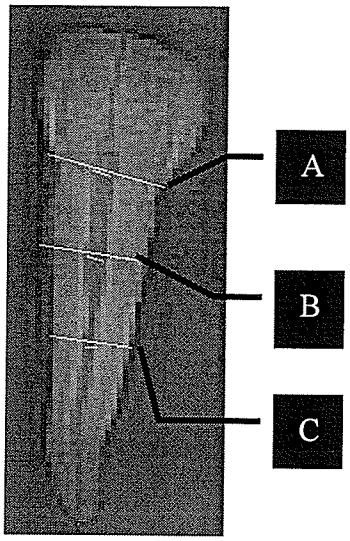


Figure 3.9: Root (A) and pulp (B) mesial length



The image below (Figure 3.10) demonstrates the **Paewinsky et al. (2005)** digital technique. This technique records the pulp:root width ratio separately at the CEJ (A), ¼ (B), and mid-root (C) levels.

Figure 3.10: Pulp and root width measurements at CEJ (A), ¼ (B), and mid-root (C) levels.



The **Cameriere et al. (2004 and 2007)** techniques have no manual analog aside from the maxillary canine pulp and root at mid-root width (which was also recorded by calipers for comparison). The area values of the total tooth and pulp from a labio-lingual orientation are each recorded separately and the ratio is calculated. The following protocol is used for the **Cameriere et al. 2004** technique (applicable only on maxillary canines):

- using the Draw Polygon tool, the labio-lingual aspect of the pulp chamber and tooth were outlined (millimeters) (Figure 3.11)
- the area values were recorded separately
- the pulp and root width at mid-root were recorded (identical to Paewinsky B) (Figure 3.12)

Figure 3.11: Cameriere et al. (2004) labio-lingual tooth and pulp area measurements.

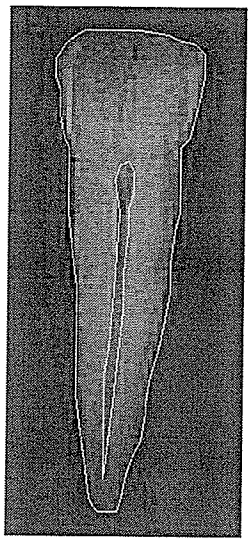
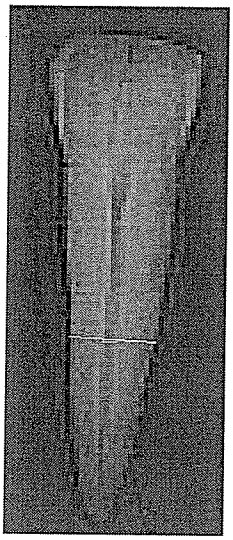


Figure 3.12: Cameriere et al. (2004) pulp and root width measurements at mid-root.



The **Cameriere et al. (2007)** technique differs from the Cameriere et al. (2004) technique in that it utilizes both the mandibular and maxillary canines and uses both the labio-lingual and mesio-distal orientations; it does not include a pulp:root width ratio. The images below illustrate these measurements (Figures 3.13 and 3.14).

Figure 3.13: Labio-lingual orientation tooth and pulp area measurements.

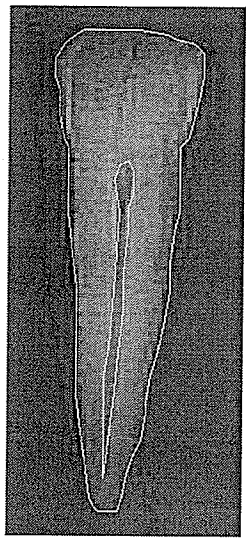
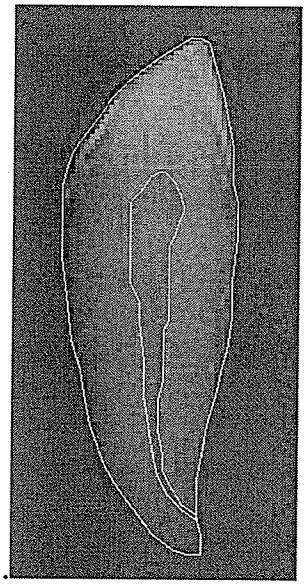


Figure 3.14: Mesio-distal orientation tooth and pulp area measurements



Age calculation

Finally, to calculate age-at-death of the individuals in this sample, the previously described measurements were input into published regression equations from the previously described techniques which provide the estimated age.

Chapter 4: Results

This chapter presents the results of the dental age assessments and comparison of techniques used on the Apollonia Pontica dental sample. Bivariate statistics (Paired *t*-tests are employed to test for intra-observer and intra-method variation). All statistical analyses were accomplished using by SPSS version 14.0.

Digital and manual methods: observer error

Table 4.1 shows all pairs of measurements used for intra-observer error analysis described in the following sections.

Table 4.1: Paired samples *t*-tests for testing intra-observer error for repeated measures. Values in bold are those measures whose mean difference is significantly different than zero.

Measurement	N	Mean Difference (mm)	*t*- value	Sig. (2-tailed)
Dig. Root Width CEJ	**4**	**0.4375**	**4.108**	**.026**
Dig. Root Width 1/4	8	0.15375	1.363	.215
Dig. Root Width Mid	4	0.0225	.243	.824
Dig. Pulp Width CEJ	4	0.0075	.075	.945
Dig. Pulp Width 1/4	8	0.05	1.461	.187
Dig. Pulp Width Mid	4	-0.0625	-1.0	.391
Dig. Mesial Root Length	8	0.03875	.182	.861
Dig. Pulp Length	8	0.03875	.205	.844
Labio-Lingual Pulp Area	7	0.00143	.003	.998
Labio-Lingual Tooth Area	7	1.75857	1.982	.095
Mesio-Distal Pulp Area	7	-0.84857	-1.218	.269
Mesio-Distal	7	.04571	.064	.951

Tooth Area				
Dig. Labial-CEJ Root Length	6	0.05	.253	.810
Dig. Labial Transparency Height	6	0.03167	.131	.901
Dig. Minimum Transparency Height	6	0.27	.974	.375
Dig. Maximum Transparency Height	6	-0.715	-1.064	.336
Cal. Root Width CEJ	4	0.0775	2.067	.131
Cal. Root Width 1/4	9	0.16	1.340	.217
Cal. Root Width Mid	**4**	**0.13**	**7.723**	**.005**
Cal. Pulp Width CEJ	**4**	**-0.0775**	**-3.564**	**.038**
Cal. Pulp Width 1/4	9	-0.03111	-1.465	.181
Cal. Pulp Width Mid	4	0.035	1.021	.382
Cal. Mesial Root Length	**8**	**0.5075**	**2.586**	**.036**
Cal. Pulp Length	8	0.225	.239	.818
Cal. Labial Root Length	8	0.21375	.688	.513
Cal. Labial Transparency Height	8	-1.25	-1.476	.138
Cal. Minimum Transparency Height	**9**	**-0.98889**	**-3.585**	**.007**
Cal. Maximum Transparency Height	**9**	**-0.69333**	**-2.759**	**.025**

Digital methods

Pulp and root measurements

Paired samples *t*-tests revealed no statically significant differences in measurements between the following digital scores: root width at 1/4 (p=0.215), root width at mid-root (p=0.824), pulp width at CEJ (p=0.945), pulp width at mid-root (p=0.391), pulp width at ¼ root (p=0.187), mesial root length (p=0.861), pulp length (p=0.844), labio-lingual pulp area (p=0.998), labio-lingual tooth area (p=0.095), mesio-distal pulp area (p=0.269), and mesio-distal tooth area (p=0.951). Digital measurements found to be significantly different were: root width at CEJ (p=0.026).

Transparency and root measurements

Paired samples *t*-tests indicated no s significant differences in measurements between the following digital scores: labial root length (p=0.810), labial transparency height (p=0.901), minimum transparency height (p=0.375), and maximum transparency height (p=0.336).

Manual methods

Pulp and root measurements

Paired samples *t*-tests indicated no significant differences in scores for the following caliper measurements: root width at CEJ (p=0.131), root width at ¼ root (p=0.217), pulp width at ¼ root (p=0.181), pulp width at mid-root (p=0.382), and pulp length (p=0.818). Manual measurements that were found to be significantly different

were: root width at mid-root (p=0.005), pulp width at CEJ (p=0.038), and mesial root length (p=0.036).

Transparency and root measurements

Paired samples *t*-tests indicate no significant differences in manual measurements of the following: labial root length (p=0.513) and labial transparency height (p=0.138). Manual measurements that were observed to be significantly different were: minimum transparency height (p=0.007) and maximum transparency height (p=0.025).

Comparison of Inter-method measurements and ratios

The following compares full sets of digital/manual measurements and pulp/tooth and transparency/root ratios (paired *t*-test) (Table 4.2).

Table 4.2: Full digital and manual measurement set comparison.

Measurement	N	Mean Difference (mm)	*t*-value	Sig. (2-tailed)
Root Width CEJ	**18**	**.19611**	**2.631**	**.018**
Root Width 1/4	**30**	**-.17267**	**-2.554**	**.016**
Root Width Mid	18	-.04333	-.600	.556
Pulp Width CEJ	**18**	**-.28000**	**-6.125**	**<0.001**

Pulp Width ¼	**30**	**-.22233**	**-7.226**	**<0.001**
Pulp Width Mid	**18**	**-.24333**	**-6.776**	**<0.001**
Root Length	30	.17067	1.003	.324
Pulp Length	30	-.35500	-7.43	.464
Pulp/Root Length Ratio	30	-.03200	-1.219	.223
Pulp/Root Width CEJ Ratio	**18**	**-.05611**	**-6.44**	**<0.001**
Pulp/Root Width ¼ Ratio	**30**	**-.04100**	**-6.264**	**<0.001**
Pulp/Root Width Mid Ratio	**18**	**-.05611**	**-5.68**	**<0.001**
Labial Root Length	**22**	**-.64545**	**-3.612**	**.002**
Labial Transparency Height	22	-.37864	-.907	.375
Minimum Transparency Height	22	-.09136	-.199	.844

Maximum Transparency Height	22	.26955	.647	.525
Labial Transparency Height/Root Length Ratio	31	2.76419	.811	.424

No significant differences in measurement sets were found between the following: root width mid-root (p=0.556), mesial root length (p=0.324), pulp length (p=0.464), pulp/root length ratio (p=0.223), labial transparency height (p=0.375), minimum transparency height (p=0.844), maximum transparency height (p=0.525), and labial transparency height/root height ratio (p=0.424). Measurement sets that were found to be significantly different were: root width CEJ (p=0.018), root width ¼ root (p=0.016), pulp width CEJ (p=<0.001), pulp width ¼ root (p=<0.001), pulp width mid-root (p=<0.001), pulp/root width CEJ ratio (p=<0.001), pulp width ¼ root ratio (p=<0.001), pulp width mid-root ratio (p=<0.001), and labial root length (p=0.002).

Age estimates and distributions

This section displays the calculated dental age estimates and distributions. The total age estimates for the entire sample, separated by individual (Cat #), method (manual and/or digital), and technique (Foti, Kvaal and Solheim, etc.) are presented in Table 4.3 and 4.4. The individuals #267, #267(2), #320, #358, #291, #233, #219/104 were omitted from age calculation as they were unusable because of taphonomic destruction.

Table 4.3: Digitally acquired dental age estimates.

Cat #	Foti	Miles	B & R	Drusini	Cam 04 AR	Cam 04 AR+C	Cam 07	K & S FL	K & S FWC	K & S FWC+T	Pae A	Pae B	Pae C
501	53.29	38.75	47.02	30.05					54.12	38.82	60.09	49.81	72.96
19	51.70	32.38	40.03	26.42					59.50	39.07	67.10	62.40	56.53
253	61.10	68.34	71.31	45.97					48.10	56.16	52.25	51.92	58.31
289	61.36	65.20	70.67	59.42					46.96	54.16	50.76	52.07	54.68
352	69.53	85.84	73.79	59.96			56.81		75.90	95.83	88.45	69.52	63.62
336	58.69	53.34	54.96	47.57								67.39	67.42
244	57.03	69.13	65.33	43.63			20.52		41.60	47.55	43.79	35.36	70.47
215	57.74	72.04	69.11	47.72					59.41	57.25	66.97	56.97	68.01
192	52.95	57.17	58.75	34.38					51.44	44.77	56.59	62.46	71.35
307									40.81		42.75	58.82	52.29
249	59.59	56.02	58.53	47.22					52.96	45.79	58.57	61.01	53.03
207									75.90		88.45	51.92	58.31
284	51.72	32.42	42.47	29.96			26.92		58.76	38.69	66.14	76.21	59.34
242a									51.02		56.06	63.88	70.34
305									53.19		58.88	67.61	72.17
250							28.50		49.96		54.67	63.94	71.35
321	51.89	39.86	49.03	30.06			24.17		52.16	36.05	57.54	66.14	72.27
304	54.04	41.29	42.44	31.36				48.68					
359	55.40	43.46	43.81	38.04	22.36	21.80	24.57	44.40					
374	55.34	49.87	45.73	42.09	20.87	21.02	32.01	50.73					
242	58.86	60.22	49.62	52.48	45.63	45.49	51.60	50.36					
309	54.10	39.58	40.10	33.92	32.99	34.59	30.90	59.80					
67	62.11	78.82	66.28	53.06				52.82					
254	68.07	84.96	73.84	72.79				55.14					
338								54.86					
354	58.28	63.82	52.03	41.93	39.38	39.31	45.12	60.03					
302	64.16	74.94	69.85	56.03				52.68					
330								63.53					
331	51.66	52.64	46.02	28.41									

Table 4.4: Manually acquired dental age estimates.

Cat #	Foti	Miles	B & R	Drusini	K & S FL	K & S FWC	K & S FWC+T	Pae A	Pae B	Pae C
301	53.61	39.63	47.24	30.70		38.69	26.91	39.97	33.05	45.68
19	51.61	33.53	40.31	25.51		43.69	27.07	46.48	43.67	32.35
253	62.90	72.27	72.47	49.21		47.34	59.08	51.24	31.87	43.47
289	62.14	65.71	68.25	57.57		41.19	54.97	43.23	55.12	54.94
352	70.34	80.21	77.84	58.79		75.90	91.07	88.45	44.94	49.51
336	58.89	54.03	59.91	46.96					46.40	61.19
244	61.31	67.70	65.56	50.80		34.56	46.08	34.59	38.31	47.88
215	64.56	71.62	69.48	66.86		75.90	79.44	56.58	54.42	40.49
192	52.95	58.79	59.71	33.60		46.62	40.84	50.30	57.72	55.55
307						30.02		28.67	39.45	52.09
249	58.05	62.02	62.49	42.92		37.28	40.83	38.13	53.75	47.51
207						75.90		88.45	41.38	52.49
284	52.02	44.93	49.15	30.62		39.91	24.51	41.56	59.71	65.52
242a						33.84		33.65	60.84	51.81
305						44.37		47.37	55.32	48.92
250						44.41		47.42	43.89	57.18
321	53.55	43.36	48.24	33.09		38.49	26.72	39.70	38.87	47.73
304	54.01	41.98	41.80	31.47	49.73					
359	55.66	44.24	44.42	39.91	52.01					
374	55.55	47.52	44.30	42.54	57.89					
242	58.92	54.17	52.01	49.91	54.12					
309	53.93	38.01	40.00	33.18	56.07					
67	62.88	81.87	66.82	54.14	49.61					
254	48.19	94.01	40.41	80.34	46.96					
338					53.67					
354	59.68	59.71	55.27	45.07	59.17					
302	54.03	44.93	41.56	36.05	73.92					
330					62.08					
331	51.99	32.47	38.63	29.14	46.96					

The age estimates and age ranges of each individual (Cat #) separated by method (digital and/or manual) and individual technique are presented graphically in (following charts) Figures 4.1 to 4.58. The bold horizontal line in each of the charts represents the mean age of the individual from all combined technique age estimates.

Figure 4.1: Digital method age range, #301.

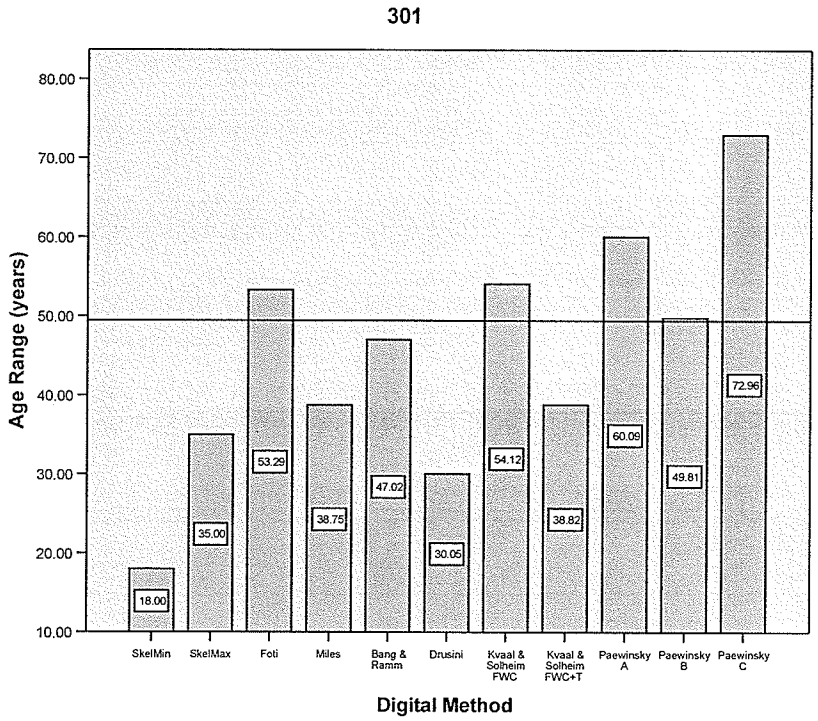


Figure 4.2: Manual method age range, #301.

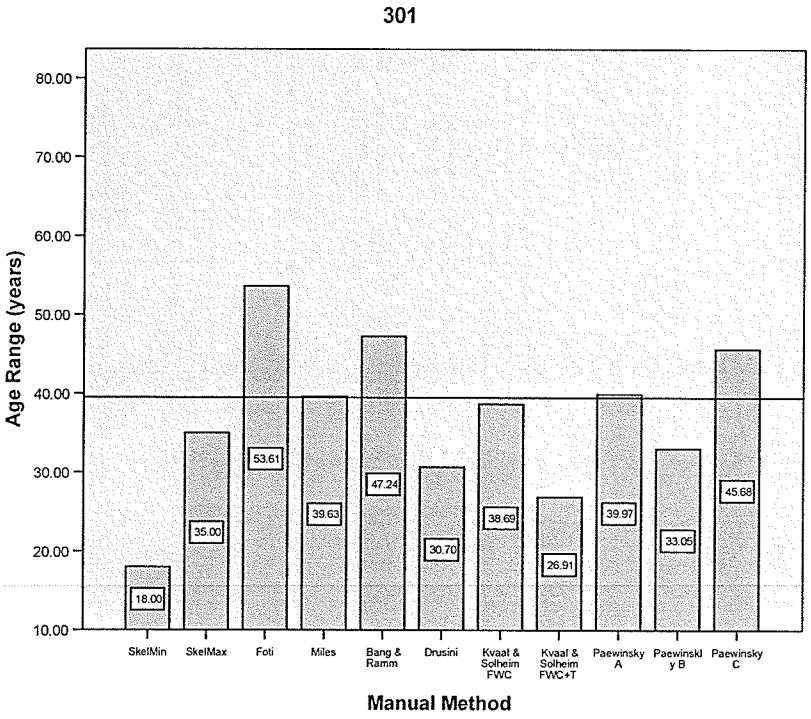


Figure 4.3: Digital method age range, #19.

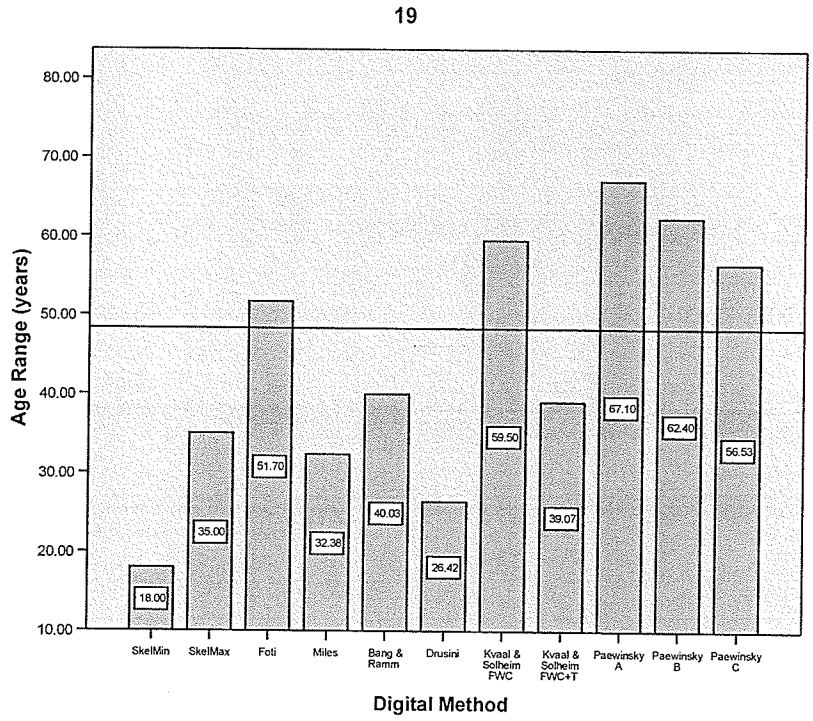


Figure 4.4: Manual method age range, #19.

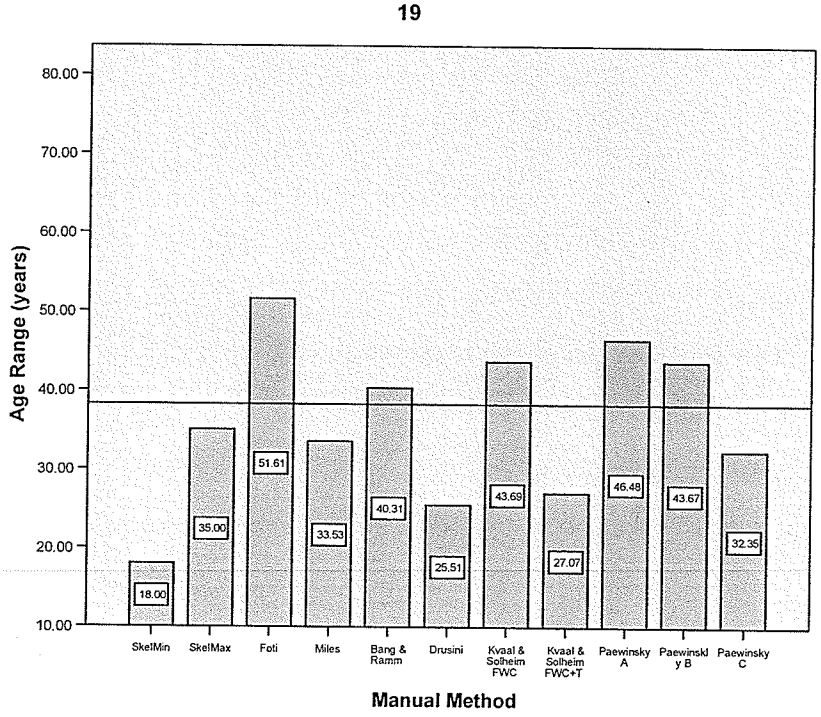


Figure 4.5: Digital method age range, #253.

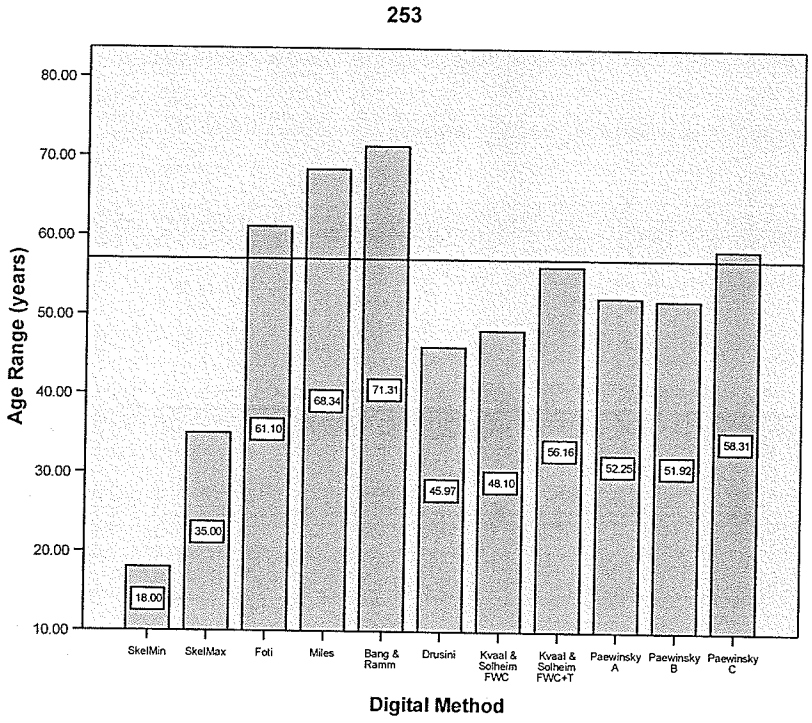


Figure 4.6: Manual method age range, #253.

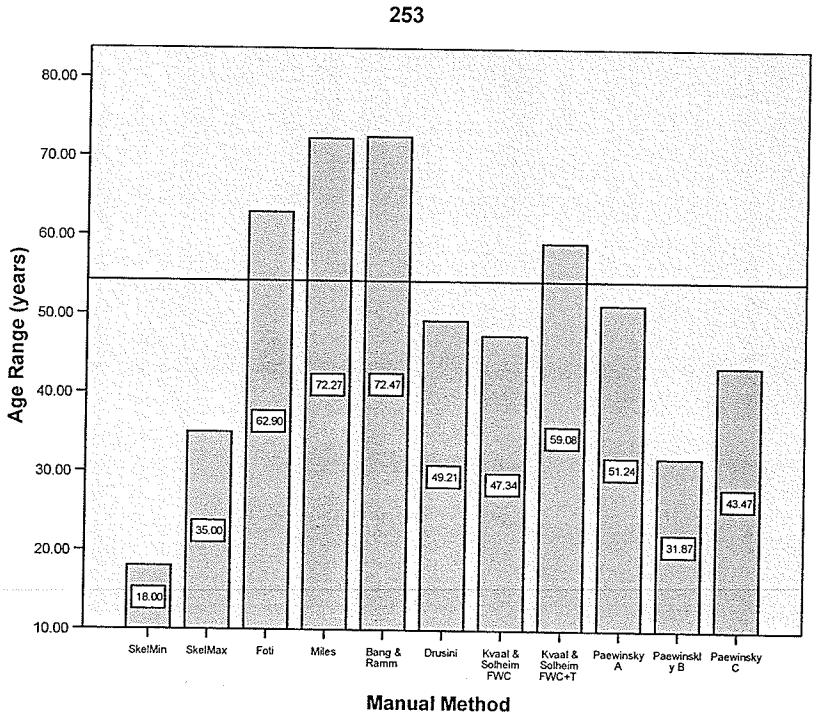


Figure 4.7: Digital method age range, #289.

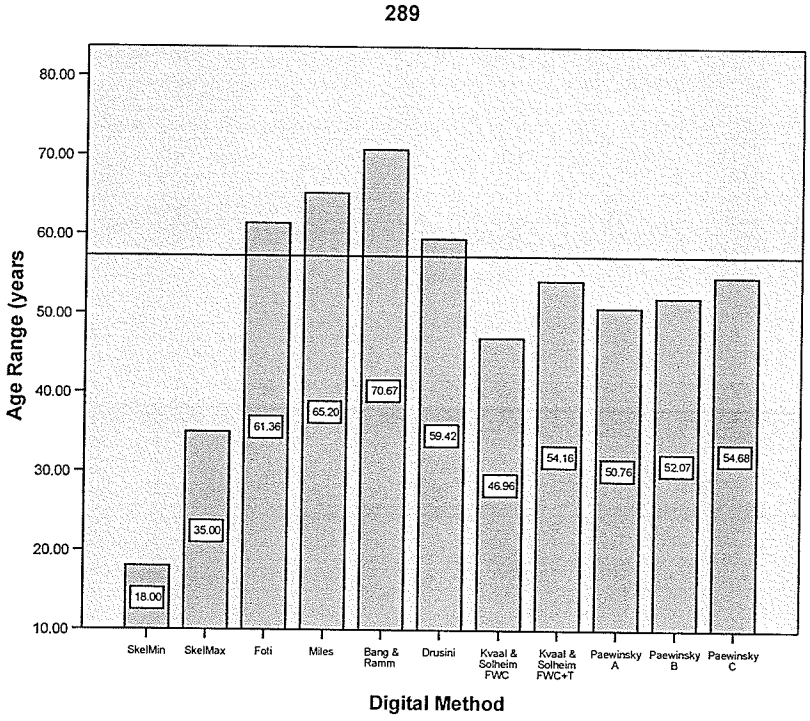


Figure 4.8: Manual method age range, #289.

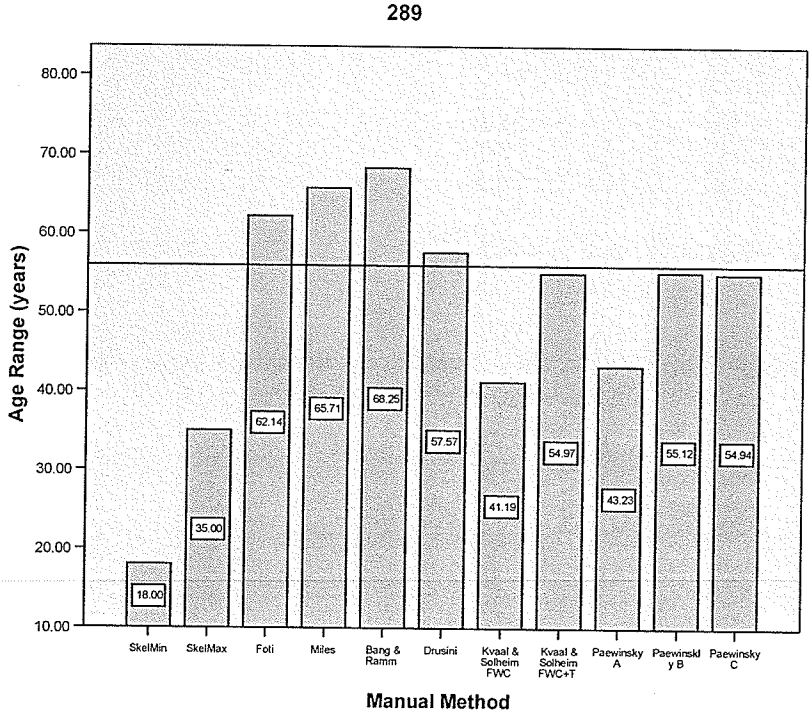


Figure 4.9: Digital method age range, #352.

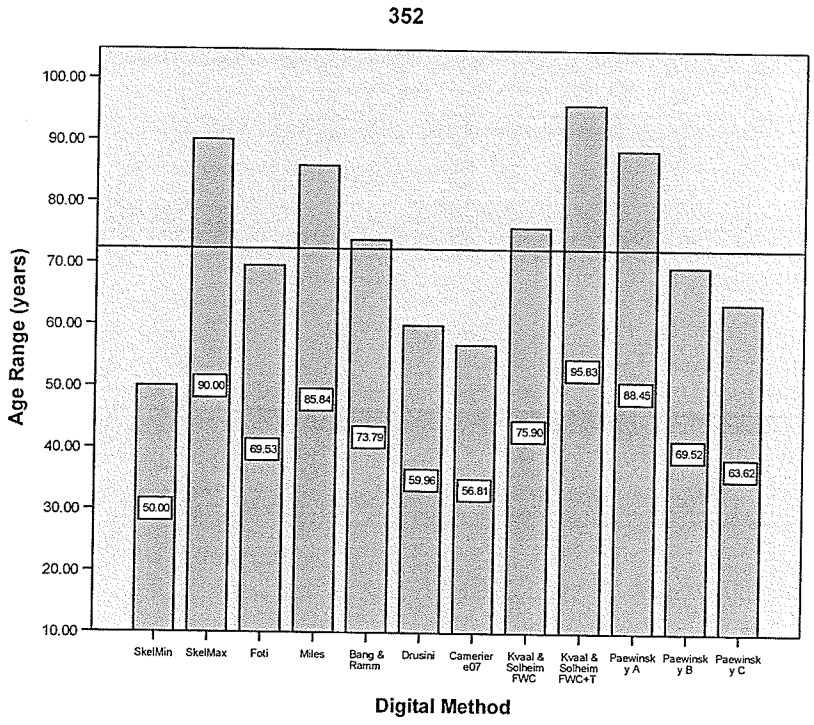


Figure 4.10: Manual method age range, #352.

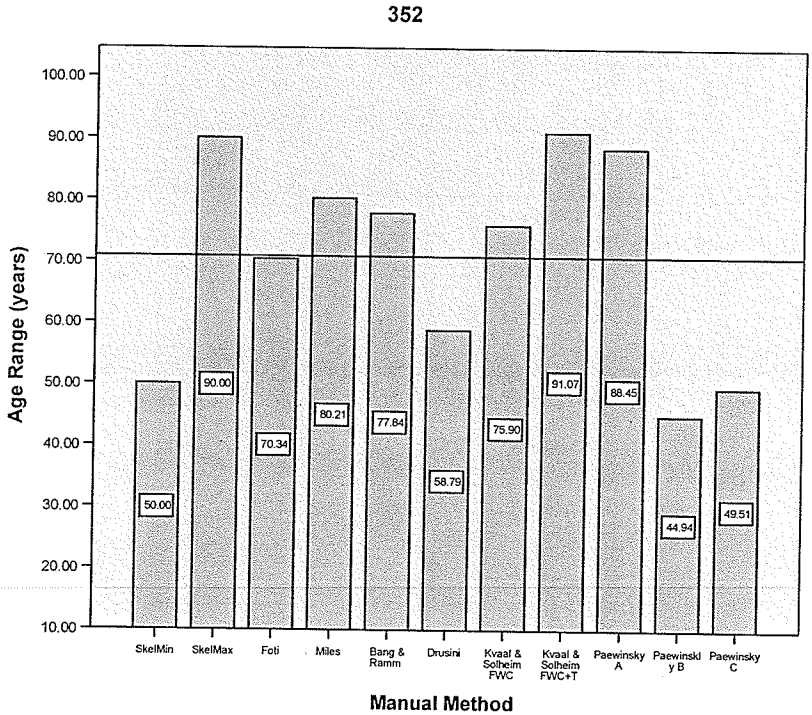


Figure 4.11: Digital method age range, #336.

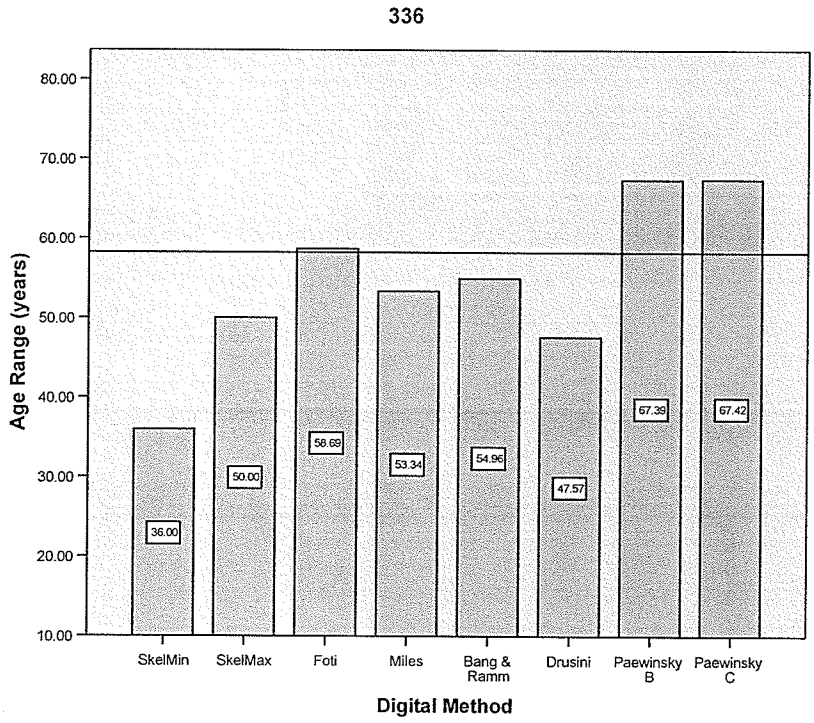


Figure 4.12: Manual method age range, #336.

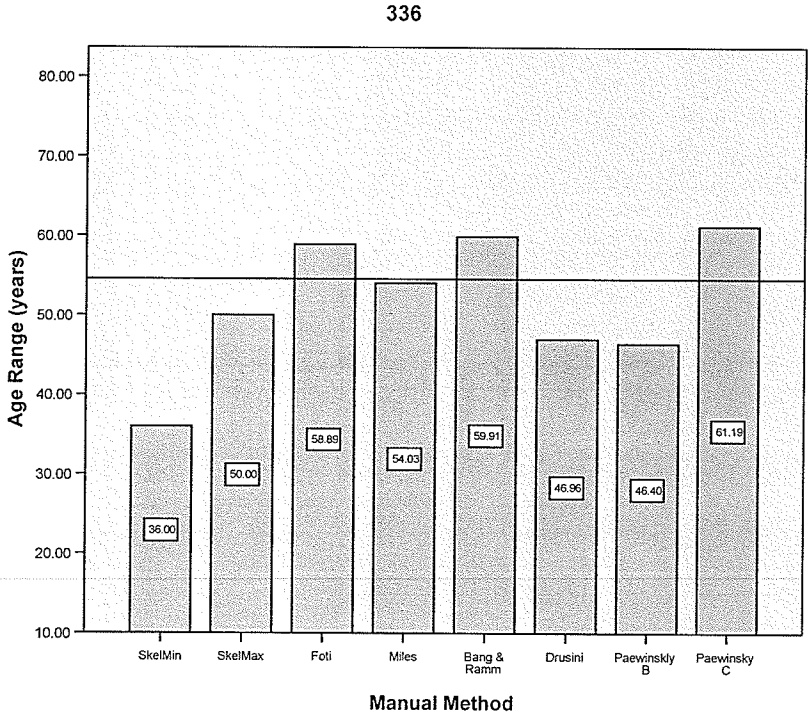


Figure 4.13: Digital method age range, #244.

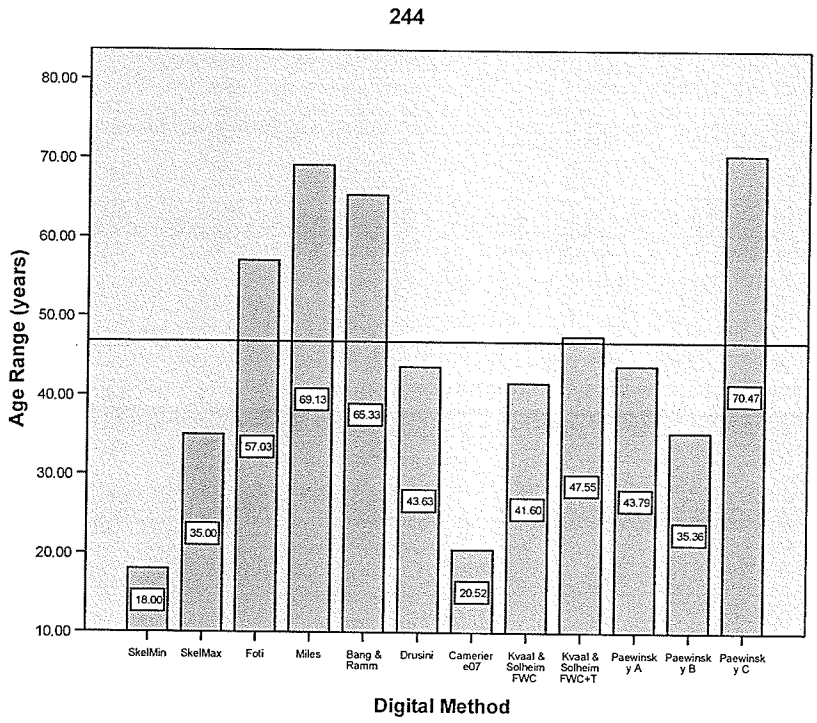


Figure 4.14: Manual method age range, #244.

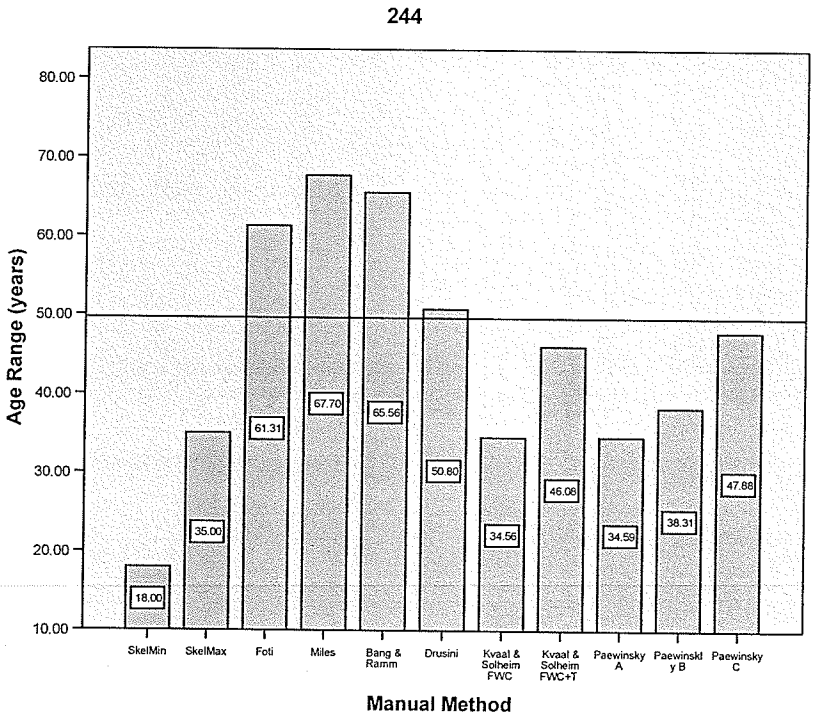


Figure 4.15: Digital method age range, #215.

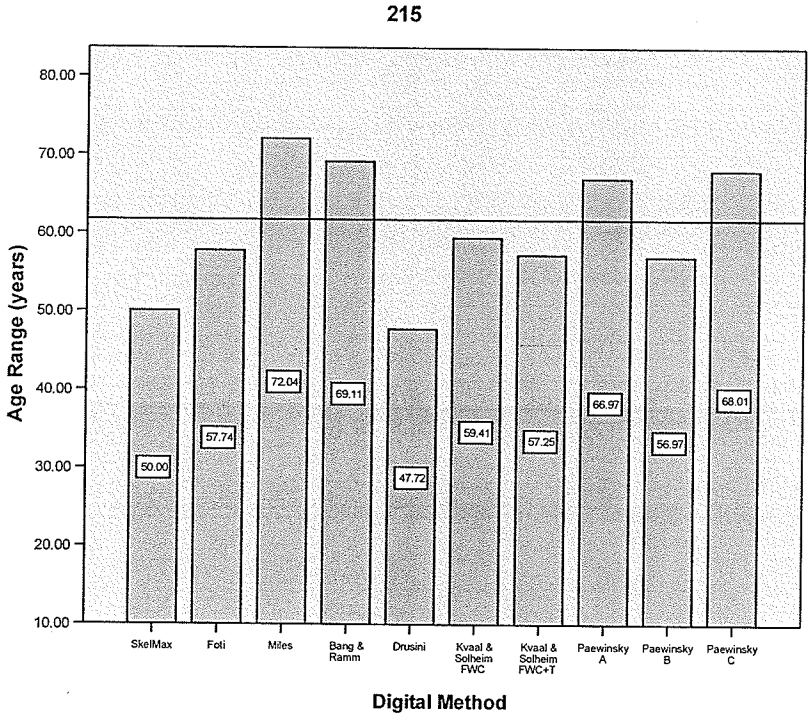


Figure 4.16: Manual method age range, #215.

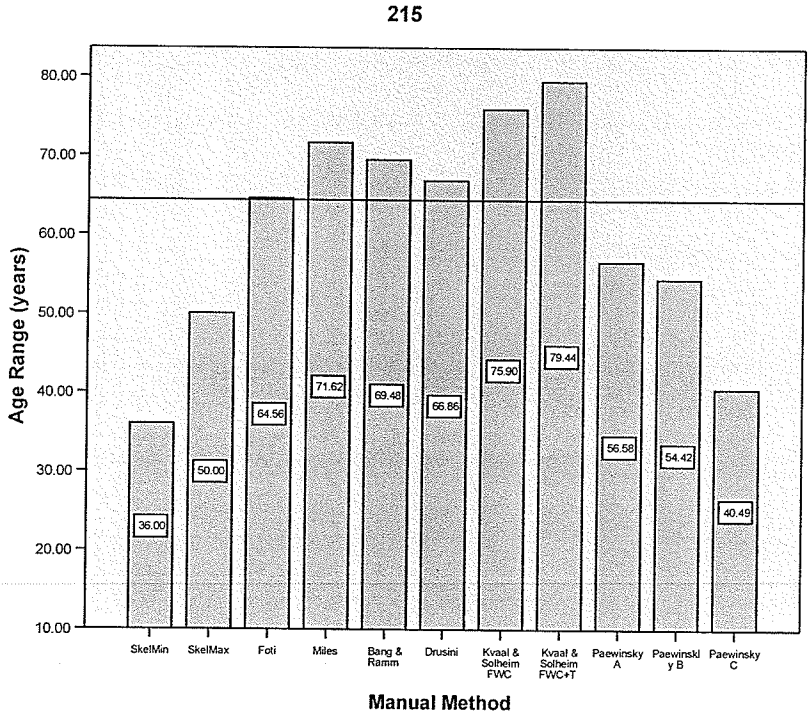


Figure 4.17: Digital method age range, #192.

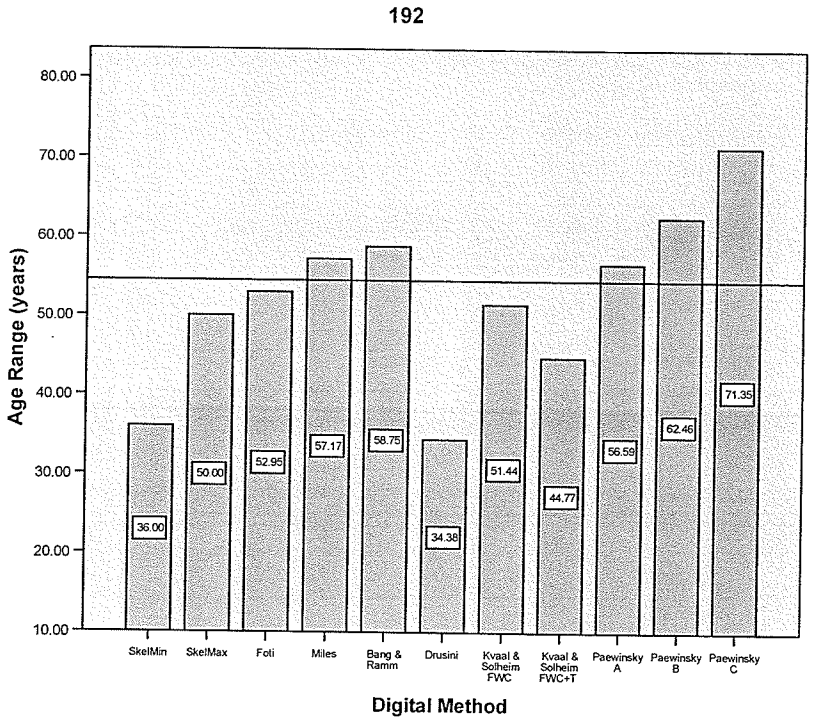


Figure 4.18: Manual method age range, #192.

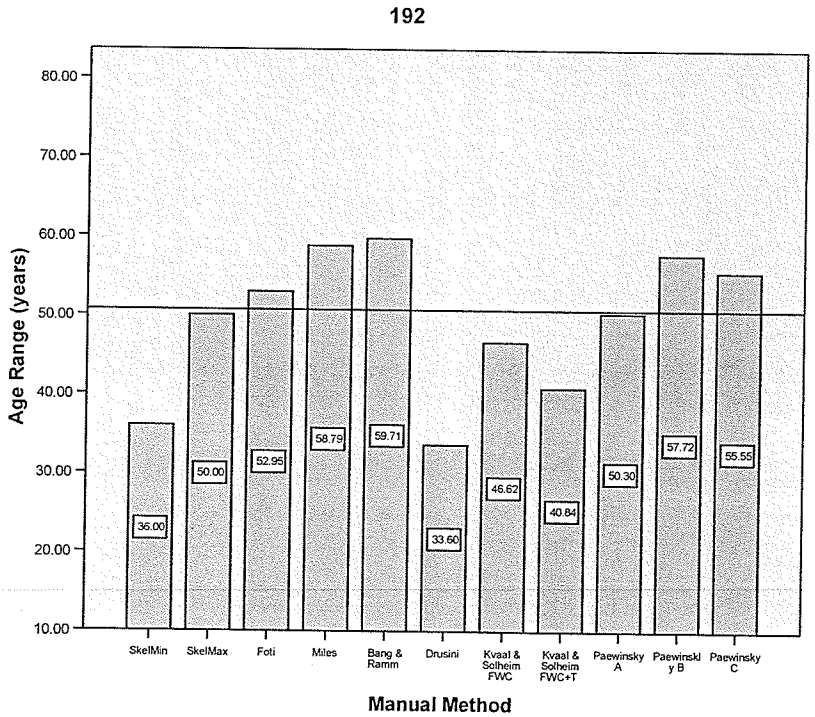


Figure 4.19: Digital method age range, #307.

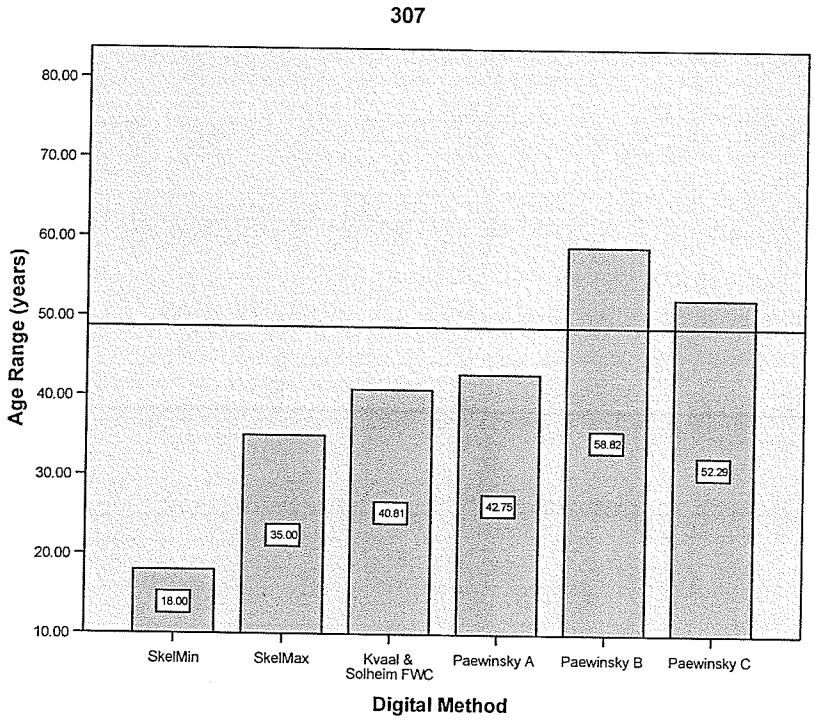


Figure 4.20: Manual method age range, #307.

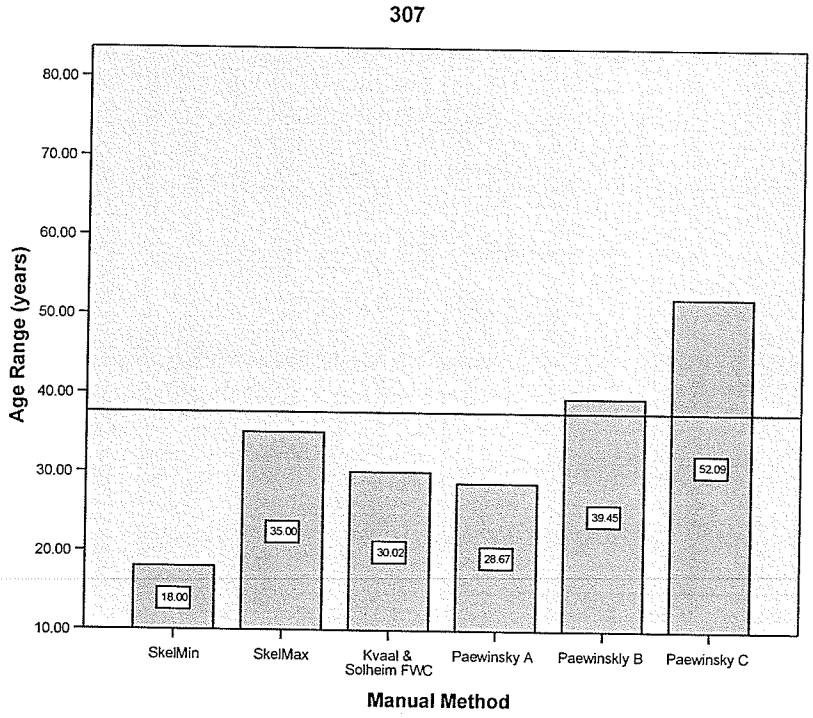


Figure 4.21: Digital method age range, #249.

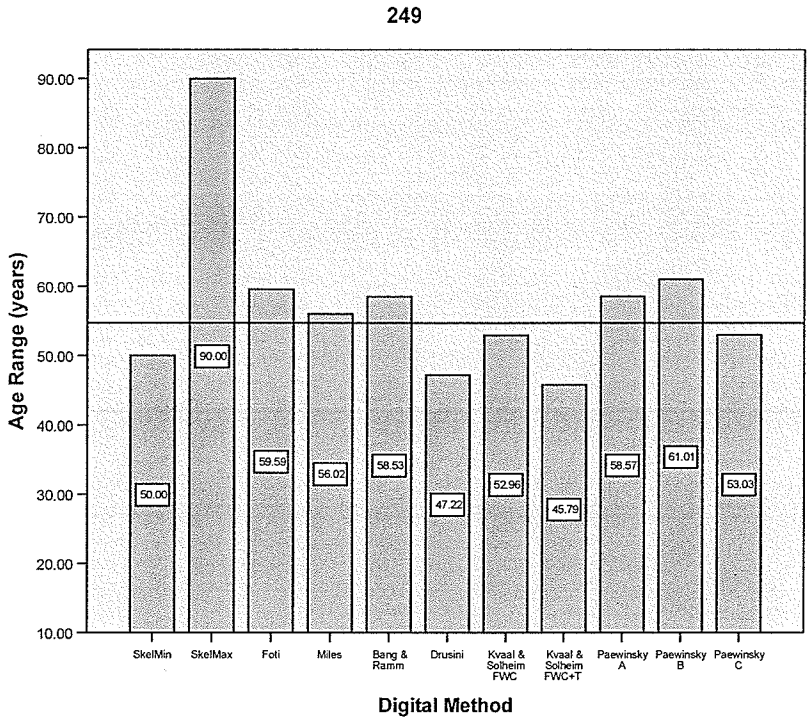


Figure 4.22: Manual method age range, #249.

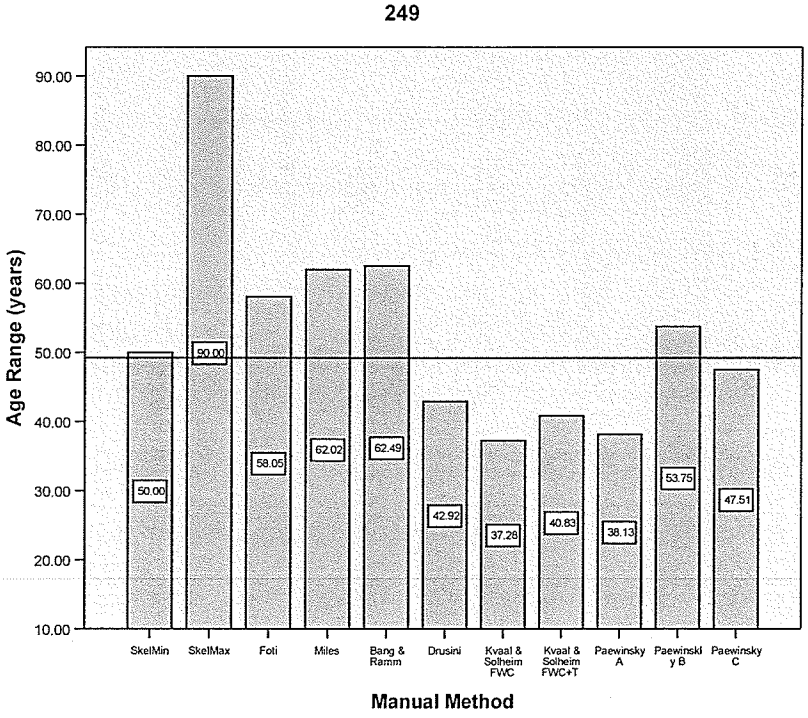


Figure 4.23: Digital method age range, #207.

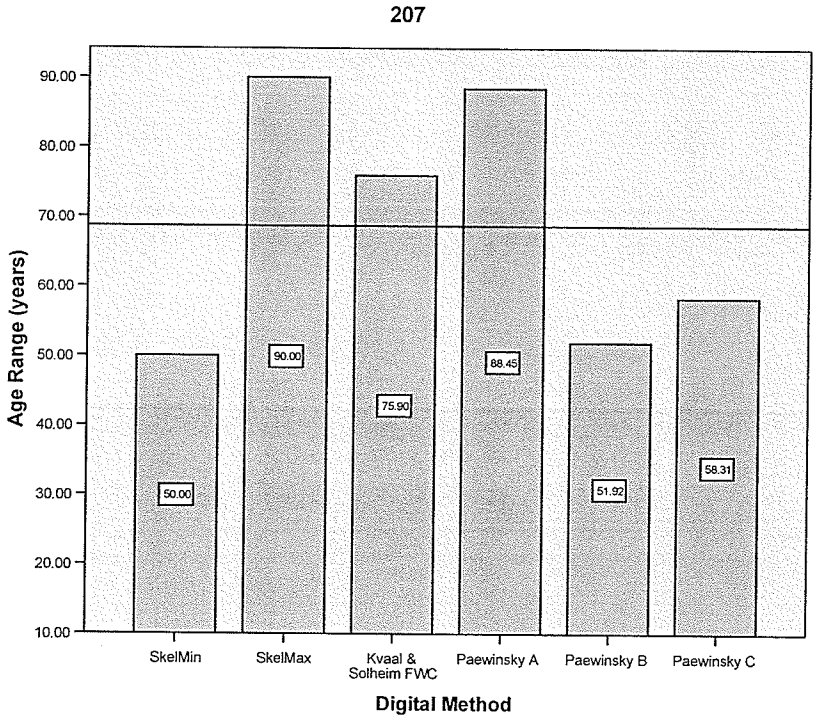


Figure 4.24: Manual method age range, #207.

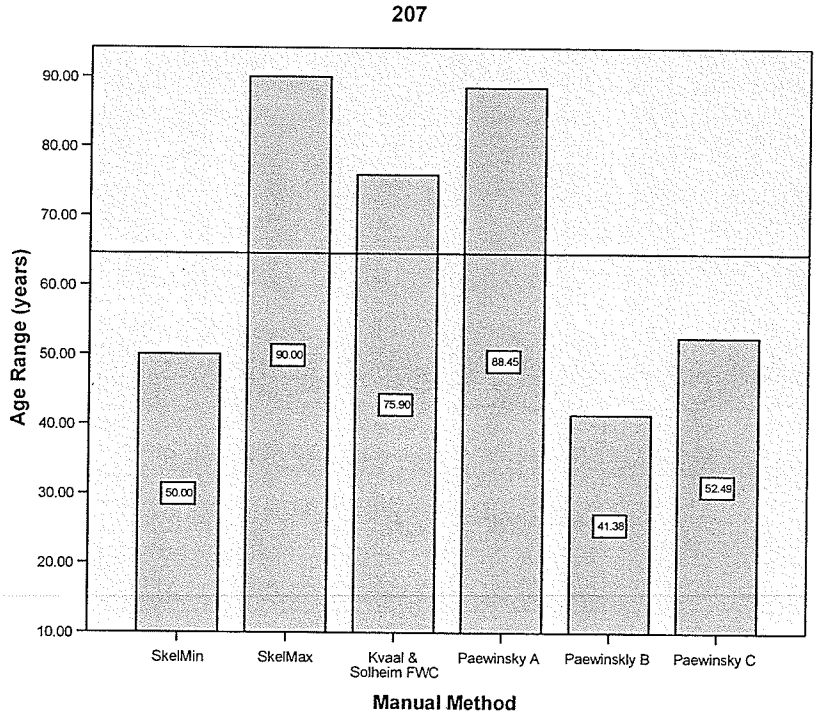


Figure 4.25: Digital method age range, #284.

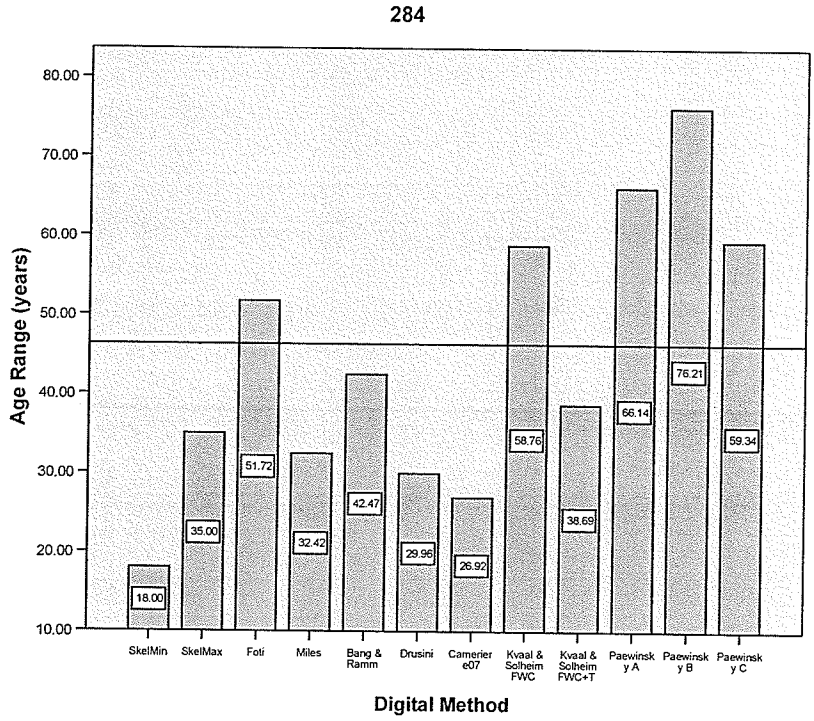


Figure 4.26: Manual method age range, #284.

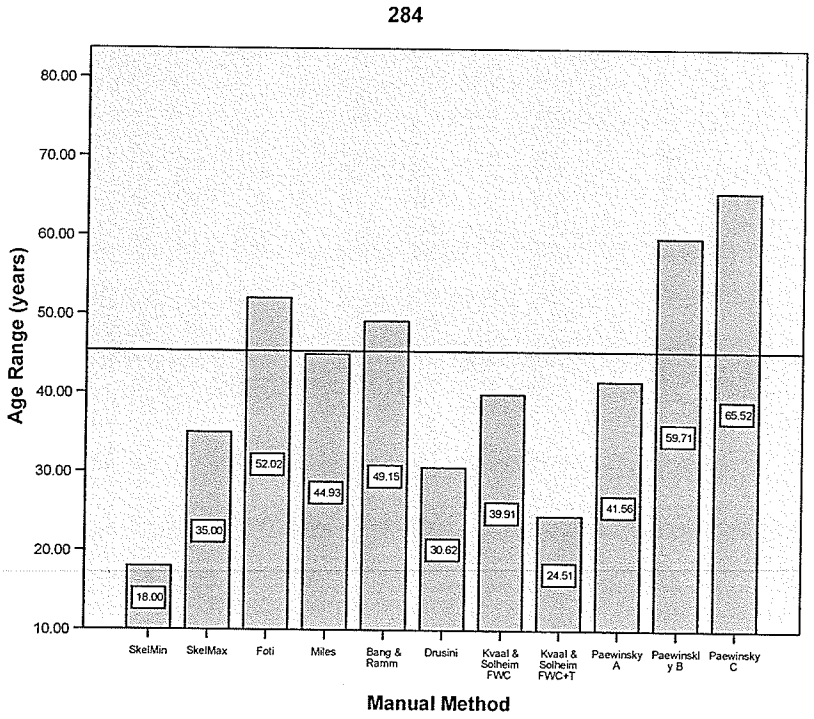


Figure 4.27: Digital method age range, #242A.

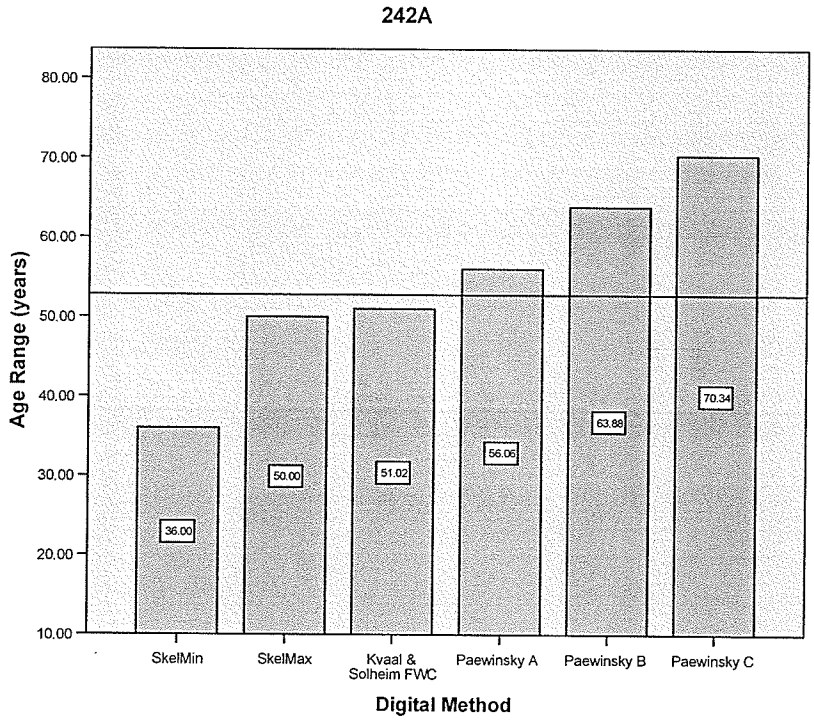


Figure 4.28: Manual method age range, #242A.

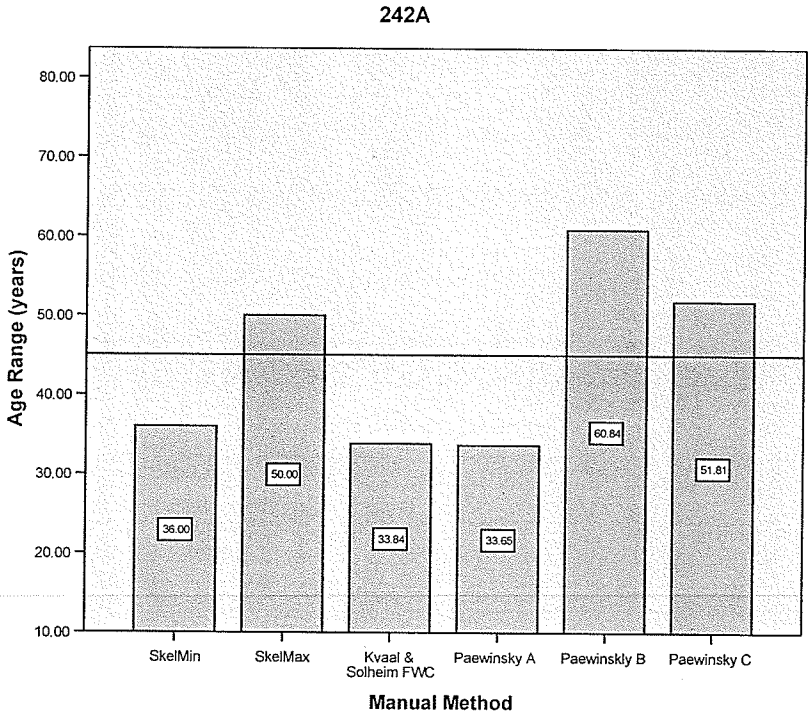


Figure 4.29: Digital method age range, #305.

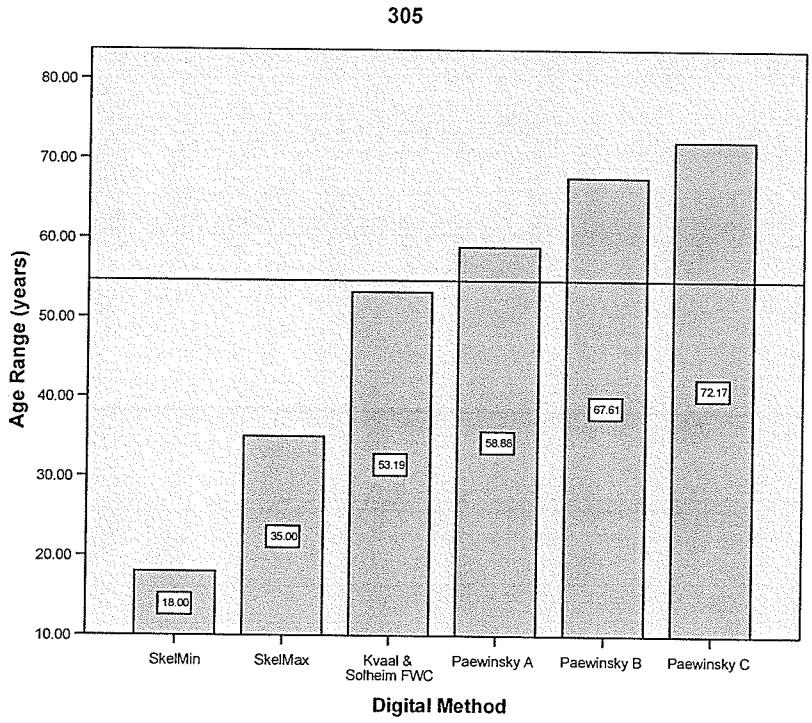


Figure 4.30: Manual method age range, #305.

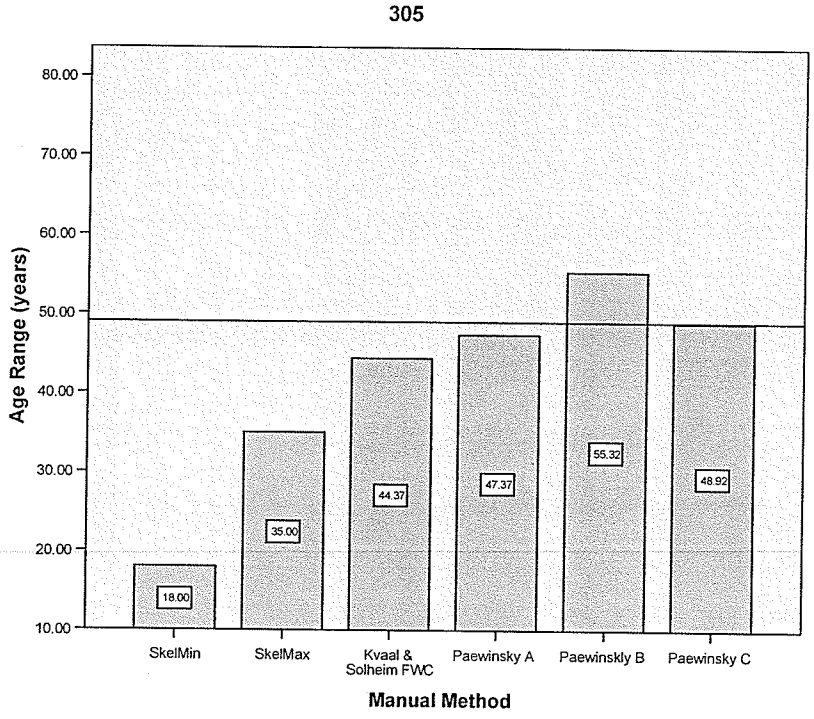


Figure 4.31: Digital method age range, #250.

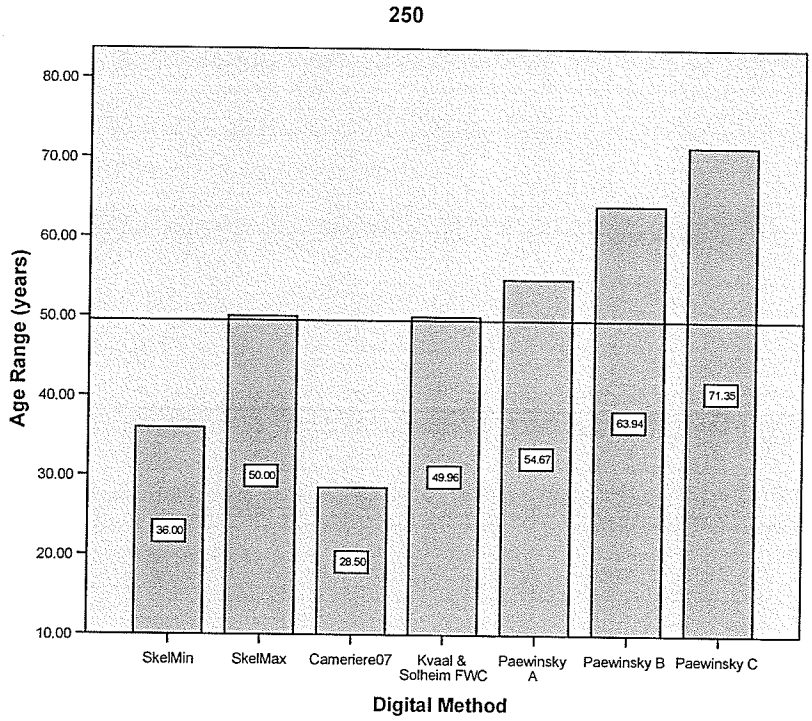


Figure 4.32: Manual method age range, #250.

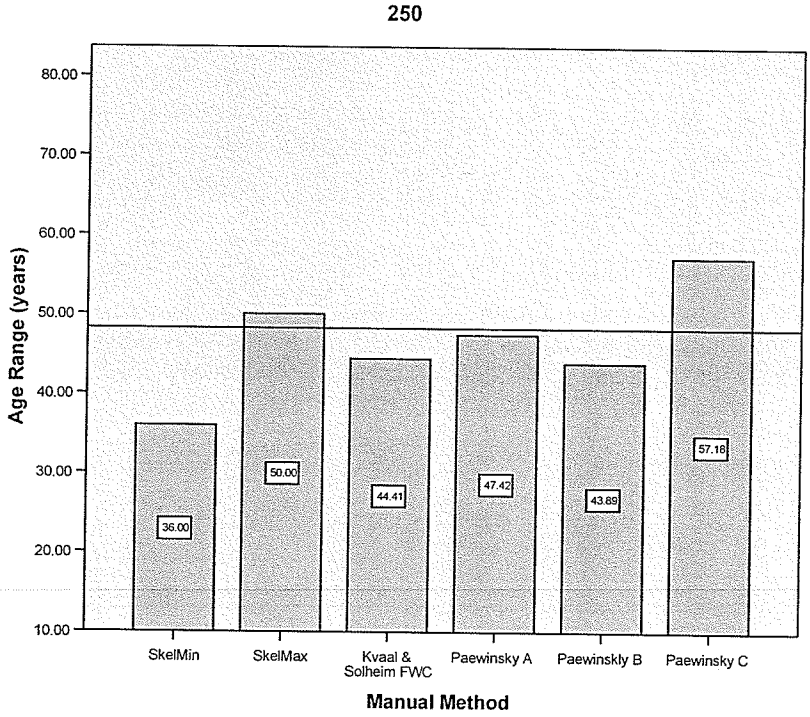


Figure 4.33: Digital method age range, #321.

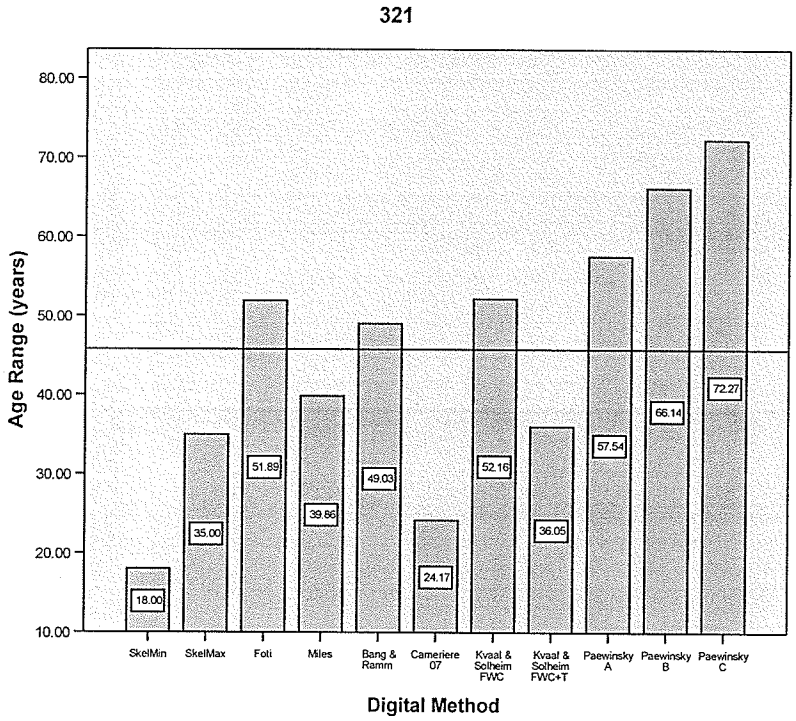


Figure 4.34: Manual method age range, #321.

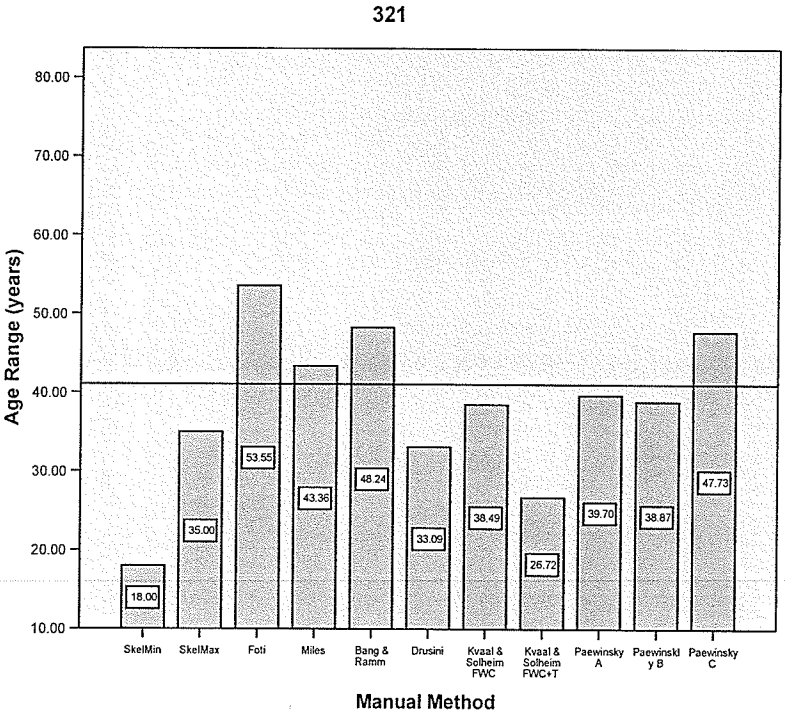


Figure 4.35: Digital method age range, #304.

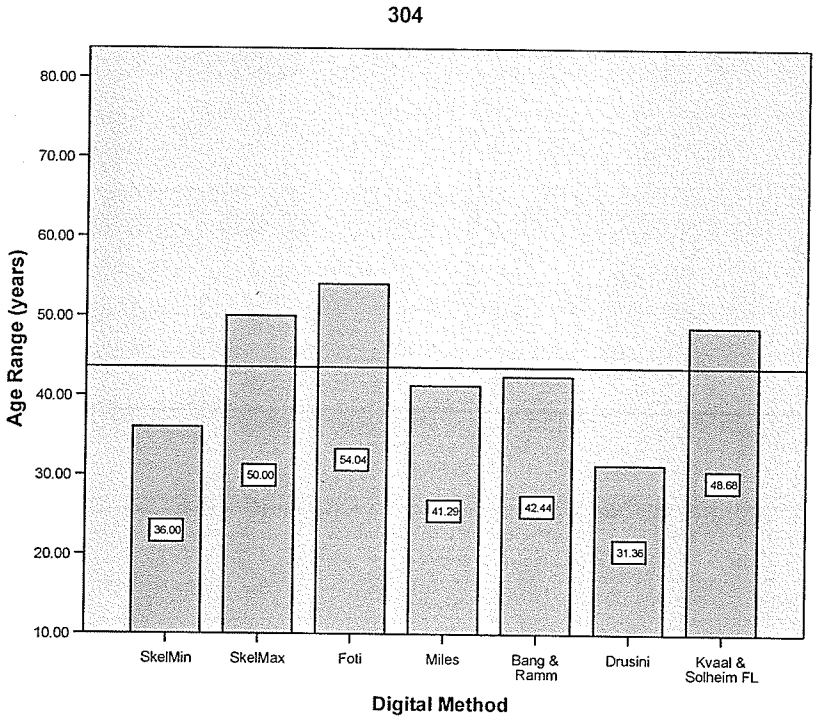


Figure 4.36: Manual method age range, #304.

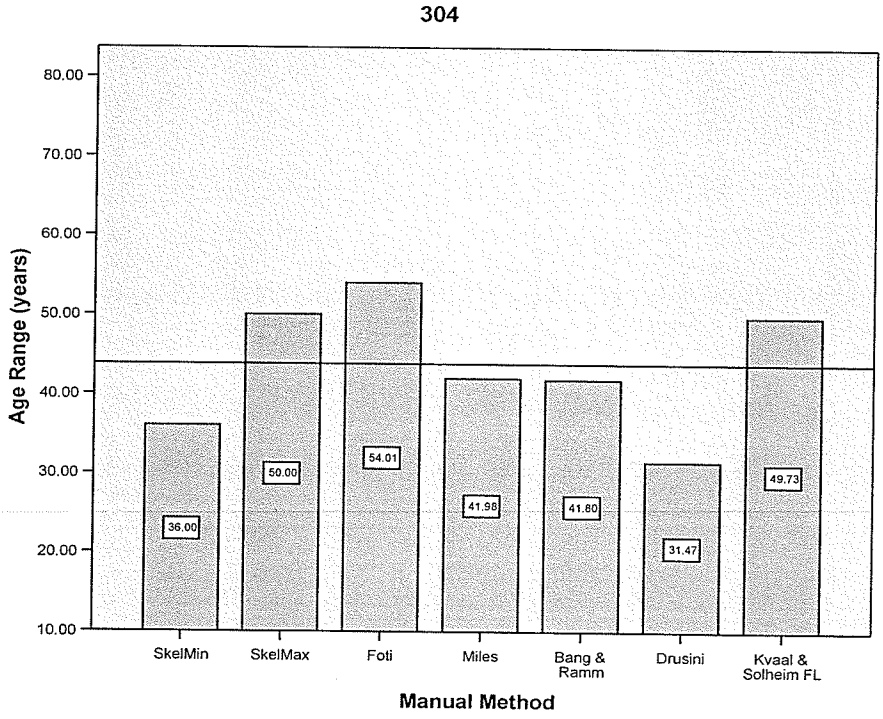


Figure 4.37: Digital method age range, #359.

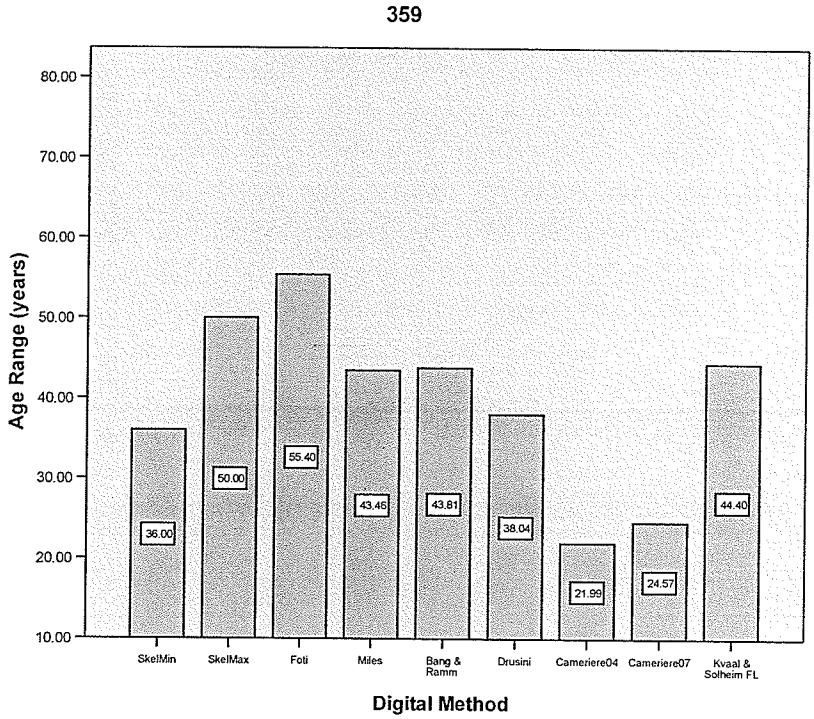


Figure 4.38: Manual method age range, #359.

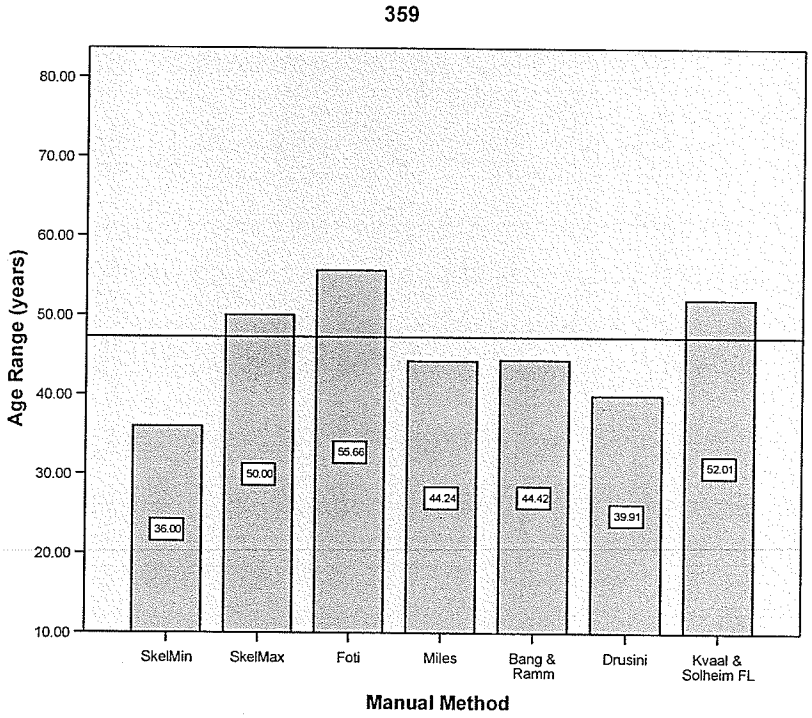


Figure 4.39: Digital method age range, #374.

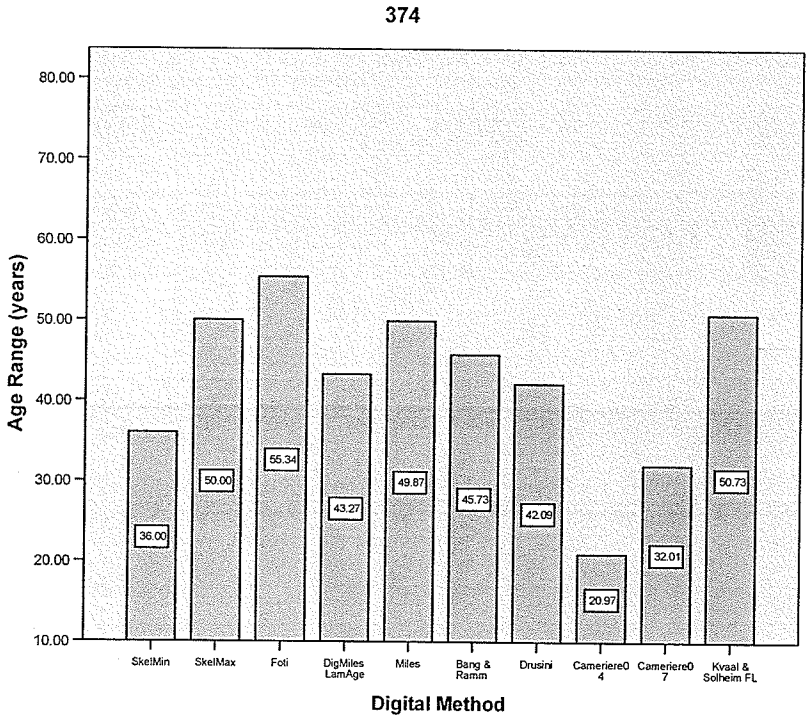


Figure 4.40: Manual method age range, #374.

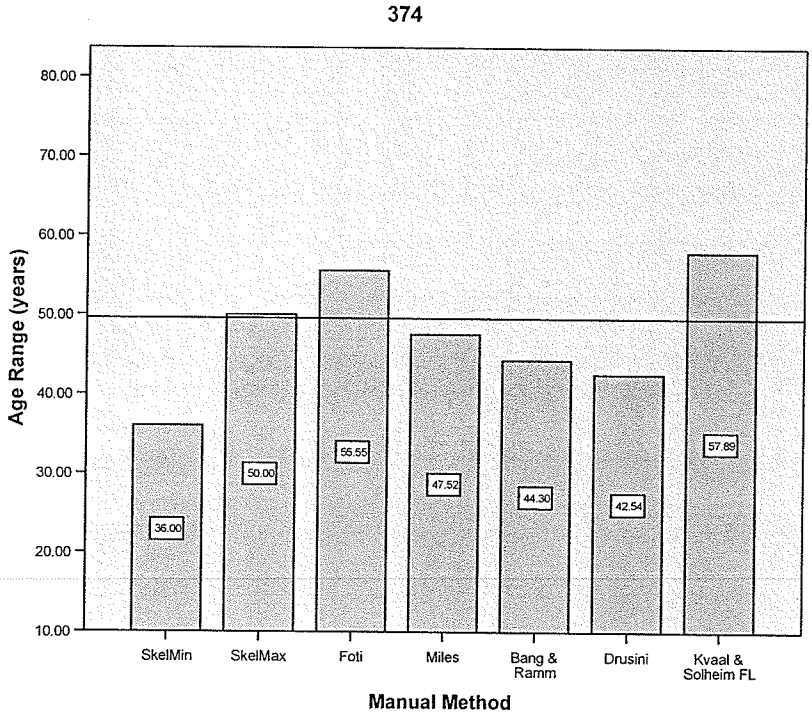


Figure 4.41: Digital method age range, #242.

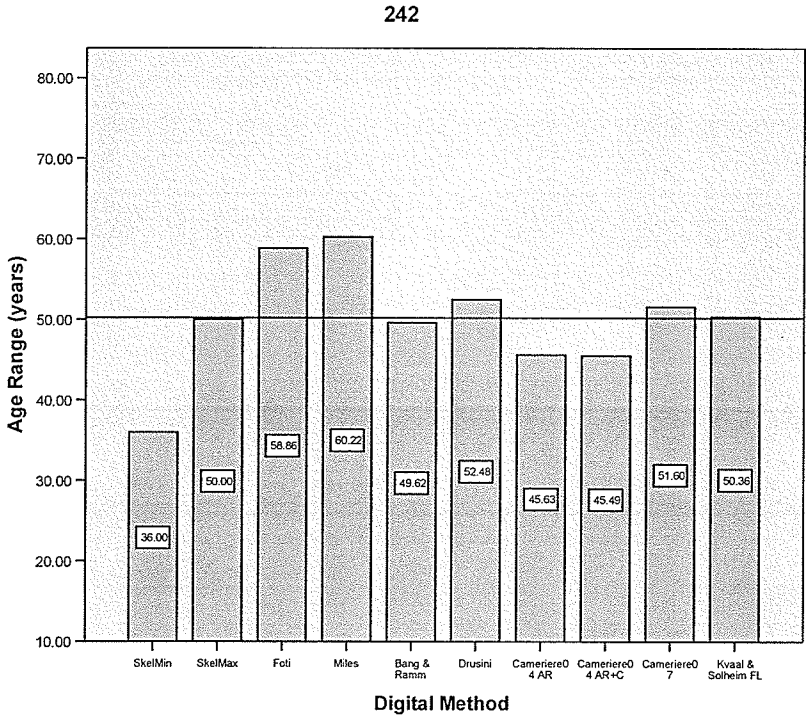


Figure 4.42: Manual method age range, #242.

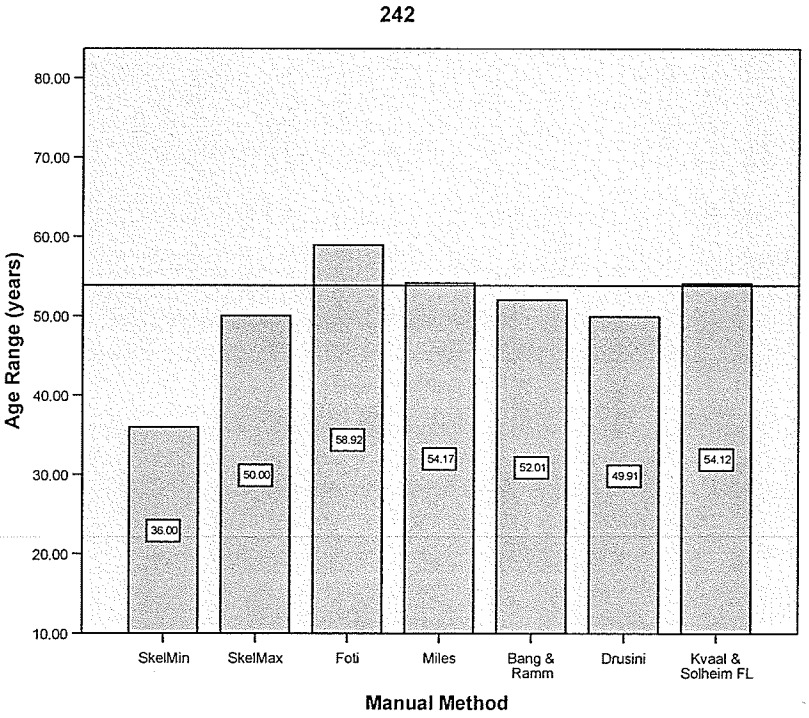


Figure 4.43: Digital method age range, #309.

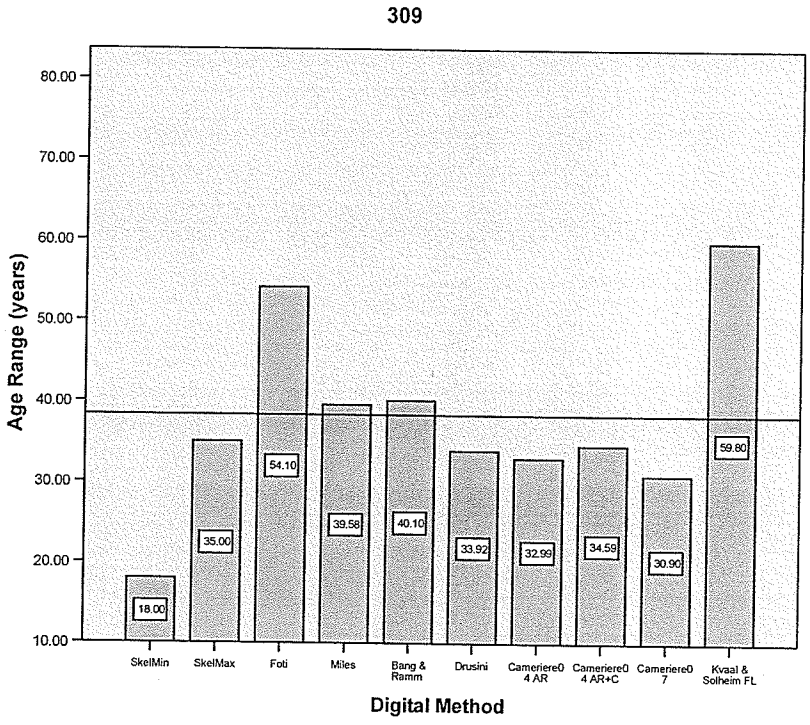


Figure 4.44: Manual method age range, #309.

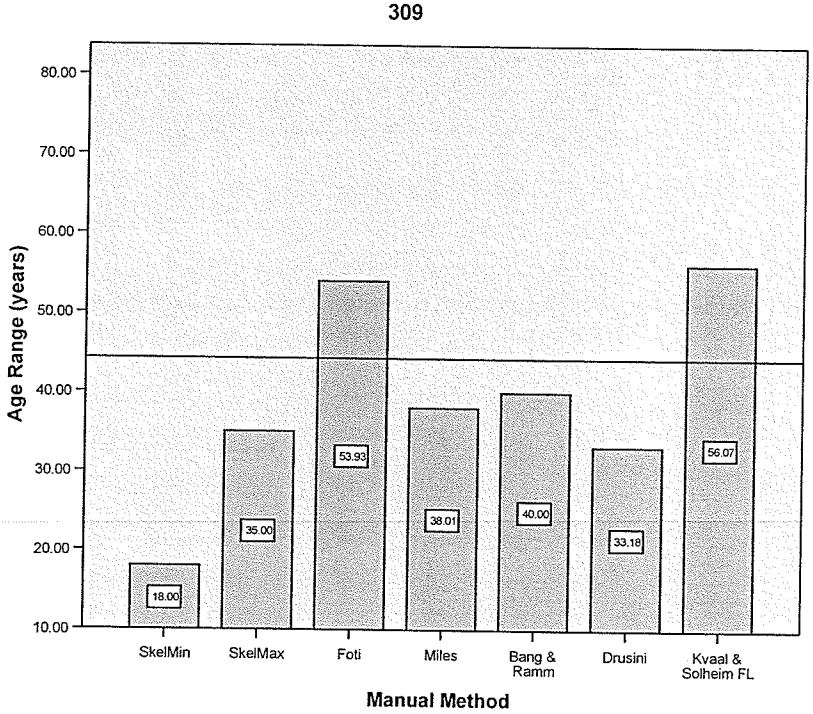


Figure 4.45: Digital method age range, #67.

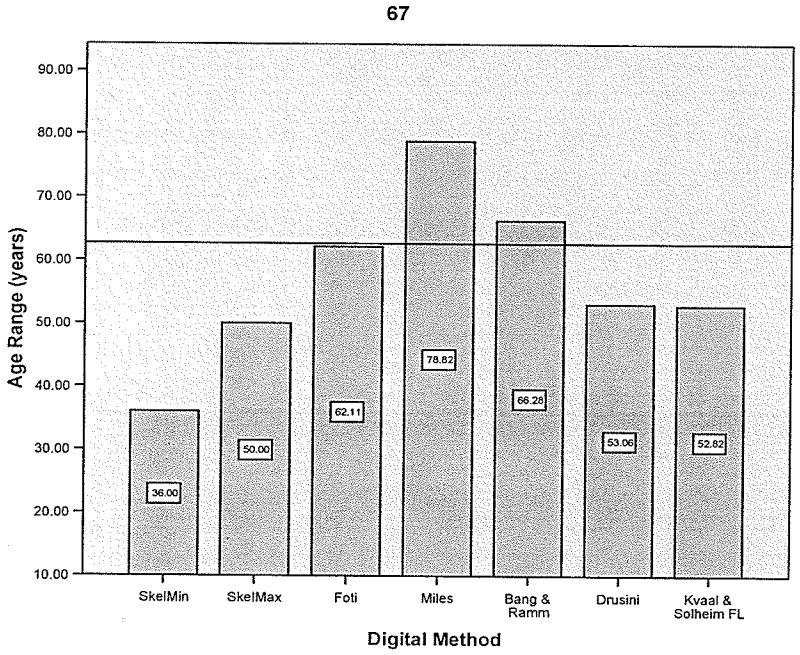


Figure 4.46: Manual method age range, #67.

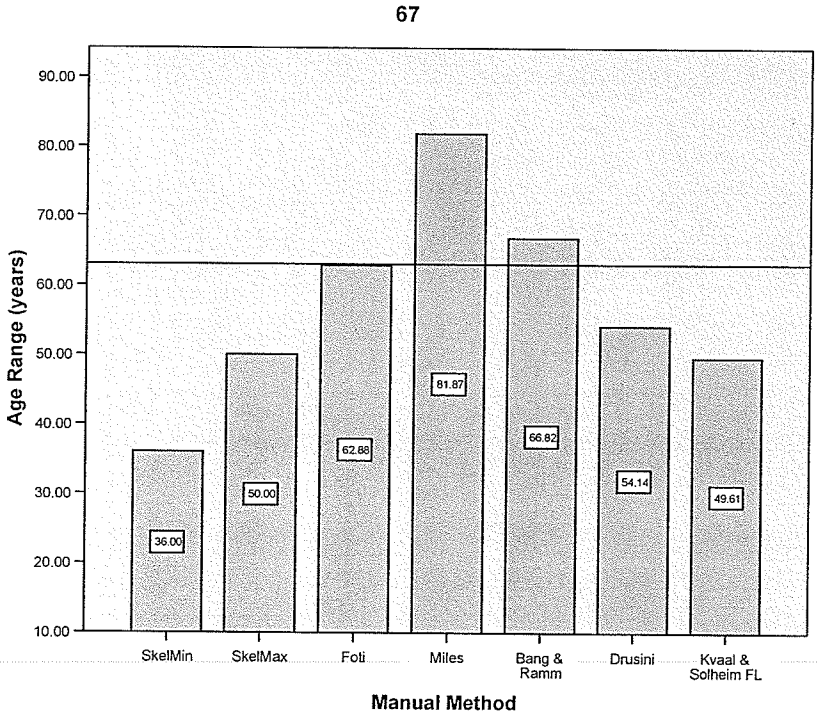


Figure 4.47: Digital method age range, #254.

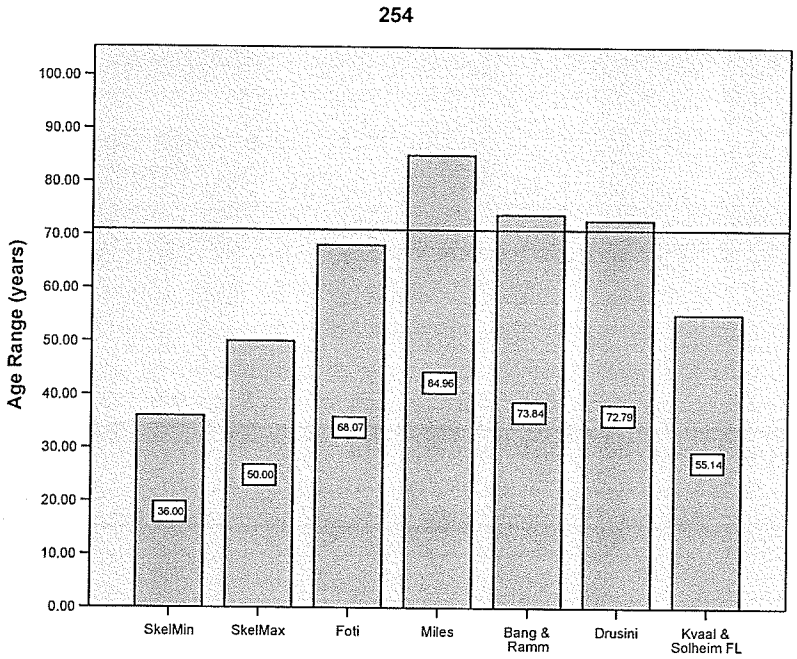


Figure 4.48: Manual method age range, #254.

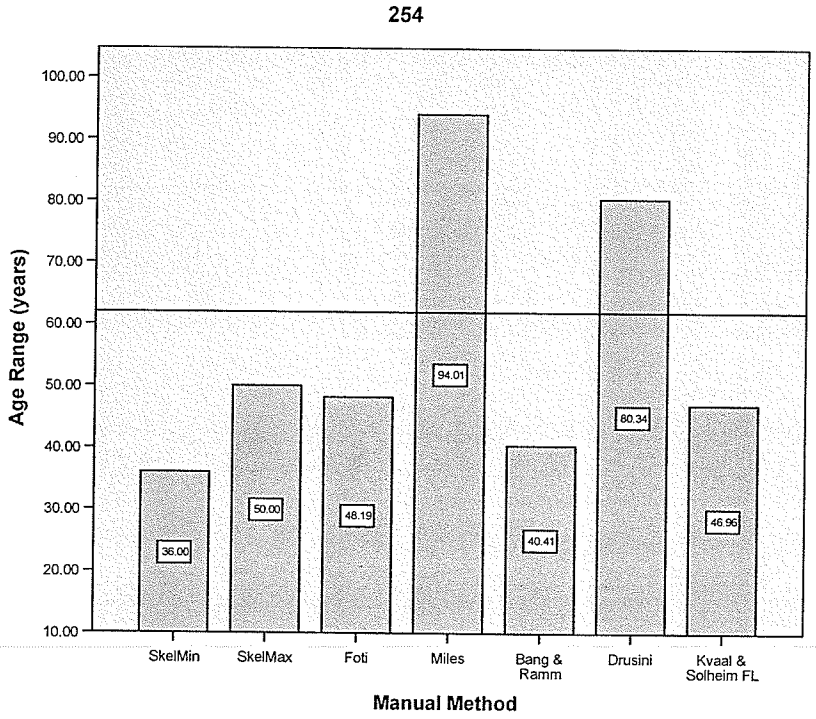


Figure 4.49: Digital method age range, #338.

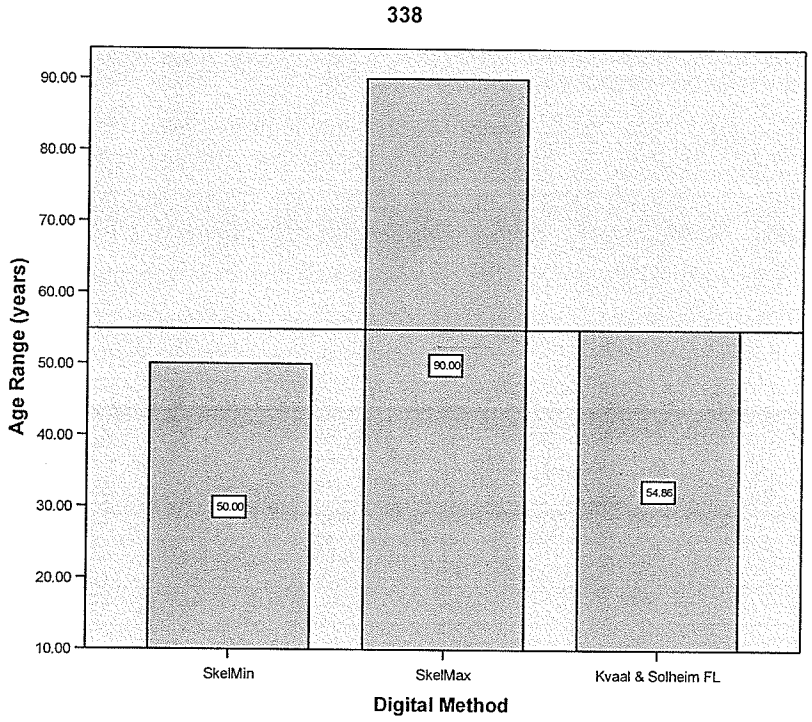


Figure 4.50: Manual method age range, #338.

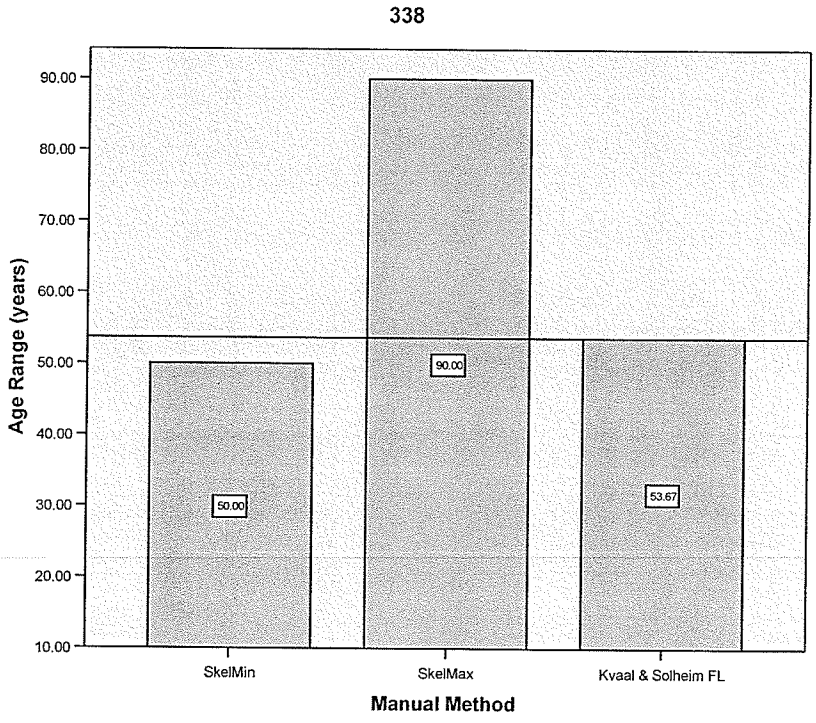


Figure 4.51: Digital method age range, #354.

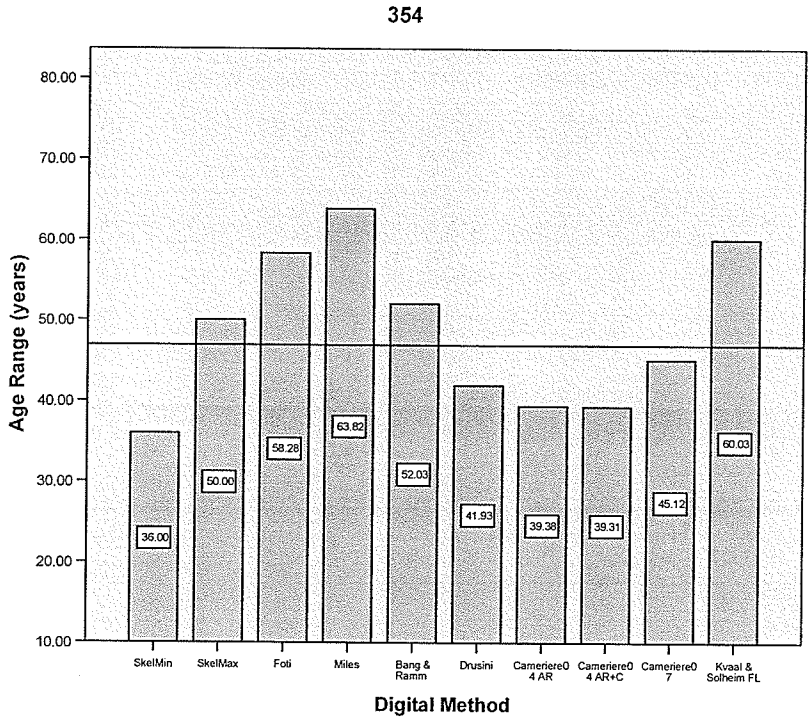


Figure 4.52: Manual method age range, #354.

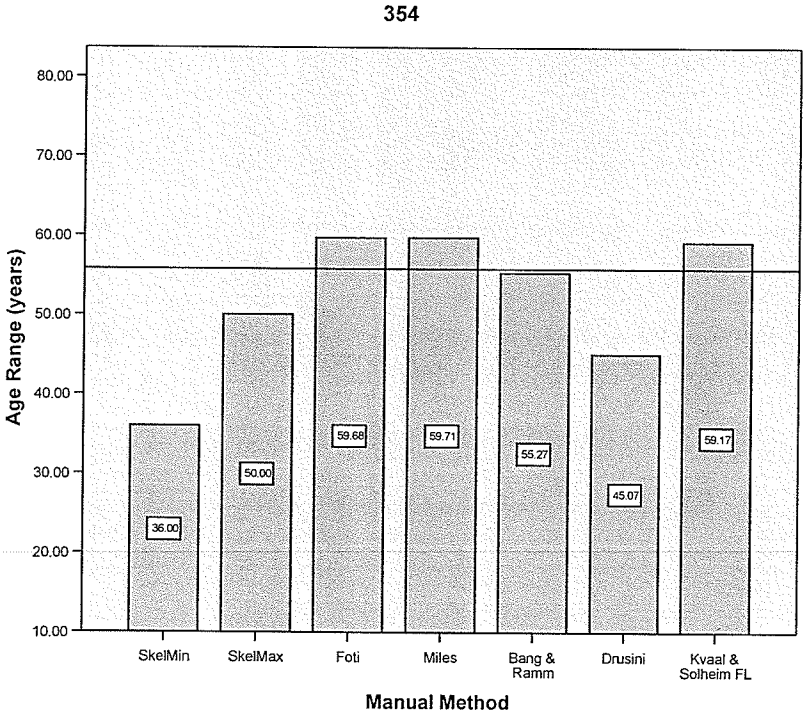


Figure 4.53: Digital method age range, #302.

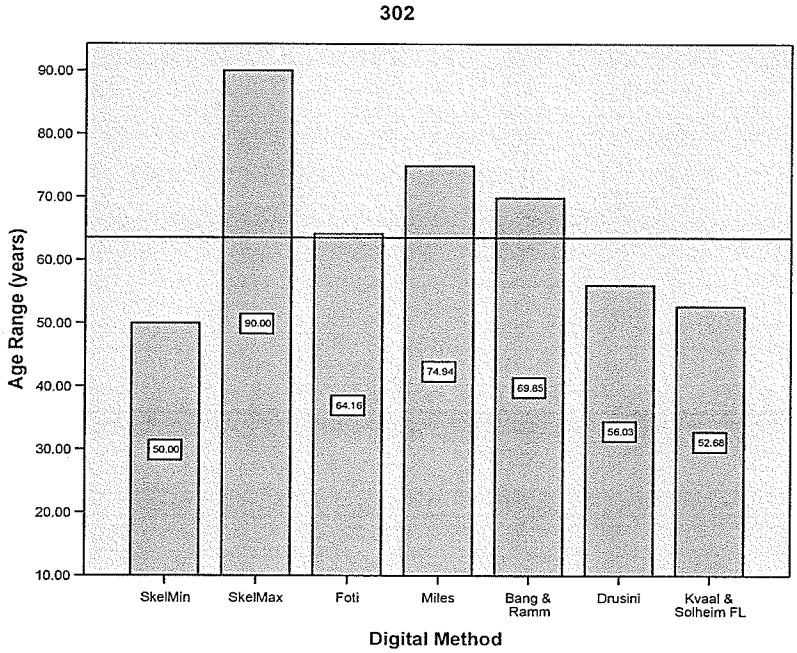


Figure 4.54: Manual method age range, #302.

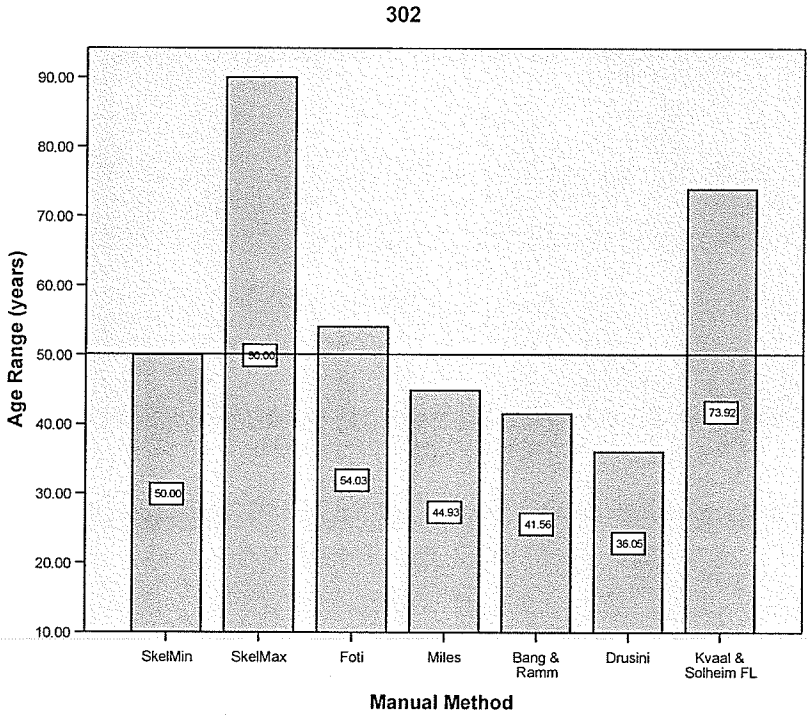


Figure 4.55: Digital method age range, #330.

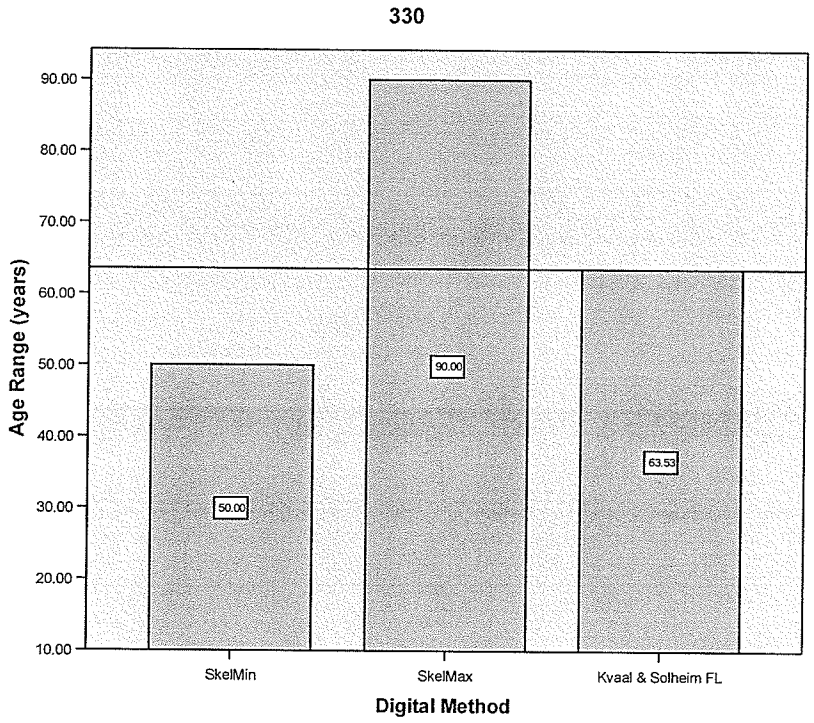


Figure 4.56: Manual method age range, #330.

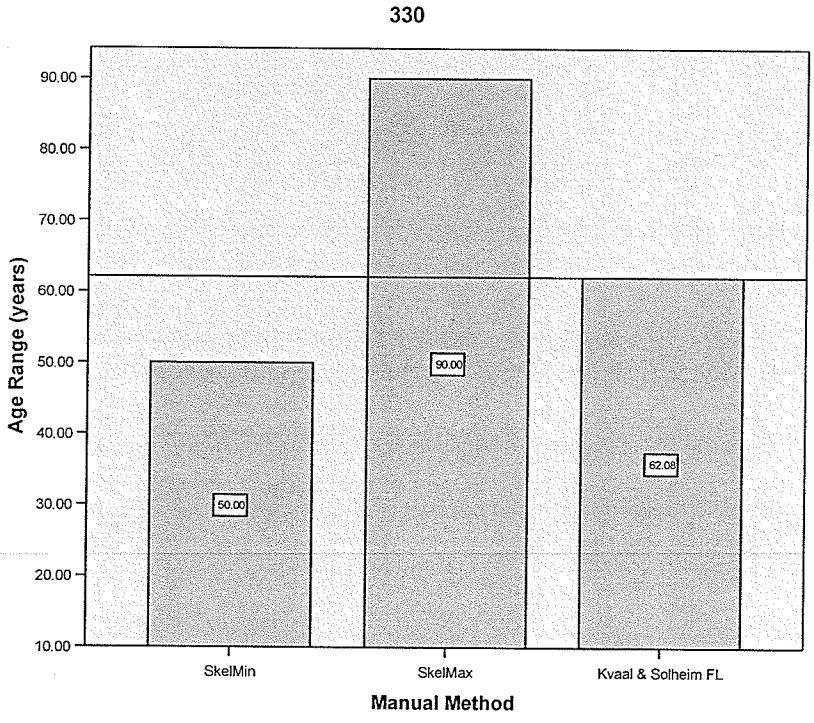


Figure 4.57: Digital method age range, #331.

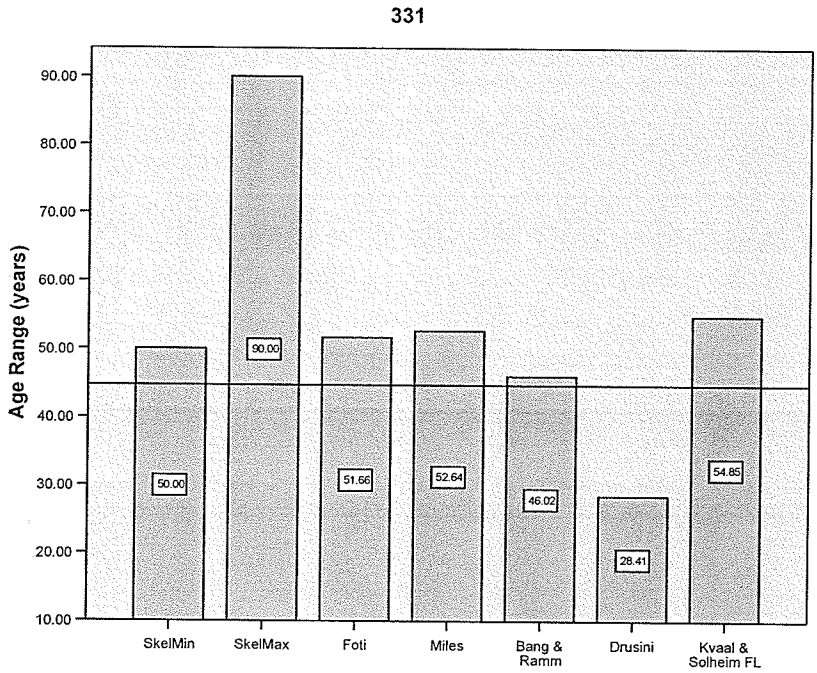
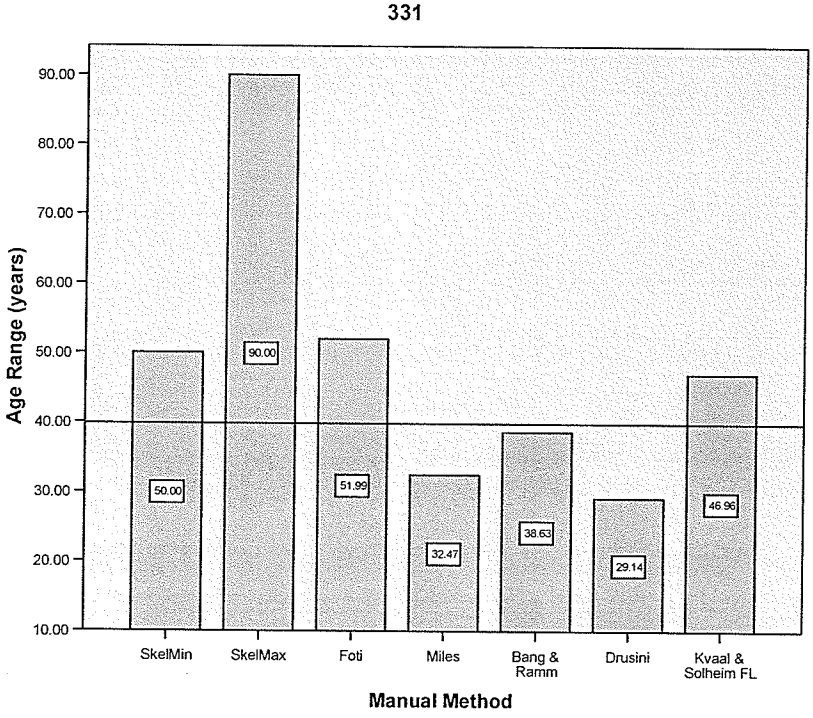


Figure 4.58: Manual method age range, #331.



The means and standard deviations of the digital and manual methods combined and separated by technique (transparency or pulp reduction) can be seen in the following

table (Table 4.5). The pooled age estimate distributions of the combined methods and techniques are presented graphically in (n the following charts) Figures 4.59 – 4.67.

Table 4.5: Mean age estimates separated by individual.

Cat #	Dig Age	Dig S.D.	Man Age	Man S.D.	Dig T Age	Dig T S.D.	Man T Age	Man T S.D.	Dig PR Age	Dig PR S.D.	Man PR Age	Man PR S.D.
301	49.44	12.81	39.50	8.48	42.28	10.09	42.79	9.88	55.16	12.63	36.86	7.15
19	48.35	14.33	38.25	9.04	37.63	10.91	37.74	11.05	56.92	10.71	38.65	8.45
253	57.05	8.67	54.43	13.48	61.68	11.32	64.21	10.95	53.35	3.98	46.60	10.06
289	57.25	7.55	55.90	9.15	64.16	4.96	63.42	4.63	51.72	3.10	49.89	7.05
352	72.37	13.18	70.78	16.41	72.28	10.73	71.79	9.64	72.42	15.22	69.97	21.61
336	58.23	7.96	54.56	6.57	53.64	4.63	54.95	5.91	67.41	0.02	53.79	10.46
244	46.81	17.51	49.64	12.82	58.78	11.29	61.34	7.51	39.97	17.24	40.28	6.33
215	61.69	7.81	64.37	12.12	61.65	11.15	68.13	3.07	61.72	5.36	61.37	16.16
192	54.43	10.54	50.67	8.88	50.81	11.22	51.26	12.15	57.32	10.20	50.21	6.81
307	48.67	8.42	37.56	10.81					48.67	8.42	37.56	10.81
249	54.75	5.43	49.22	10.11	55.34	5.61	56.37	9.19	54.27	5.90	43.50	7.00
207	68.65	16.65	64.56	21.46					68.65	16.65	64.56	21.46
284	46.32	17.11	45.33	13.07	39.14	9.98	44.18	9.50	50.43	19.60	46.24	16.49
242 a	52.77	18.44	45.03	13.55					52.77	18.44	45.03	13.55
305	54.63	20.05	48.99	4.62					54.63	20.05	48.99	4.62
250	49.48	17.86	48.23	6.17					49.48	17.86	48.23	6.17
321	45.76	16.29	41.08	8.21	42.71	9.87	44.56	8.71	47.50	19.59	38.30	7.51
304	43.56	8.53	43.80	8.64	42.28	9.28	42.32	9.21	48.68		49.73	
359	32.93	12.29	47.25	6.41	45.18	7.31	46.06	6.73	25.93	8.24	52.01	
374	35.61	13.60	49.56	6.83	48.26	5.69	47.48	5.76	28.38	11.15	57.89	
242	50.63	5.20	53.83	3.35	55.29	5.07	53.75	3.86	47.96	3.06	54.12	
309	38.68	9.59	44.24	10.16	41.93	8.59	41.28	8.91	36.83	10.26	56.07	
67	41.47	26.24	63.06	12.53	65.07	10.70	66.43	11.58	22.59	16.99	49.61	
254	45.62	31.04	61.98	23.69	74.92	7.15	65.74	25.57	22.18	18.54	46.96	
338	54.86		53.67						54.86		53.67	
354	47.60	9.32	55.78	6.27	54.01	9.38	54.93	6.90	43.94	7.60	59.17	
302	63.53	9.28	50.10	14.83	66.25	8.11	44.14	7.54	52.68		73.92	
330	63.53		62.08						63.53		62.08	
331	44.68	11.23	39.84	9.60	44.68	11.23	38.06	10.09			46.96	

Figure 4.59: Combined digital and manual method age distribution.

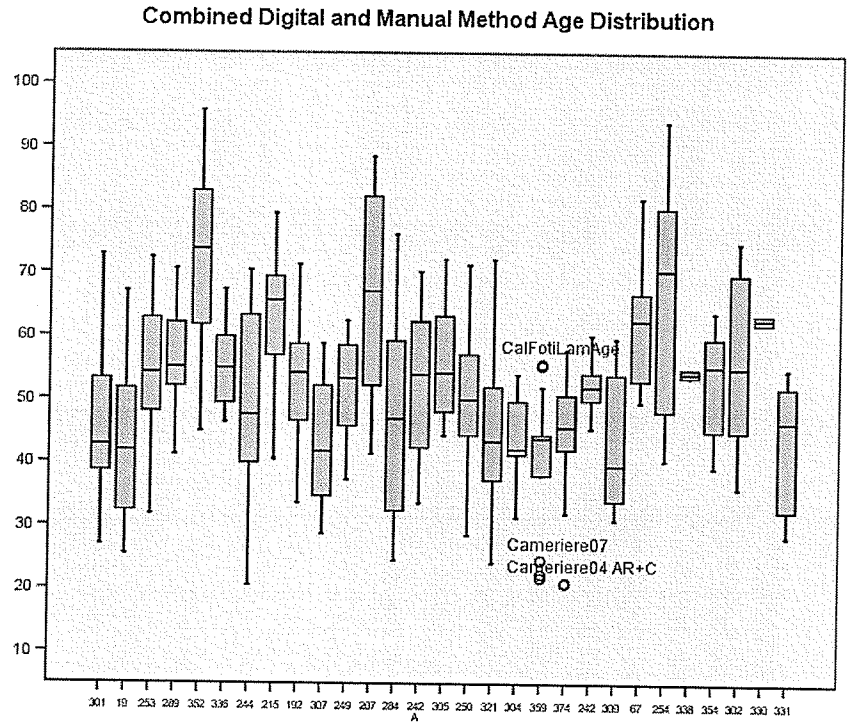


Figure 4.60: Combined digital method age distribution.

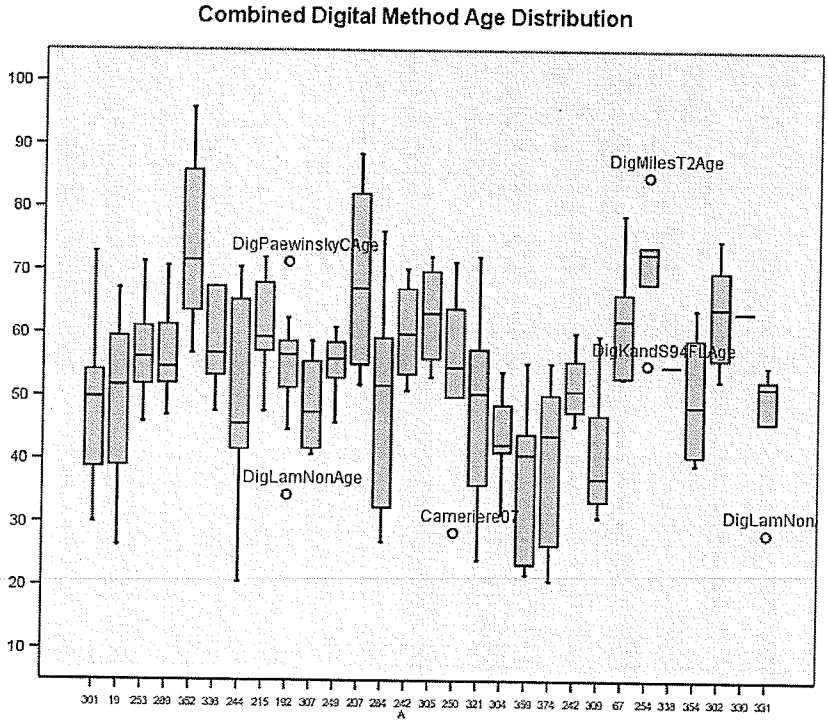


Figure 4.61: Combined digital method age distribution (w/o Cameriere techniques).

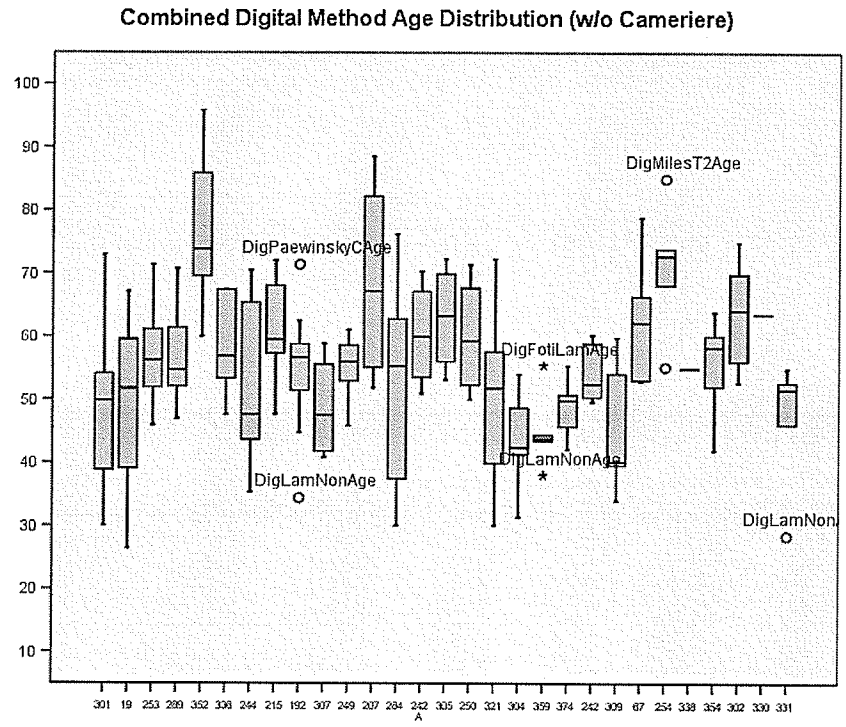


Figure 1.62: Combined manual method age distribution.

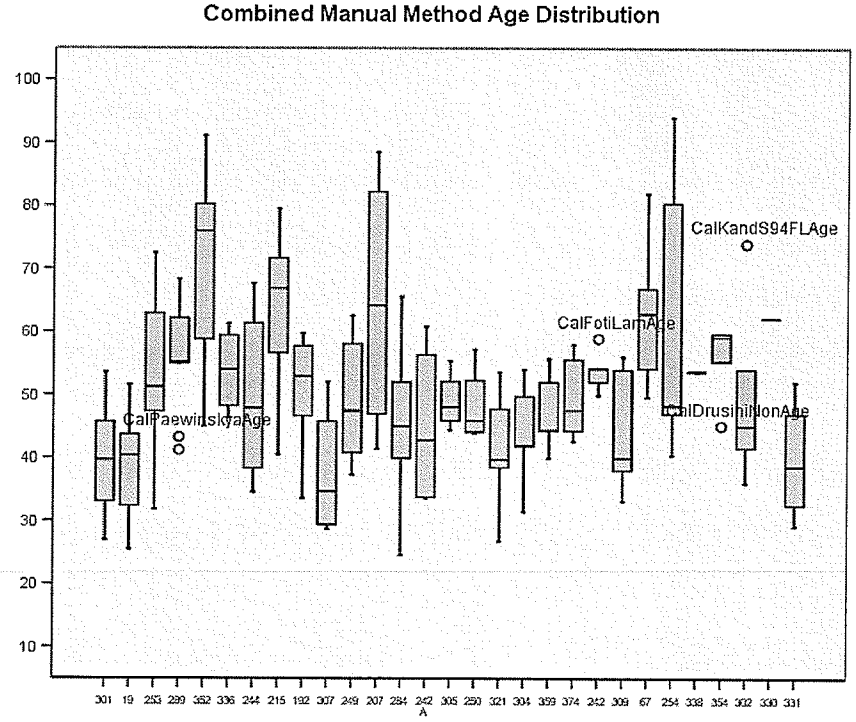


Figure 4.63: Combined digital method transparency age distribution.

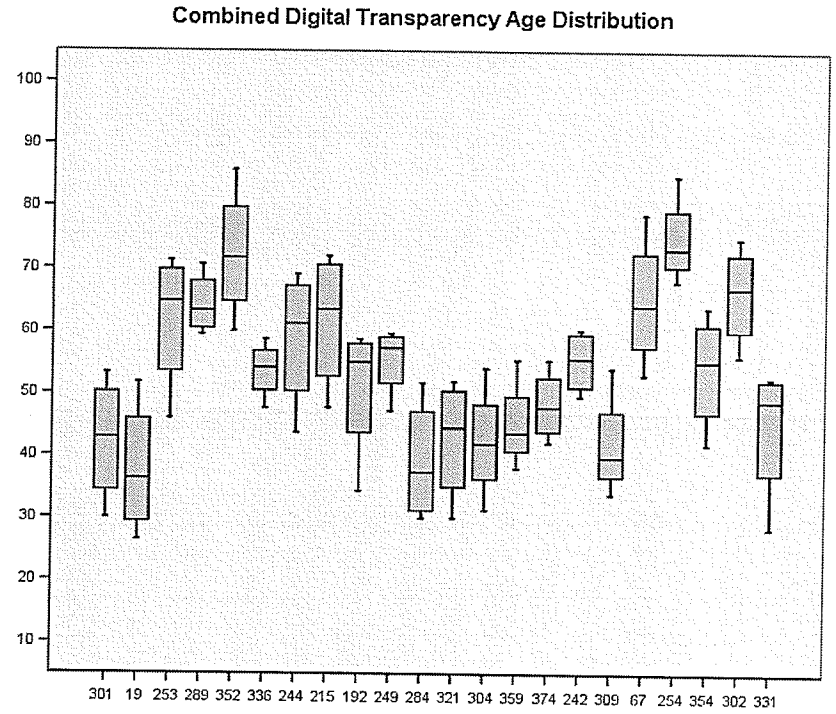


Figure 4.64: Combined manual method age distribution.

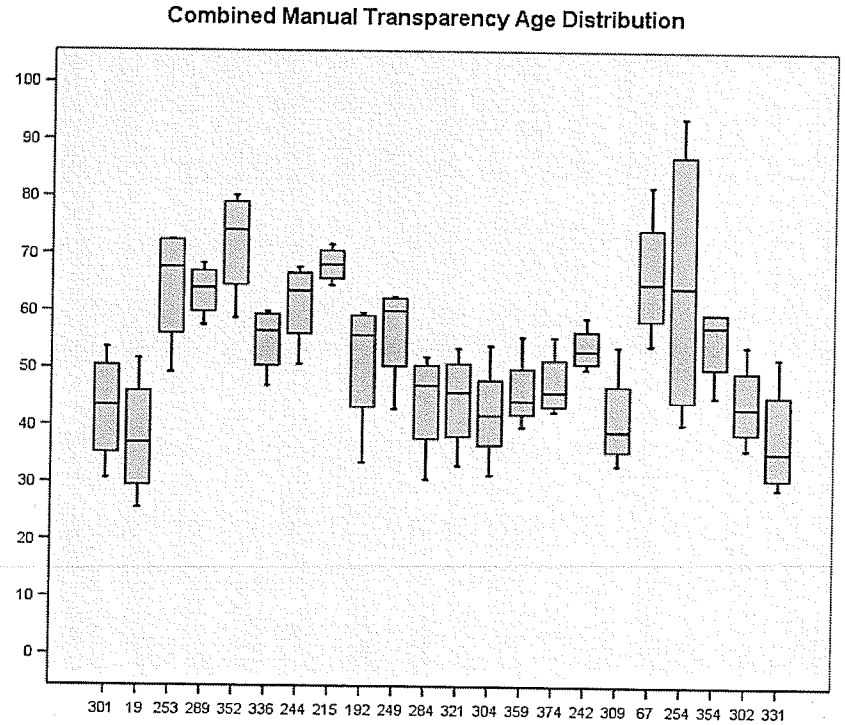


Figure 4.65: Combined digital method pulp reduction age distribution.

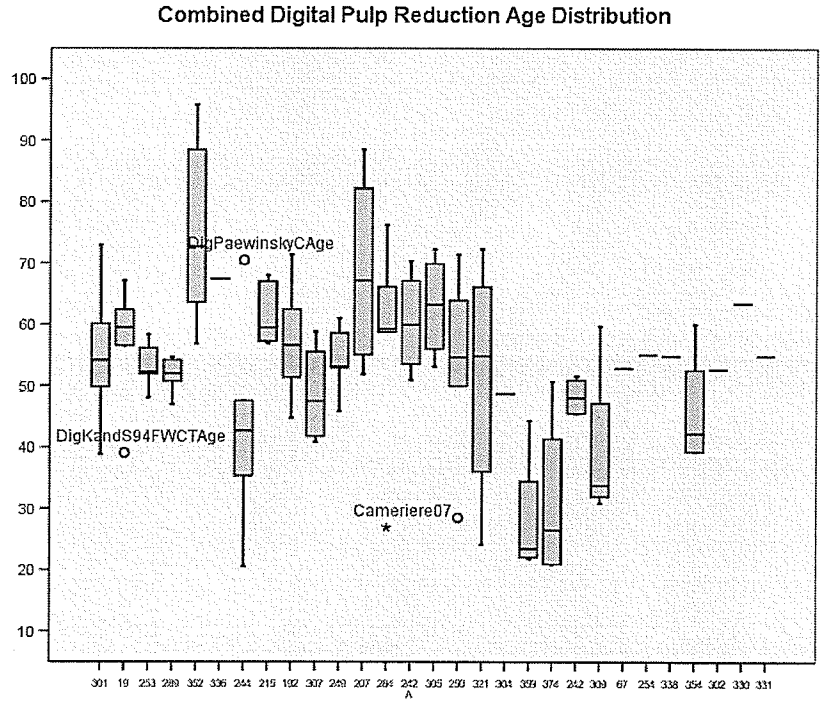


Figure 4.66: Combined digital method age distribution (w/o Cameriere techniques).

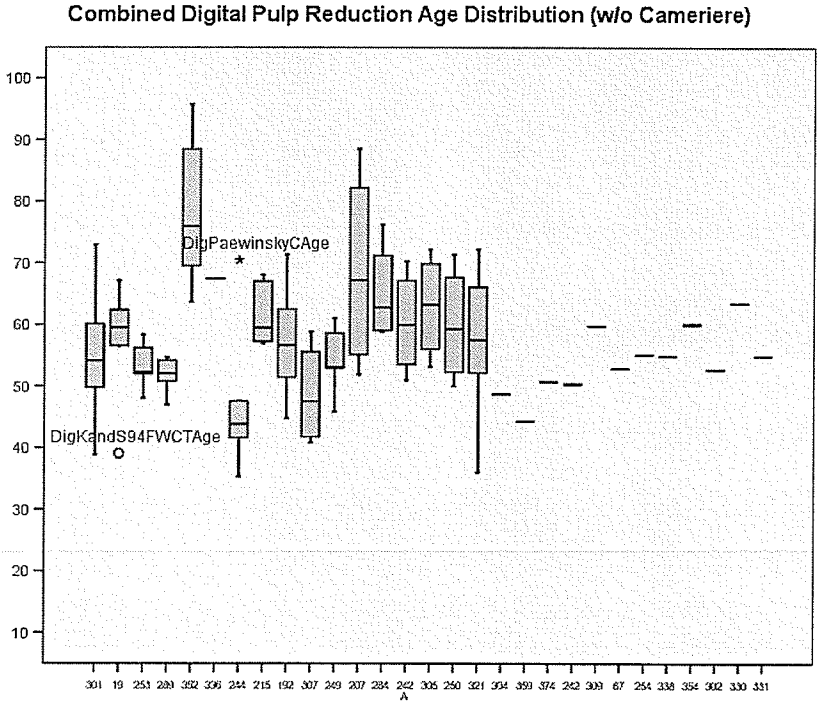


Figure 4.67: Combined manual method pulp reduction age distribution.

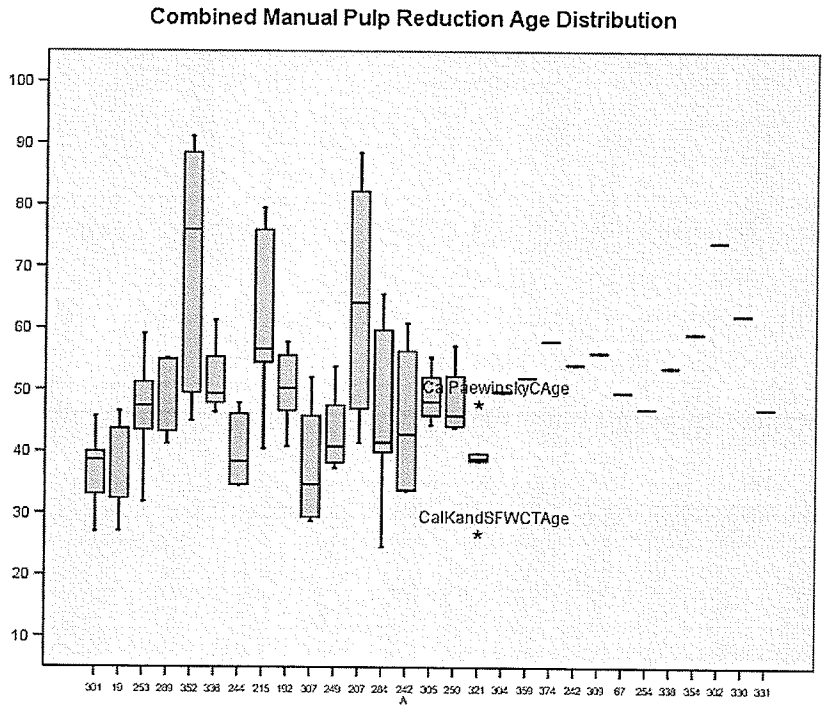


Table 4.6 presents the mean age estimate and standard deviation, separated by method and technique for all individuals.

Table 4.6: Mean age estimate and standard deviation of techniques

Method/Technique	Tech Mean Age	Tech S.D.
Digital		
Foti	57.66	5.17
Miles	57.28	16.46
Bang & Ramm	55.94	12.09
Drusini	43.29	12.32
Cameriere 04 AR	32.25	10.70
Cameriere 04 AR+C	32.44	10.79
Cameriere 07	34.11	12.54
Kvaal & Solheim FL	53.91	5.57
Kvaal & Solheim FWC	54.49	9.96
Kvaal & Solheim FWC+T	50.38	16.81
Paewinsky A	60.57	12.96
Paewinsky B	59.85	9.56
Paewinsky C	64.26	7.58
Manual		
Foti	57.13	5.31
Miles	56.03	16.86

Bang & Ramm	53.90	12.33
Drusini	44.02	13.75
Kvaal & Solheim FL	55.18	7.58
Kvaal & Solheim FWC	46.76	15.20
Kvaal &Solheim FWC+T	47.05	22.36
Paewinsky A	48.49	17.16
Paewinsky B	46.98	9.30
Paewinsky C	50.25	7.77

All respective pairs of age estimates were evaluated for differences by paired samples *t*-tests (see Appendix 1 and Table 4.7). No significant differences in age estimate sets were found between the following manual and digital techniques: Foti (p=0.633), Miles, (p=0.522) Bang and Ramm (0.340), Drusini (p=0.615), Cameriere 2004 AR and 2007 (p=0.105), Cameriere 2004 AR+C and 2007 (p=0.142), Cameriere 2004 AR and AR+C (p=.624), Kvaal and Solheim FL (p=0.619), and Kvaal and Solheim FWC+T (p=0.493).

Age value sets found to be significantly different were: Kvaal and Solheim FWC (p=0.004.), and Paewinsky A, B, and C (p=<0.001).

Figure 4.7: Digital and manual full age estimate set comparison.

Age Method Pair	N	Mean Difference (years)	*t*-value	Sig. (2-tailed)
Foti	22	.53789	.485	.633
Miles	22	1.24497	.652	.522
Bang & Ramm	22	2.03853	.977	.340
Drusini	22	-.72365	-.510	.615

Cameriere 04 AR & 07	5	-4.5940	-2.092	.105
Cameriere 04 AR+C & 07	5	-4.3980	-1.823	.142
Cameriere 04 AR & AR+C	5	-.19600	-.531	.624
Kvaal & Solheim FL	12	-1.19244	-.512	.619
Kvaal & Solheim FWC	**16**	**7.73226**	**3.430**	**.004**
Kvaal & Solheim FWC+T	10	2.24339	.714	.493
Paewinsky A	**16**	**12.07836**	**5.885**	**<0.001**
Paewinsky B	**17**	**12.86506**	**5.613**	**<0.001**
Paewinsky C	**17**	**14.00942**	**5.511**	**<0.001**

Table 4.8 illustrates the average method and technique age estimates and associated standard deviations.

Table 4.8: Average of means and standard deviations of methods and techniques.

Method/Technique Mean		S.D.
Total Mean Dig Age	52.84	11.87
Total Mean Man Age	51.15	10.64
Total Mean Dig Trans Age	53.54	8.83
Total Mean Man Trans Age	52.77	9.02
Total Mean Dig Pulp Age	51.80	11.05
Total Mean Man Pulp Age	51.11	10.69
Total Mean Trans Age	53.16	2.16
Total Mean Pulp Age	51.37	6.52
Total Combined Age	51.99	8.95

Comparison of dental and skeletal age estimates

The data below illustrates the correspondence between the respective dental and skeletal age estimates. Appendix 1 shows each technique separated by method with the particular dental age estimate highlighted as it corresponds to the associated skeletal morphological age (green indicates a direct dental–to-skeletal age correspondence, yellow indicates a correspondence between dental and skeletal age estimate ±5 years in correspondence, and red indicates a correspondence between dental and skeletal age estimates ±10 years. Table 4.9 illustrates the numerically tallied correspondences as fully described in Appendix 1.

Table 4.9: Dental and skeletal age estimates and correspondence

Tech.	Usable #	Dir. Corr.	±5 Years	±10 Years	% Direct	%≥±5Years	%≥±10 Years
Digital Method							
Foti	22.00	4.00	2.00	6.00	0.18	0.27	0.55
Miles	22.00	9.00	4.00	1.00	0.41	0.59	0.64
Bang & Ramm	22.00	7.00	5.00	2.00	0.32	0.55	0.64
Drusini	22.00	12.00	5.00	1.00	0.55	0.77	0.82
Cameriere 04 AR All	5.00	3.00	0.00	0.00	0.60	0.60	0.60
Cameriere0 4 AR+C All	5.00	3.00	0.00	0.00	0.60	0.60	0.60
Cameriere 07 All	10.00	6.00	2.00	1.00	0.60	0.80	0.90
Kvaal & Solheim FL	12.00	5.00	4.00	1.00	0.45	0.82	0.91
Kvaal & Solheim FWC	16.00	4.00	3.00	2.00	0.25	0.44	0.56
Kvaal & Solheim FWC+T	10.00	2.00	5.00	1.00	0.20	0.70	0.80
Paewinsky A	16.00	3.00	1.00	4.00	0.19	0.25	0.50
Paewinsky B	17.00	3.00	1.00	1.00	0.18	0.24	0.29
Paewinsky C	17.00	3.00	0.00	0.00	0.18	0.18	0.18
Manual Method							
Foti	22.00	5.00	2.00	5.00	0.23	0.32	0.55
Miles	22.00	6.00	4.00	5.00	0.27	0.45	0.68
Bang & Ramm	22.00	6.00	2.00	5.00	0.27	0.36	0.59
Drusini	22.00	11.00	3.00	1.00	0.50	0.64	0.68
Kvaal & Solheim FL	12.00	6.00	3.00	2.00	0.55	0.82	1.00
Kvaal & Solheim FWC	16.00	6.00	5.00	2.00	0.38	0.69	0.81

Kvaal & Solheim FWC+T	10.00	6.00	0.00	1.00	0.60	0.60	0.70
Paewinsky A	16.00	5.00	5.00	2.00	0.31	0.63	0.75
Paewinsky B	17.00	6.00	5.00	2.00	0.35	0.65	0.76
Paewinsky C	17.00	3.00	3.00	3.00	0.18	0.35	0.53

Chapter 5: Discussion and Conclusions

Methodological Considerations

Digital method: intra-observer error

Pulp reduction: linear and area measurements

All digital linear pulp and tooth measurements contained no significant differences except for the root width at CEJ. However, despite the fact that this score was significantly different, it is worth examining the magnitude of error (mean difference) to determine just how much variation existed. The root width CEJ magnitude of error was modernly low (0.43 mm), which implies a small but substantial amount of variation between measurement pairs.

It is peculiar that the difference occurred at the root CEJ and not at any other region, especially since the root and pulp usually (but not in all cases) become increasingly narrower as the apex is approached. Logically, the region that should be expected to have the largest amount of difficulty in accurate assessment is the mid-root, as this is the smallest width while, the CEJ should be considerably easier to measure because of the greater width. As will be explained below, however, the software had difficulty correctly capturing proper *width* measurements and this may contribute to the unexpected location of the observer error. The low rate of intra-observer error in the digital method is attributed to the software's inability to truly place lines where desired. This will be elaborated upon below.

The pulp and tooth area measurements contained no significant differences for intra-observer tests. Although only a minor proportion of the sample was useable (due to destruction) for these techniques (Cameriere 2004 AR n=5; Cameriere 2004 AR+C n=5; Cameriere 2007 n=10), the simpler and more rapid means of measurement compared to the linear techniques presented above, reinforce the technique as a more advantageous quantification. However, the utility of area quantification is severely compromised by the specimen condition (as demonstrated in this research) it may not always be the choice technique for practical reasons.

Root transparency: transparency and root measurements

No significant differences were detected with the digital method for any transparency or root length scores. This is in accordance with studies such as Ermenc (1997), Sengupta et al. (1998), Whittaker and Bakri (1992) who found no significant intra-observer errors between repeated measurements of transparency and root length using an image analysis system. Drusini and associates (1991b) also found no significant differences between transparency and root measurement evaluated with both caliper and image analysis software. They do not report measurement correspondence, but state that the ages estimated between their two methods were equally predictive of known-age. Lopez-Nicolas and coworkers (1990) evaluated transparency in dental half-tooth sections with the IBAS image analysis system. They do not report intra-observer errors but state that the image analysis system proved superior in correlating with chronological age because the method improved measurement accuracy. Lopez-Nicolas and Luna (1991) and Lopez-and coworkers (1993) again tested the IBAS image analysis system alone on dental thick-sections and recorded transparency values (among other age parameters).

One interesting note is that the images used for digital transparency analysis were in color as opposed to black and white images used by Sognnaes and colleagues (1985). It is probable that using either image style would be equally sufficient in delineating the limits of transparency.

Manual methods: intra-observer error

Pulp reduction: linear measurements

The manual measurements that were found to be significantly different between pairs were root width at mid-root, pulp width at CEJ, and mesial root length. Although these measurements were observed to be significantly different, the magnitude of mean error was low. The mean difference between observer error measurements was negligible for root width at mid-root (0.13 mm) and pulp width CEJ (-0.0775 mm), but larger for mesial root length (0.5075 mm). The intra-observer errors associated with the root and pulp width scores can be considered negligible and the error with the mesial root length may be attributed to reproducing reference points and variation in angulation of measurements that is difficult to avoid.

Root transparency: transparency and root measurements

The manual intra-observer measurements that were observed to be significantly different were the minimum and maximum transparency height. The mean difference of the minimum transparency scores was markedly high (-.98 mm), while the maximum transparency height mean error was lower (-0.69 mm).

Although the magnitude of error did not generate serious error, the difficulty surrounding manually measuring the minimum and maximum height of transparency has

methodological as well as age estimate implications for choice of technique (i.e. Bang and Ramm (1970) versus Drusini et al. 1989). This will be elaborated upon below.

Full measurement sets

Significant differences were found between several of the digital and manual measurements sets and ratios when compared. However, the mean error was negligible for all of these. The mean difference was less than 0.30 mm for the root width CEJ (0.19 mm) and ¼ root (-0.17 mm), pulp width CEJ (-0.28 mm), ¼ (-0.22 mm), and mid-root (-0.24 mm) and slightly larger for the labial root length (-0.64 mm). In addition, several ratio sets were also found to be significantly different but the mean difference of error was negligible for pulp/root width CEJ (-0.05 mm), ¼ (-0.04 mm) and mid-root (-0.05 mm).

Possible sources of error

Digital methods

Pulp and root measurements

There are a number of possible reasons for of the intra-observer differences and difficulty in the digital method of linear pulp and root width measurements: 1) the quality of the image, 2) the format of the image, 3) the resolution size of the image, 4) the accuracy and versatility of the linear lines used for measurement, 5) the reproducibility of measurements, and 6) tooth root morphology. All of these factors, at some level, influenced the observer's ability to properly ascertain the target structures. It should be

noted that the digital linear width measurements are not as accurate as the manual measurements, despite fewer intra-observer errors.

The main cause of error was the limitation in the linear lines plotted on the target image structures. In many cases the graphical lines could not be positioned exactly where desired and often could not adequately link anatomical target points.

This inaccuracy encompasses both linear target points (the ends of the lines) and the adjustment angle of the line (see Figures 3.8 and 3.10). The other major linear measurement problem was the angle of the line. In many cases the angle was either not parallel with a respective line (i.e. the pulp width line angle is not parallel to the root width line or vice versa) and could not be adjusted to the desired location (see Figure 3.10). The root and pulp length measurements seem to be free of these types of problems, possibly due to the larger dimensions as opposed to the smaller width dimensions. The angle error has several implications for erroneous age estimation as it would influence the pulp:root width ratio. It would appear that the total root and pulp length measurements are less prone to the software limitations compared to the width measurements (visually compare the position consistency and accuracy between width measurements in Figures 3.8 and 3.10 and length measurements in Figure 3.9). In addition, when trying to define the ¼ root and mid-root positions it was noticed that the length measurements could not divide the root exactly as calculated and therefore another source of artificial measurements.

The second most influential factor contributing to error was the image quality, size/resolution, and format. Compromised image quality most likely originated in the format of the scanned images. The x-rays were first a film or 'hard copy', scanned (on a

professional medical x-ray scanner), and then converted from a DICOM to a TIFF format for measurement. As previously mentioned, upon first examination of the converted x-ray images, the baseline image quality was noticeably 'blurry' and/or highly 'pixilated', and the junctions of many anatomical features could not be easily delineated in some cases. For this reason, the sharpen filter was applied to make features and borders (i.e. pulp from dentin and/or root from air) more visible (see Figure 3.7). It is unknown if the sharpen filter introduced error at some level (although doubtful), but was necessary as this drastically improved visualization for assessment. The image *resolution* also has considerable influence on the software's ability to maintain clarity when magnified (for plotting lines). The scanned x-rays were a 1:1 size ratio of the film and when magnified, the teeth were noticeably 'pixilated' which created some difficulty in deciding where, exactly, anatomical features were. It should be noted that these problems in image magnification, clarity, and resolution were much less problematic than the software's inability to properly plot linear lines at the desired points.

The last two factors that affected measurement accuracy were the inability to reproduce *some* reference points and marked variability in tooth root morphology; more specifically the relationship between these two variables. These two variables introduced the least amount of error compared to the others.

The recreation and maintenance of reference point locations related to the software, such as accurately dividing the root into three levels, may have introduced errors when the root length and subsequent sub-divisions were made. As already stated, the mesial edge of a crown is used as a benchmark and often the root is curved to some degree, presenting some subjectivity when trying to correctly position the horizontal

width lines (but, again the software introduced more error than root morphology). This still does not totally explain why only the root width at CEJ was as significantly different and the other pulp and root width measurements were not. One explanation may be that sometimes when trying to define the limits of the root width at CEJ, the compromised image quality and limitations of the software made it difficult to correctly acquire data (i.e. this particular width lies somewhere outside the pixel threshold for plotted lines). The lack of errors at all other width locations may, again, stem from the artificially created measurement accuracy of the software and inter-tooth root morphology, as these locations should logically be more difficult to assess (visually compare Figures 3.8, 3.10, and 3.12).

Unlike Bosmans and coworkers (2005), Kolltveit and colleagues (1998) experienced similar problems to the present research, but the two studies differ slightly in respect to systemic problems when they evaluated digitally converted x-rays and measurement software. The authors comment that the "main source of errors in measurement seemed to be difficulties in reference points on radiographs when viewed on the monitor" and when "the observers viewed the radiograph on an illuminating table, the structures in the radiograph seemed to have more sharply defined edges and fewer grey levels when viewed on a monitor than in large scale" (Kollotveit et al. 1998:94). They do state that both width and length measurements had significant differences between manual and digital methods, probably due to uncalibrated digital x-rays. This was not found in the present research when all measurement sets were compared for mean difference in error.

Kolltveit and coworkers (1998) also comment that another source of error may be the x-ray machine configuration as a "low kilovoltage produces a short-scale contrast on the radiograph with fewer shades of grey (giving the radiograph high-contrast) whereas a high kilovoltage, as used in dental radiography, produces a high-contrast leaving more details on the radiograph, but with lower contrast" (Kolltveit et al. 1998:94). Their x-rays were taken in 60-70 kV but the exposure time is not stated. The present research found that 80 kV for 10 minutes was the ideal configuration for the archaeological teeth and a lower or higher kV and exposure time produced unsatisfactory contrast between the dental tissues. It is entirely possible that non-archaeological teeth (as used in Kolltveit et al. 1998) require a different x-ray configuration than archaeological dental material or possibly x-ray machine configuration variation. However, in the present research it should be stressed that it was not an issue of contrast with the digital method but a limitation in measurement tools.

Manual method

Pulp and root measurements

The cause of measurement differences in the manual method of linear scoring of tooth dimensions has six sources: 1) level of magnification, 2) limitations in illumination of the x-ray, 3) root morphology, 4) the ability to reproduce reference points, 5) proper delineation of dental tissues, and 6) skill of the observer.

The primary contributing cause of problems in the manual width and length linear measurements was the difficulty in reproducing *some* reference points. The main contribution to this error was dividing the root into segments and having these divisions

in the exact spots as the originals, although not at every level of division did problems occur. In other words, when the mesial root length was determined and the ¼ and mid-root levels were calculated, a slight difference in these two spots caused this discrepancy from first and second measurements even when spots were marked on the x-ray. Root curvature may also have contributed to the error in mid-root width as the root tends to bend sharpest at this location. However, the mean difference in error of the manual root width mid-root and pulp width CEJ is negligible; the small intra-observer error test sample size may be the cause. Specifically, the pulp length presented the largest mean difference. Some pulps were difficult to assess as the contrast (the illumination from the light box) did not satisfactorily allow delineation of the tissues in the coronal portion. The level of illumination the light box produced in the majority of cases was adequate to delineate the pulp from the crown dentin at the coronal limit and the pulp tip at the apex, but some teeth had intrusions within the pulp chambers. This caused some pulps to appear faint as the intrusions posed problematic in decisive evaluation. These crystalline intrusions within the pulp chamber could be separated from the pulpal walls in some cases, but in other cases only an approximation in measurement could be made.

Another possible source of error may have been the level of magnification (10x) the x-rays were scored in, but this factor contributes less error than those previously described. It is entirely possible that 10x magnification was not appropriate for these techniques, although doubtful as the x-ray films were additionally illuminated by the light box and was it the same magnification as the stereomicroscope available. Lastly, the measurement skill of the observer is a significant factor in attaining proper measurement

scores. There is no doubt that the present author is not as highly skilled as more experienced scientists which may have contributed error in some form.

Willems and colleagues (2002), testing the Kvaal et al. 1995 technique, found significant differences between three examiners' measurements: examiner A used a stereomicroscope, examiner B used no magnification, and examiner C used a "low power magnifying lens" (Willems et al. 2002:222); exactly what level of magnification this lens was set at is unknown. Their radiograph films were illuminated by a light box for all measurements. But, as the low magnitude of error between the first and second measurement sets illustrates, the problem is probably in reproducing exact reference points and not an issue of equipment. It should be stressed that this low magnitude of error only demonstrates the more constant scores representative of the graphical measurement limitation of the digital method, and not a true assessment of pulp or root dimensions.

In contrast to Willems and associates (2002), Woods and coworkers' (1990) results are more in accordance with the current research when mean error is considered. They also evaluated canines and found no significant inter-observer error (<3%) between measurements of three observers and all cases (n=234) using an identical setup as the manual setup used in the present research. They even comment that the "measurements were made directly on the transilluminated radiographs using electronic sliding calipers; it was helpful to view the radiographs through a low-powered lens" (Woods et al. 1990:42).

Transparency and root length measurements

The cause of errors with the manual method of assessing the minimum and maximum heights of transparency has six sources: 1) the morphological expression of transparency, 2) complexity of quantification, 3) location of the measurement, 4) the condition of the root surface, 5) root morphology (amount of root curvature), and 6) brightness level of the light box (which causes eye strain).

The chief sources of error were the morphological expression, measurement location, and complexity in quantification of transparency. Olze and associates (2004) are in agreement in that in some cases the border between transparent and non-transparent dentin is difficult to manually assess because of idiosyncrasies in morphological expression. In other words, the junction is not always well-defined and is sometimes 'blurry' or 'patchy', which makes manual assessment only an approximation. The complexity of the transparency quantification (i.e. technique) is undoubtedly a contributing factor to intra-observer and mean errors as the manual method produced different values between the minimum and maximum transparency heights. Determining the labial transparency height is a more objective and more time efficient (and better for age estimation) than deciding the minimum and/or maximum transparency (i.e. the Bang and Ramm 1970 technique is more complex and more prone to error than the Foti et al. 2001, 2005 or Drusini et al. 1989 techniques). Because the minimum and/or maximum transparency height can be recorded anywhere on the root, it also creates more margin for subjectivity. Willems and coworkers (2002) support the current findings and found difficulty in the Bang and Ramm (1970) method as their scores between intra-observer sets of measurements of one observer were difficult to reproduce. On the other hand,

Bang and Ramm (1970) found no significant differences between first and second sets of measurements for intact roots and all transparency scores. Drusini and colleagues (1989) also do not report any significant intra-observer differences between transparency and root measurements. Many authors do not report intra-observer error statistics for caliper evaluation of transparency parameters as this method is generally accepted to have a low rate of error.

The degree of root condition also presented some problems in that rough root surfaces added some subjectivity in manual assessment as this made the transparent/non-transparent junction difficult to ascertain at times. The root curvature also created measurement problems as a more sharply curved root is not as easy to measure as a straighter one. However, the degree of root condition and amount of curvature contributed much less error and subjectivity than those problems previously mentioned.

Lastly, when illuminating the tooth roots, the level of light brightness introduced eye strain and fatigue (at some level) during scoring. This was attempted to be remedied by taking breaks in-between teeth (i.e. one tooth at a time) for several minutes at a time, recording data over several days, and using a magnification lupe. Despite these precautions, it was an unavoidable fact that even after scoring one tooth, the observer did accumulate eye strain/fatigue. The manual method of transparency measurement, although much more efficient (i.e. requires less time and simpler equipment) and accessible than the digital method, is not at all as pleasant during data acquisition. This would especially be the case if a larger sample size were to be evaluated.

Comparison of measurements

The total digital method produced fewer intra-observer errors than the total manual method. Nevertheless, the individual measurements must be separated and examined individually in relation to the errors previously explained to truly assess which method and techniques reflect the ascertained dental parameters.

The digital method of root and pulp width measurements and ratios is biased because of software limitations and small horizontal tooth dimensions. In contrast, the root and pulp *length* scores appear to be free of these problems which can be attributed to the much larger longitudinal measurement required of these dental parameters. When the full measurements sets between these pulp reduction parameters are compared, several of the root and pulp *width* scores are significantly different from one another but the mean error is negligible; this does not apply between pulp and root length sets. Therefore, to err on the side of caution, the root and pulp *length* values for the digital method probably reflect the true tooth dimensions to a greater degree than the manual method, while the manual method of root and pulp *width* values are more reflective of real tooth dimensions.

The digital method produced fewer transparency and root length measurement intra-observer errors than the manual method. More specifically, the manual method produced more intra-observer errors and larger mean differences for the *minimum* and *maximum* height of transparency while the digital method contained no intra-observer errors. The labial transparency height and root length appear to be more objective in scoring than the minimum and/or maximum limits of transparency. When the inter-measurement sets of ratios for transparency and/or root scores are examined, no

significant differences are detected. Therefore, both digital and manual measurement methods can be considered comparable in accuracy for transparency limit assessment (the age estimates also demonstrate this). It is interesting to note that the neither the digital nor the manual method produced significantly different intra-observer values for the labial root length, but the inter-method set was found to be significantly different and had a mean error of -0.64 mm - this can be attributed to the differences in manual caliper measurement and the calibration bar used for the digital method. There is bound to be some variation between the two methods, although nominal in scale.

Age estimates and distributions

The age estimate ranges (Figures 4.1 – 4.58), regardless of digital or manual method, usually follow a similar pattern. However, the digital age ranges are almost always higher to a certain degree than the manual age estimate ranges. When the transparency and pulp reduction techniques are separated, a better indication of which one contributes more to this method variability is apparent. The pulp reduction techniques contribute much more to the higher mean ages of the digital method. This, as previously stated, is due to the limitation of the digital technique in creating an artificially higher age estimate.

The transparency techniques are closer at the inter-method level where many of the pulp reduction techniques are not (see Tables 4.3 and 4.4). Kolltveit and colleagues (1998) also found this discrepancy between the digital and manual methods and respective estimated ages. The digital method produced significantly different age estimates for some pulp reduction techniques (Kvaal and Solheim FWC, and Paewinsky A, B, and C) as the computer program could not measure the pulp and tooth width

dimensions as accurately as the manual caliper and x-ray film method. It should be noted that the Paewinsky techniques have the largest inter-method age estimate discrepancy (approximately 12 to 14 years). The simplicity in measurement of the transparency techniques (specifically the Drusini and Foti techniques) exemplifies the closer similarity of the inter-method age estimates (Tables 4.3 and 4.4) and also when age sets are compared (Table 4.5). For decades, transparency age data has been successfully collected by caliper means and light box illumination (Bang and Ramm 1970; Drusini et al. 1989; Foti et al. 2001; Lamendin et la. 1992; and Miles 1963), but more recently many researchers who have assessed transparency digitally (Ermenc 1997; Sengupta et al. 1998; Sengupta et al. 1999; Whittaker and Bakri 1996) have indicated that computer-assisted methods produce comparable age estimates, although some (Drusini et al. (1991) have not been in such agreement. Likewise, pulp reduction age data has been traditionally acquired by caliper measurement and light box illumination (Kvaal et al. 1995; Paewinsky et al. 2005) and also by digital methods (Bosmans et al. 2005; Cameriere et al 2004, 2006, and 2007) with good age prediction results.

One interesting finding is that the standard deviations for the estimated ages of the manual method technique means are larger while the digital method technique means produce fewer and smaller standard deviations (Table 4.6). Generally, the inter-method transparency techniques have *much* closer standard deviations than the pulp reduction techniques. However, when the pulp reduction techniques are separated, it is those techniques that utilize the pulp and root *width* measurements (Kvaal and Solheim FWC and all Paewinsky techniques) that have the largest inter-method standard deviations and not *length* measurements. The Kvaal and Solheim FL technique (that uses pulp and root

length scores) also has a smaller standard deviation than all other *width* based pulp reduction techniques regarding inter-method estimated age. Even with the addition of the area-based Cameriere techniques (the manual method does not include this) that often give the lowest age values, the digital method age estimate means are still higher than the manual method means for most individuals. The Cameriere techniques were applicable to only nine individuals (#352, #244, #284, #250, #321, #242, #309, #354, #302) with only four individuals (#244, #321, #354, #309) having a slightly higher mean manual digital and manual age. The main contribution of the greater mean age of the digital method is largely attributed to the Paewinsky techniques (compare Tables 4 and 5). Generally, the Paewinsky A, B, and C techniques (Table 4.6) experienced the greatest inter-method variation with the digital method grossly producing higher mean age estimates than the mean manual method.

The inter-method and inter-technique age estimate results are somewhat unexpected (Table 4.6). The digital method transparency values are slightly higher for the Foti, Miles, and Bang and Ramm techniques but not for the Drusini technique (which may be an indication of the transparency:tooth root ratio used, allocating more conservative age estimates). It should be noted that although the mean digital method transparency age estimates are higher, it is only by a very slight degree and they are equal for all intents and purposes. As expected, the digital pulp reduction age estimates means are approximately five to ten years higher for the digital method except the Kvaal and Solheim FL technique. The manual Kvaal and Solheim FL technique scored slightly higher and may be a by-product of the caliper measurement technique (reduced observation of pulpal limits).

The distribution of the mean standard deviations for each technique is interesting in that the manual method has higher values as opposed to the digital method (Tables 4.6 and 4.8). The digital method produced more consistent age estimates with less age variation across all techniques besides the Paewinsky B technique. The digital method usually had a mean standard deviation of approximately 2 years less than the manual method besides one technique. This was the Kvaal and Solheim FWC+T technique which had the most inter-method variation with the manual method yielded age estimates about five years higher than the digital method. The transparency technique standard deviations were much closer between methods than the pulp reduction techniques. The overall higher pulp reduction standard deviations for the manual method, again, illustrate the digital method's limitation in linear width measurements.

Aside from methodological problems influencing the age estimates, the individual age markers themselves (transparency or pulp reduction) have also been demonstrated to be congruent (Kvaal and During 1999; Kvaal et al. 1994b) and incongruent (Soomer et al. 2003) to one another in age prediction similarity; this phenomenon undoubtedly adds some inter-age estimate variation. Of course, the condition of the sample is also important to consider regarding archaeological remains (Mandojana et al. 2000), as this may influence age estimates.

Generally, the combined methods (combined manual and digital, combined digital, and combined manual) age distributions follow a similar pattern (Figures 4.59 – 4.62). However, the combined manual age distribution estimates differ slightly with respect to age ranges and outlying age estimates in that they usually fall lower than the digital age estimates. The combined digital method age distribution (Figure 4.61) versus

the mean combined manual method age distribution (Figure 4.62) follows the age range pattern in age estimates in that it (combined digital age distribution) usually has higher values but more conservative (narrower) maximum and minimum estimates (whiskers). The combined digital and combined manual method *technique* age distributions also follow a similar pattern.

The combined manual transparency technique generally produced slightly lower age ranges, tighter age distributions, narrower minimum and maximum age estimates (whiskers), and no outlying estimates, while the combined digital method transparency age distribution gives slightly larger age ranges, wider age distributions, and wider extremes. Interestingly, the median of the ages tends to cluster in the middle-to-upper third for both methods

The combined digital method transparency age distribution has a slightly higher age distribution but has lower variability (tighter mean age ranges and minimum and maximum estimates) than the combined digital transparency technique. Interestingly, the combined manual transparency technique age distribution produced a very wide age range for individual #254 while the combined digital method did not. This is possibly an indication of the advantage in the digital transparency analysis method in minimizing error associated with eye fatigue and taphonomic influences. The combined manual pulp reduction technique yielded an overall lower age distribution, tighter age ranges, and smaller maximum and minimum estimates (whiskers) than the digital counterpart. Although some combined inter-method differences are notable, the combined digital and combined manual method transparency age distributions produce age distributions very

uniform when compared to another which gives another indication of the similarity in digital versus manual measurements.

Unlike the transparency technique age distributions, the pulp reduction techniques, when separated by combined method, produce pronounced differences in age distributions and numbers of outlying age estimates (with or without the Cameriere techniques) (Figures 4.65 and 4.66 versus 4.67). The most apparent observation is the high degree of variation in age estimates in that the digital method produced higher ages versus the manual method, especially the Paewinsky techniques. In addition, several individuals had significantly tighter age ranges with the manual method than the digital method (also with and without the Cameriere techniques).

The combined digital pulp reduction age distributions *with* the Cameriere technique (Figure 4.66) when compared to the combined manual pulp reduction techniques (Figure 4.67) generally follow a similar pattern in age distribution. However, there are many apparent differences. The combined manual method pulp reduction techniques (Figure 4.65) produced slightly lower age distributions, narrower whiskers, wider age ranges, and fewer outlying estimates. Generally, the combined digital method (*with* Cameriere techniques) had higher age ranges, more outlying age estimates, and wider minimum and maximum estimates (whiskers). The combined digital method pulp reduction techniques (*without* Cameriere techniques) and the combined manual method pulp reduction techniques had an interesting and unexpected and contrasting pattern: the age distribution pattern of the combined digital method pulp reduction techniques (*with* Cameriere) was *closer* in age distribution pattern to the combined manual method pulp reduction techniques. The combined digital method pulp reduction techniques without the

Cameriere age estimates produces fewer outlying estimates when included; this is probably because the Cameriere techniques often underage individuals compared to all others. Generally, the combined digital method pulp reduction techniques produced slightly higher age ranges, tighter age distributions, and wider whiskers. The higher age distributions of the combined digital pulp reduction techniques without the addition of the Cameriere age estimates lends evidence to the artificially created older age estimates produced by the limitation of the software (discussed previously), namely the width scores. This is apparent as the Paewinsky age estimates utilize the most pulp and root width data and it is logical that the combined digital method pulp reduction would have more variation in age distribution and higher age estimates.

The age markers themselves have a degree of variability when comparing a certain equation to another. Marcsik and associates (1998) and Kvaal and company (1994b) state that the Bang and Ramm (1970) method usually gives higher age values than some other techniques; this is also found in the present research. On the other hand, the Lamendin (1992) method tends to produce lower age estimates compared to other methods (Whittwer-Backofen et al. 2008). This phenomenon is due to: 1) the quantification measurement technique and 2) the age distribution the reference sample was derived from.

Lastly, one interesting observation is that the averages for both the method means and individual techniques center around 50 years of age (Figure 4.8). This phenomenon is termed 'attraction to the middle' and is reflective of the regression formula capturing the predicted 'population' mean (Prince and Konigsberg 2008; Soomer et al. 2003).

Comparison with skeletal age estimates

This section will discuss the comparison between the skeletal and dental age estimates (Figure 4.9 displays the tallied correspondences and Appendix 1 shows the direct, ±5 year, and ±10 year correspondences). Below is a conglomerate ranking of the top seven techniques that scored highest to lowest:

1. Drusini (either method, digital preferred)
2. Miles (digital method)
3. Bang and Ramm (either method, digital preferred)
4. Cameriere 2007
5. Kvaal and Solheim FWC (manual method)
6. Kvaal and Solheim FL (digital method)
7. All Paewinsky techniques (manual method)

Overall, the manual method proved to fall more frequently within direct correspondence to the respective skeletal age indicators than the digital method. Specifically, all transparency techniques were more consistent (with skeletal age ranges) than the pulp reduction techniques. Specifically, the Drusini et al. (1998) technique, followed by Miles (1963), and Bang and Ramm (1970). The Cameriere and company (2007) technique was the best pulp reduction technique in terms of corresponding to the skeletal ages followed by the manual Kvaal and Solheim (1994) techniques.

The transparency techniques could be used on a greater number of specimens than the pulp reduction techniques. In fact, the transparency techniques could be used on almost twice as many specimens as some of the pulp reduction techniques. This is due to

a high proportion of damage to the pulp canal and root body (cracking and intrusions) that did not pose a problem to the transparency age marker.

The present research is in agreement with studies such as Lucy and colleagues (1995) and Whittwer-Backofen and associates (2008) who found that using multiple dental age indicators on the same individual produces multiple age estimates. For the present research, the Drusini and company (1989) technique *overwhelmingly* provided the most useful, consistent, reliable, and precise correspondences with the respective skeletal age estimates. Soomer and colleagues (2003) also found that transparency age estimates matched closer to respective skeletal age estimates than did pulp reduction age estimates. There are three possible reasons why the Drusini et al. 1989 technique was so successful over all other techniques: 1) the technique records the labial surface transparency height as this area is said to reflect chronological age better than measurement from other areas and is less influenced by pathological damage (Lamendin et al. 1992), 2) the quantification expresses transparency as a ratio of the total root height which accounts for inter-individual, inter-tooth, and inter-population variation, and 3) the equation was *specifically* developed on a known-age archeological sample (which may better reflect non-modern human biology than regression equations based on modern humans) and may be more appropriate to be used on such samples.

The best pulp reduction technique was by far the Cameriere and coworkers (2007) technique. One major disadvantage of this technique is that it was applicable to the least amount of usable specimens of any technique. This technique may be useful in terms of corresponding well with skeletal age, but post-mortem damage seriously reduces its utility on the sample of canines from the Apollonia Pontica/Kalfats necropolis sample

used in the present research. This technique is useful for two reasons: 1) it accounts for the total overall reduction of the pulp compared to the total tooth expressed as an area ratio, and 2) because of this quantification, it is useful for archaeological samples (on damage-free specimens). However, as previously stated, the tooth must be free from damage as any absent portions of the tooth would bias age estimation. The manual Kvaal and Solheim (1994) FWC technique was nearly as useful but probably more widely applicable than the Cameriere et al. (2007) technique because conventional equipment can be more widely used over the somewhat more specialized software (Auto CAD) used in the latter's method.

It should be stressed that for archaeological samples where known-age is not available, that age estimates from dental age indicators should be compared with skeletal age estimates as a large window of error is potentially present (population variation and post-mortem factors). In cases where only isolated teeth are available, such as in the present research, more than one well established and robust dental age marker with several techniques and regression equations should be utilized to ensure confidence in estimated age as the true ages of the skeletons is impossible to be ascertained.

Other factors to consider

Aside from the methodological limitations which introduce systemic error, there are other inherent sources of error contributing to limitations. These two main sources of error are taphonomic influences and idiosyncrasies of the canine tooth-type. The canine (maxillary) generates the least amount of secondary dentin of all tooth types (Philippas and Applebaum 1968), which means a smaller pulp width. In addition, all canines generate more reparative secondary dentin, as opposed to regular secondary dentin, in the

crown portion than other tooth types (Phillipas and Applebaum 1968). Linear measurements of canine pulp and root in both length and width quantifications have generally been demonstrated to be less correlated to chronological age than other tooth types (Kvaal and Solheim 1994; Paewinksy et al. 2005). Research on dentin transparency also shows that the canine tooth type is moderately correlated to chronological age as opposed to some other tooth types (Bang and Ramm 1970; Drusini et al. 1991; Monzavi et al. 2003). Conversely, some authors (Drusini et al. 1991) have stated that the canine is the ideal tooth type as it is the most resistant to damage and often the straightest, largest, and simplest tooth root to score.

Split roots and crowns, crystalline intrusions, and transparency mimicry or obliteration critically reduced the usability of certain measurements and techniques on many specimens when the sample was examined as a whole. The decision to use the appropriate technique was carried out with painstaking effort omit those specimens which were damaged. Many specimens had a piecemeal of applicable techniques. Despite efforts to filter out taphonomically compromised specimens and only apply the appropriate technique, some specimens could not be used while a small number of others appeared suitable but the estimated ages did not correspond to the respective skeletal morphological age. In these cases it is unknown to what extent influences from the burial environment imposed upon the specimen. Individual biological variation may also be another source of age discrepancy. The dental material for the current research was excavated from a sandy, and potentially moist marine burial environment and it is typical that pulpal intrusions and/or physical destruction at some level are observed (Chandler and Fyfe 1997).

Generally, the x-ray dental images showed a high proportion of cracked root structures, foreign intrusions of the pulp chamber, and/or obliterated tooth portions (also found by Doberenz and Wyckoff 1967, Falin 1961, Kvaal and During 1999, and McGowan and Prangnell 2006). Only teeth with 'hairline' cracks that did not artificially alter the physical dimensions of the tooth and/or destroy anatomical reference points were used; even though some of these specimens did have unobtrusive cracks it was decided that they could be used for age estimation as the sample was small. Regarding the pulp reduction techniques, the burial environment rendered a significantly higher proportion of the sample unusable than the transparency techniques. The most difficult taphonomic alteration that presented a challenge in scoring were the 'hairline' cracks as subjectivity was introduced, especially for the pulp width and in some cases length assessments. The digital method allowed a clearer observation and delineation of the 'hairline' cracks than the manual method. The adjustment of contrast especially aided in this. However, possibly because the canine has small pulp widths, the digital method was lacking whereas the manual method proved superior in measurement but not visualization of the features.

Overall, the transparency measurement techniques proved much more universally applicable to the sample than the pulp/root/tooth techniques did (as Table 4.7 demonstrates). In the present sample, the most frequently identified forms of burial influences on transparency were 'chalky' dentin, dark staining, and deeply pitted root surfaces; Beyer-Olsen et al. 1994, Kvaal et al. (1994), Kvaal and During (1999), Lucy et al. (1995), Robbins 2005, and Sengupta et al. (1998 and 1999) also found these problematic in measurement. These damages are attributed to invading particulates in the

tubules (Marcsik et al. 1989). In many cases, isolated patches of 'chalky' spots were located on the transparent and/or non-transparent root portions; these areas were avoided and the closest region of transparency was scored. For example, if the 'chalky' area was located on a diagnostic location of measurement, then it would be avoided. Dark patches were similarly avoided. Pitted roots often presented the greatest challenge in proper transparency assessment as the uneven surface sometimes made the junction between transparent and non-transparent dentin more difficult to pinpoint than roots with less damage. Root surface pitting usually affected the entire surface when compared to the 'chalky' or dark stained dentin, which was usually found on small, isolated portions. The digital method of transparency made assessment easier with less eye fatigue and delineation of features, as opposed to the manual method.

As will be illustrated below, the transparency techniques (specifically Drusini et al. 1989) were better suited for this sample than any other techniques for both manual and digital methods. However, despite the suite of techniques employed, age estimates of a few individuals such as #253 and #289 who did not fall directly (but did nearly within ±10 years) within the skeletal age estimates. Taphonomic forces may have influenced the age estimates of thee individuals.

Probable age estimates (AKA the 'best guess' scenario)

As stated in the previous section the Drusini and company (1989), Cameriere and coworkers (2007), and manual Kvaal and Solheim FWC and FL (1994) techniques were by far the best age indicators for the entire sample. Since the 'true ages' of the individuals will never be known, the only option is to estimate age using a reliable dental age indicator (as carried out in the present study) and compare these estimates to skeletal

ages. The success of the aforesaid dental techniques, compared to other dental techniques, is not surprising as they express tooth dimensions as a metric ratio and not as an absolute measurement. This accounts for inter-individual, inter-tooth, and inter-populational variation in tooth size and/or age tempo, where other techniques fall short. The probable age estimates, age ranges, and age distributions should prioritize the Drusini and coworkers' (1989) method. For those specimens that are taphonomically compromised and have only pulp reduction techniques available (#307, #242A, and #305), then the Cameriere and associates (2007) or Kvaal and Solheim (1994) FWC technique should be prioritized. Two specimens (#338 and #330) only had one technique, Kvaal and Solheim (1994) FL, applicable due to destruction. In these cases the morphological skeletal and dental age corresponded and the dental age estimate should be used as the predicted age. The following (Table 5.1). displays the amalgamated single dental age estimates (the mean dental age estimates of digital Drusini et al. 1989, Cameriere et al. 2007, and manual Kvaal and Solheim 1994 FWC and FL techniques that corresponded with the skeletal morphological age estimates; for individuals #338 and #330 the manual Kvaal and Solheim 1994 FL was used as it was only available) and skeletal morphological age estimates for each individual Regarding the aforementioned mean combined dental age estimates, all individuals fell within ±10 years of the skeletal morphological age estimates except individual #331. This particular tooth from this individual (skeletally aged as 50+) was quite damaged and only transparency techniques could be applied. Most transparency techniques (see Appendix 1) estimated the age to be below the skeletal morphological age except for the Foti and Miles techniques which determined the individual to be just slightly above 50 years old. This specimen may be a

perfect example of transparency obliteration from taphonomic influence and/or a idiosyncratic anomaly that represents individual variation in the expression of an age marker.

Table 5.1: Final age estimates of the Apollonia Pontica individuals.

Cat #	Min Morph	Max Morph	Mean Dental Age
301	18	35	34.37
19	18	35	35.05
253	18	35	46.66
289	18	35	50.30
352	50	90	64.22
336	36	50	47.57
244	18	35	32.90
215	36	50	61.81
192	36	50	40.50
307	18	35	30.02
249	50	90	42.25
207	50	90	75.90
284	18	35	32.26
242a	36	50	33.84
305	18	35	44.37
250	36	50	36.45
321	18	35	30.90
304	36	50	40.02
359	36	50	35.67
374	36	50	41.61
242	36	50	51.48
309	18	35	41.54
67	36	50	52.94
254	36	50	63.96
338	50	90	54.86
354	36	50	49.03
302	50	90	54.36
330	50	90	63.53
331	50	90	28.41

Contributions to the study of Apollonia Pontica

The canine dental sample used for the present research presents several hurdles in terms of archaeological age estimation.. As Klein and Cruz-Uribe (1984) caution, the

portion of skeletal elements available for study in archaeological samples is usually biased to some degree. In addition, the age indicators themselves are biased at the population and individual levels (Bocquet-Appel and Masset 1982; Hoppa 1999). It is apparent that this bias and degree of error is multi-layered and not every specimen, because of this complexity of bias mixture, will be available for accurate age estimation.

Idiosyncratic specimens like #301, #19, #253, and #309 all have the lowest skeletal age estimates of the sample. However, the respective dental age estimates, for nearly all techniques, tend to place the predicted age at the high end (or exceed it) of the morphological age range. This phenomenon has been noticed by several researchers (Marcsik et al. 1989; Kvaal and During 1994; Whittwer-Backofen et al. 2008), who note a marked discrepancy between the age markers; some specimens simply are difficult to correctly age. Again, this may be due to influences by the post-mortem environment in that age mimicry may be occurring.

Mandojana and coworkers (2000) demonstrated that the burial environment can have serious consequences on the inter- and intra-congruency of skeletal and dental age indicators. This taphonomic influence appears to be somewhat random in terms of time since interment and how it affects the dental tissues (Bell et al. 1996; Hillson 2005). Nevertheless, teeth are very useful element for use in archaeological age estimation because of their hardiness and abundance over other hard parts (Martin 1999). Both transparency and pulp reduction have been stated to be unsuitable as archaeological age markers (Sengupta et al. 1999; Mandojana et al. 2000), although other researchers have had success (Bang 1989; Kvaal and During 1999).

Regarding the pulp reduction techniques, pulpal intrusions (Chandler and Fyfe 1997) and split/cracked/broken roots (Hillson 2005) were responsible for virtually all teeth being unusable in some form (i.e. technique). Specifically, individuals #267 and #267(2) had crystalline intrusions observable by radiograph and individuals #19, #289, and #307, for example, had cracks or absent tooth portions which negated the use of several pulp techniques. These types of damages are not suppressing considering the marine environment the Kalfata necropolis is located on.

As previously stated, the transparency techniques were overwhelmingly more applicable to the current sample than pulp reduction techniques. However, some individuals such as #267, #267(2), and #307, for example, were not suitable for transparency evaluation and/or gave questionable dental age estimates (i.e. #331). For this sample, the main cause of destruction was not 'chalky' dentin (Sengupta et al. 1999) or 'dark' patches (Kvaal and During 1999), but simply broken dental material. It should be noted that every archaeological sample will be unique in terms of taphonomic influence (Bell et al 1991, 1996) and what age marker is most appropriate. Kvaal and coworkers (1994b) and Beyer-Olsen and associates (1994) compared the inter-age estimates of transparency, pulp reduction, and skeletal indicators of known-age archaeological individuals, and found that transparency allocated more people into older age categories than pulp reduction techniques. However, they demonstrated that the pulp estimates followed the known ages of the individuals most closely, but the transparency estimates followed the skeletal age estimates closest of all. This means that in instances where known age is not determinable in an archaeological sample, transparency corresponds better with skeletal age than pulp reduction, also found in the present study.

Nevertheless, in archaeological and/or paleodemographic situations where known age is unavailable, the only option is testing several age markers and comparing them for similarity as demonstrated in the current research.

The three specimens that exhibited signs of pathological lesions (i.e. caries) were #338, #336, and #309. It should be noted that discussing pathology is mainly outside the scope of this research as caries have not been found to substantially interfere with transparency and/or pulp reduction to a considerable degree (i.e. several publications have incorporated pathological teeth) (Beyer-Olsen et al. 1994; Phillipas and Appelbaum 1968). These individuals had (although at the high range) correspondence between dental and skeletal age indicators. Olze and associates (2004) state that the health status of the individual has some bearing on the correspondence of transparency and known age. Interestingly, the present study did not control for the health status (skeletal or dental pathological evidence) of the individual, yet still found excellent agreement between transparency and skeletal age estimates.

Keenleyside (2005, n.d.) and Keenleyside and Panayotova (2005 a, b) established the first age profiles for the adult skeletal remains in the Apollonia Pontica, Kalfata necropolis skeletal sample. These age estimates were based on skeletal morphological age markers. As previously stated, morphological skeletal age indicators are 1) phase oriented, 2) produce overlapping and often wide age estimates, 3) are more influenced by pathological and taphonomic insults, and 4) subjective in assessment which requires a skilled worker for evaluation. The present research has applied two well documented and robust dental age indicators that lack the aforementioned criticisms and have achieved

excellent results (correspondence with skeletal morphological age estimates) for the majority of cases.

Table 5.2, presented below, illustrates the comparison of the young (18-35 years), middle (36-50 years), and old (50+) age cohorts (age estimates) of the total (n=158) adult Kalfata skeletal sample examined by Keenleyside (n.d.), the usable dental sample for the current research, and the most effective dental age estimates (Table 5.1); the age categories are expressed as a percentage of the total sample. The total Kalfata adult skeletal sample and associated skeletal morphological age estimates for the current research are compared to the selected dental age estimates. Interestingly, when the percentage of age categories compared to the total number of specimens is examined, all three have very similar distributions of young, middle, and older aged individuals. The present research uses a dental sub-sample from the sub-sample of skeletons used in Keenleyside's (n.d.) analysis which is a sub-sample of the entire Kalfata cemetery and still the percentages of age categories are all similar. The major difference in age distribution is that the selected dental estimates (Table 5.1 and 5.2) place slightly more individuals into the middle (36-50 years) and old (50+) age categories compared to Keenleyside's (n.d.) (Tables 3.1) sample and/or the skeletal morphological age estimates used in the current study (Table 3.2). Since known age is not available for the Apollonia sample, the exact reason why the selected mean dental age estimates allocate slightly more individuals to the old age category is unknown, several reasons for this may be that: 1) dental parameters often estimate higher age than skeletal indicators, 2) the selected dental techniques are more effective at capturing the oldest old (teeth are more resistant to metabolic retrograde than respective osseous morphological expression of age), the 3)

skeletal and dental age estimates are a product of the reference population from which they were created from and reflect the particular age distribution/morphological expression, or 4) or a complex combination of all these variables. The similarity in age cohort distribution as demonstrated in Table 5.2 may lend evidence that it is possible to create a normally distributed paleodemographic profile given the specifications used in the present research, however, it is beyond the scope for further exploration of this topic.

Table 5.2: Age distributions comparing % of age categories of Kalfata sample (skeletal age), usable thesis sample (skeletal age), and thesis dental sample (mean selected dental techniques).

Kalfata Sample (skeletal age) (Keenleyside n.d.)	Total	% of total sample
18-35	60	38%
36-50	60	38%
50+	38	24%
Total adults	158	
Dental Sample (skeletal age)		
18-35	10	34.5%
36-50	12	41.5%
50+	7	24%
Total usable specimens	29	
Mean Age of Selected Dental Techniques (Table 5.1)		
18-35	9	31%
36-50	10	34.5%
50+	10	34.5%
Total usable specimens	29	

The major contributions of the current research are as follows: 1) the dental age techniques that produce a single point age estimate instead of an age range, 2) the dental techniques (especially dentin transparency) that produce an efficient and simple means of age estimation instead of several techniques, 3) dental age techniques that correspond well with the skeletal morphological age markers, meaning that the dental techniques may be used alone if necessary, and 4) the age distributions, when separated by young,

middle, and old age cohorts are similar between the total analyzed Kalfata adult sample (Keenleyside n.d.), the skeletal morphological age estimates used in the current research, and the selected mean dental age estimates.

Perhaps the most useful contribution to our knowledge of the Apollonia Pontica, archaeological age estimation, and to paleodemography is that the techniques, as demonstrated in the present research, indicate that many people lived to middle or old age. The older age groups are the most difficult to accurately capture using skeletal morphological age markers (Hoppa and Vaupel 2002; Miles 1958; Schmidt 2008). The dental age estimates indicate that the majority of the individuals in the present sample died in middle to late adulthood with younger age groups being slightly underrepresented, although not to a market degree (at least for the present dental sample based on dental age estimates). This has obvious implications for the overall health and well being of the population. It is beyond the scope of the current research to delve into this matter further.

The most significant finding produced by the current study is that a moderate portion (depending on the technique) of the Kalfata necropolis population (at least in the sample used in the current research) may have been living, on average, into old age (Table 5.2 illustrates this). Some techniques estimated certain individuals living into the 6^{th} or 7^{th} decade of life. This may not be surprising given the ancient Greeks' technology, living conditions, environment, and diet (Angel 1947, 1972; Batrinos 2008; Trichopoulou 2004) but further comment is beyond the scope of this research. The strategy of techniques employed in this study has allowed all usable canine dental material (non-

friable specimens and/or crowns) to be evaluated with maximum acquisition of age estimation data.

Conclusions and directions for future research

This study demonstrates that a small sample of adult canines is suitable (in the majority of cases) for age estimation specifically with archaeological applications. The two well-established age markers, root dentin transparency and pulp reduction, have proven to correspond very well with respective morphological macroscopic skeletal age indicators. This research concentrates on the comparison of two methodologies: traditional manual caliper and digital measurement techniques. The manual method produced comparable measurement scores, age values, and standard deviations to the digital method. The manual linear pulp reduction method produced lower measurement values and age estimates but larger standard deviations than the digital method. Overall, the transparency techniques were more useful than the pulp reduction techniques. However, in a few cases no transparency was observable and in those cases one of the pulp reduction techniques sufficed for age determination. Since it was expected that both techniques could not be used on all specimens, several published age techniques and age equations were utilized to acquire the most data with the minimal amount of specimens. The techniques employed for the current study are non-destructive and are applicable across populations separated by time and space (since they strongly reflect biological age). The real ages of the individuals will never truly be known, but the present research demonstrates that a more refined and simpler method of age estimation in archaeological situations is possible.

The Drusini and colleagues (1989) technique unquestionably achieved the highest correspondences with the respective skeletal morphological age estimates. The Drusini and associates (1989) technique and regression equation is the choice for this study as it is the most applicable to burial remains because it considers tooth size and is designed for use on non-modern archaeological samples.

Presented below are the study's primary findings that the present study accomplished:

- The age ranges and age distributions were in agreement between the two methods in the majority of cases. However, the transparency techniques were more uniform than the pulp reduction techniques.
- The manual method produced lower age estimates but slightly larger standard deviations than the digital method.
- The inter-method measurement differences were most pronounced with the linear pulp width reduction techniques. This is due to the limitation of the digital method.
- The transparency techniques were more similar in age distribution than the pulp reduction techniques and contained less variability in inter-method measurements, age estimates, and standard deviations.
- The transparency techniques were much more robust and significantly less influenced by the burial environment than the pulp reduction techniques (specifically the root and pulp width techniques)
- A small sample of randomly selected adult canines using one tooth per individual, regardless of sex, health state, pathological state, and/or

condition can match respective skeletal morphological age indicators in the majority of cases and produce a single age estimate and not an age range using two dental criteria

- The strategy of using two dental age criteria and several techniques maximizes minimally invasive analysis on small sample sizes and produces satisfactory results despite issues with the canine or the post-mortem environment
- The people occupying the Apollonia Pontica colony were living, on average, into old age which has obvious implications on the socio-economic status, health, and well-being of the population

The two dental age indicators were largely in agreement with skeletal age indicators. Specifically, the Drusini and coworkers (1989) technique has established itself as the most useful archaeological age estimation technique. Finally, for practical reasons, the manual measurement methods were overall more effective and time-efficient than the digital methods. However, one major advantage that the digital method has over the manual method is a slightly more objective means of analysis and more importantly, a universally transferable and reproducible media format that meets the increasing needs of the global scientist. In the future, each researcher should carefully consider the aims of his/her research goals, time-frame for data collection, and accessibility to the skeletal collection, then carefully decide upon an appropriate and logical data acquisition method.

Generally, the major problem found with the linear width dimensions measurement techniques for the digital method. The digital method can be improved by employing the following: 1) high resolution digital x-rays over scanned x-ray film, 2)

larger, higher definition images of the teeth, and 3) depending on the measurement type/technique (pulp/root length versus pulp/root width), the tooth image may need to be rotated for easier visualization. The manual method of linear pulp dimension measurement can be improved by the following specifications: 1) the dental images on the x-rays should be evaluated on a stereomicroscope that allows for transillumination of the film, 2) the aperture of the stereomicroscope should be large enough to easily see the entire tooth structure, and 3) a millimetric ruler may be used in conjunction with digital calipers for added measurement stability. Lastly, another improvement may be the linear measurement technique itself. By measuring the pulp-to-root width ratio, the amount of secondary dentin is *indirectly* evaluated, and perhaps the pulp should be *indirectly* measured as well. For example, a linear line was placed across the pulp width, but as previously stated, the graphical lines were not precise enough (due to image resolution). To circumvent this problem, the indirect measurement of pulp should be attempted by measuring 1) the root width, 2) either the mesial or distal root-to-pulp junction, and 3) subtracting the two values. This may give a more precise measurement although it has not been yet attempted.

The dental age scores (of the present sample) of the adult Apollonia Pontica individuals have now been established and compared to the associated skeletal age indicators. This research has demonstrated that the dental age estimates produce a single age score using metric analysis instead of phase-oriented, overlapping, and wide age intervals with associated the skeletal age markers.

The next step in future research is to establish a palaeodemographic profile as the dental age markers produce one single age estimate which will further increase the

current understanding of fertility and mortality patterns of the Apollonia Pontica sample as they capture more precise age estimates of the oldest individuals. In addition, it will also offer insight into the efficacy of using a small sample size and one tooth-type to create a 'normally' distributed palaeodemographic profile. In addition, the newly created dental age estimates can now be applied to achieve a more concise age estimate of non-dental skeletal material by inversely calibrating elements that may lack dentitions and/or are only represented by a few isolated remains. Furthermore, a more holistic picture of the skeletal biology of the colony is now created. The osteological analyses undertaken in the present study have further contributed to the understanding of the ancient life-ways, health, and well being of the Greek Colonial world during this time of occupation.

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Appendix 1: Total Dental and Skeletal Age Estimates