GEOMETRIC MORPHOMETRICS ASSESSMENTS OF STERNUM BY AGE

by

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LIST OF ABBREVIATIONS

GMM	= Geometric Morphometrics
CT	= Computed Tomography
PACS	= Picture Archiving and Communication System
MITK	= Medical Imaging Interaction Toolkit (MITK)
IDAV	= Institute for Data Analysis and Visualization
PCA	= Principal Component Analysis
2D	= Two-Dimensional
3D	= Three-Dimensional
MRI	= Magnetic Resonance Imaging
GPA	= Generalized Procrustes Analysis
TPS	= Thin-Plate Spline
VRT	= Volume Render Technique
ITK	= Insight Toolkit
VTK	= Visualization Toolkit
MPR	= Multi-Planar Reformatting (MPR)
*.stl	= Stereolithography File Format
*.ply	= Polygon File Format
*.dta	= Data File Format
*.pts	= 3D Points File Format
ANOVA	= Analysis of Variance
PC	=Principal Component

ABSTRACT

Geometric Morphometrics (GMM) is one common approach to shape analysis that utilizes the coordinates of landmarks to record the relative positions of morphological points, boundary curves and surfaces as the basis of shape quantification. It is usually accomplished through a series of steps that can be called the Procrustes Paradigm. Landmark-Based GMM uses sets of two- or three-dimensional coordinates of biological landmarks. In this retrospective project, a total of 70 CT-scan images samples (35 male and 35 female) were selected for the geometric morphometrics assessment of sternum by age and age groups (7 groups). This project was conducted by employing several software which include Picture Archiving and Communication System (PACS), Medical Imaging Interaction Toolkit (MITK), MeshLab, Institute for Data Analysis and Visualization (IDAV) Landmark Software, Notepad++, and MorphoJ. The results of this project suggest that GMM assessment of sternum by age is more reliable rather than by age group. However, the level of significance is not really high at only at 45.28% and the Principal Component Analysis (PCA) on the samples did not show any positive correlation between shapes of sternum and the age or age group. Thus, it can be concluded that GMM assessment is not compatible for the classification of sternum shape based on age or age group.

ABSTRAK

Morphometrik Geometri (GMM) adalah salah satu pendekatan lazim yang diaplikasikan untuk menganalisa bentuk dengan menggunakan koordinat mercu tanda untuk merekod kedudukan relatif titik-titk morphologi, keluk sempadan, dan permukaan sebagai asas kuantifikasi bentuk. Kebiasaannya, GMM dilaksanakan menerusi satu siri langkah-langkah yang digelar sebagai paradigma Procrustes. Morphometrik geometri (GMM) berdasarkan mercu tanda menggunakan mercu-mercu tanda biologi dua atau tiga dimensi. 70 sampel imej CT-scan (35 lelaki dan 35 perempuan) telah dipilih untuk pentaksiran atau penilaian GMM ke atas tulang dada (sternum) berasaskan umur dan kumpulan umur (7 kumpulan) untuk projek retrospektif ini. Projek ini dikendalikan dengan menggunakan beberapa perisian, iaitu Picture Archiving and Communication System (PACS), Medical Imaging Interaction Toolkit (MITK), MeshLab, Institute for Data Analysis and Visualization (IDAV) Landmark Software, Notepad++, dan MorphoJ. Keputusan projek menunjukkan bahawa pentaksiran GMM keatas sternum berdasarkan umur lebih signifikan berbanding kumpulan umur. Walaubagaimanapun, tahap signifikan tersebut agak rendah dengan hanya 45.28% dan hasil daripada analisis komponen utama ke atas sampel-sampel tersebut tidak menunjukkan sebarang hubungkait positif antara bentuk tulang dada dan umur atau kumpulan umur. Sehubungan dengan itu, dapat dirumuskan bahawa pentaksiran GMM bukanlah teknik yang sesuai untuk pengelasan tulang dada berdasarkan umur atau kumpulan umur.

CHAPTER 1

INTRODUCTION

1.1 Age Estimation

Estimation of age is major forensic consideration and required in civil and criminal matters. It is also one of the main parameters in the evaluation of skeletal remains in forensic anthropology casework (Chandrakanth *et al.*, 2012) and is a major issue in forensic medicine, for several reasons. It can help to establish the biological profile of a decomposed body or a skeleton, and provide important information in cases of assault, accident, or natural disaster. Estimating the age of death is an essential aspect of forensic anthropology (Barres *et al.*, 1989; Brogdon, 1998; Cox, 2000; Ritz-Timme *et al.*, 2000; Cunha *et al.*, 2009 in Oldrini *et al.*, 2016). Today, forensic age diagnostics is an established research sector of legal medicine in its own right (Ohtani *et al.*, 2003; Olze *et al.*, 2004, 2005; Paewinsky *et al.*, 2005; Ritz-Timme *et al.*, 2003; Schmeling *et al.*, 2004; Takasaki *et al.*, 2003 in Schulz *et al.*, 2005).

The estimation of bone age is also an important part of physical and forensic anthropology. It can be carried out in two ways, either by macroscopic or by radiological study (Moskovitch *et al.*, 2010). Macroscopic study of bones is commonly utilized to estimate age at death. Several indicators, such as the pubic symphysis, the fourth right rib, and the auricular face of the ilium, have yielded good results (Bass, 2005; Bedford *et al.*, 1993; Martrille *et al.*, 2007; Katz & Suchey, 1986; Iscan, Loth & Wright, 1984; Lovejoy *et al.*, 1985). Radiological studies are useful for living persons, in particular when age estimation is requested by legal authorities for foreign nationals whose identity is uncertain (Schmeling *et al.*, 2001; Schmeling et al., 2003). Besides, it also avoids the destruction of materials during bone preparation.

On the other hand, Rosing and his associates (2005) proposed that the methods available for estimating the age of skeleton can also be divided into two categories. The categories are simple but inaccurate methods on the one hand, and complex but accurate methods on the other. The simple methods are utilized as a first stage of examination, whereas the more complex methods are applied in the second stage. The decision whether the second stage should be initiated at all is taken on the basis of the findings from the first stage. For example, if it is found that the skeletal remains are historic or are scattered remains from a modern cemetery, the second stage is superfluous.

The estimation of skeletal age falls into various groups, which have marked differences in both method and accuracy. At present generally, skeletal age determination is considered to be one of the best methods. It includes age determination from the skull and pelvis, the sternum and clavicle, the long bones and the vertebrae (Tayal *et al.*, 2013). Other criteria that are involved for determining the age also include the secondary class characteristics, dental status, appearance and fusion of ossification centre of bones and various skull sutures (Tailor *et al.*, 2013). In contrast, based on a review by Cunha and associates (2009), literature has pointed out that age estimation based on dental methods is more reliable than from skeletal analysis; in detail, dental methods are supposed to be less influenced by racial and environmental factors.

One of the crucial aims of a medicolegal investigation is to establish the identity of a deceased, especially in cases where skeletal remains and dismembered or mutilated body parts are recovered and brought for examination. In these cases, it is vital to estimate age, sex, stature, as well as ancestry and thus help in establishing the biological profile of the remains. Age estimation is one of the foremost criteria in the evaluation of unknown and commingled human remains. According to Tayal and associates (2013), the age of the subject at death is usually more important than the stature and the sex as it is the most vital indication of identity which excludes a sizeable portion of the population and narrows down the field of investigation whether the context is bioarchaeological, paleontological or forensic in nature. Unless the age of a person is determined, the identity of a person, live or dead stands incomplete (Rao Nageshkumar, 2000 in Tayal *et al.*, 2013).

In recent years, it has become increasingly important to determine, in particular, the ages of living persons (Schmeling *et al.*, 2001). From a legal perspective, such age estimations are carried out to determine whether a suspect without valid identification documents has reached the age of criminal responsibility and whether general criminal law in force for adults is to be applied (Schulz *et al.*, 2005). Age estimation *in vivo* (in living persons) is also of interest in a judicial context and is becoming increasingly important in forensic affairs related to migration of foreigners without valid identity papers (Schmeling *et al.*, 2003; Schmeling *et al.*, 2004; Bassed, 2012 in Oldrini *et al.*, 2016).

A significant innovation in the analysis of skeletal maturation concerns the way in which size and shape are characterized (Braga & Treil, 2007). New concepts and methodologies collectively known as geometric morphometrics (GMM) are increasingly being used. Geometric Morphometrics is the statistical analysis of shape variation and its covariation with other variables (Bookstein, 1991). Geometric Morphometrics allows the quantitative analysis of variation in organisms from the 3-Dimensional coordinates of anatomical landmarks collected with specialized devices by digitizing the surface of body parts (e.g. hand-held laser scanner) or from computed tomography (CT).

Geometric morphometrics (e.g., Bookstein, 1991; Rohlf and Marcus, 1993) are powerful statistical and analytical tools for shape analysis that have become increasingly widespread in anthropological research (Adams et al., 2004). Based on an article by

Cramon-Taubadel, Frazier, & Lahr (2007), examples of applications in physical anthropology are numerous and include:

a) *systematic and taxonomic analyses* (Delson et al., 2001; Singleton, 2002; Frost *et al.*, 2003; Guy *et al.*, 2003; Harvati, 2003; Harvati *et al.*, 2004)
b) *morphological evolution and phylogenetic assessment* (Lockwood *et al.*, 2002, 2004; Bastir and Rosas, 2005; Nicholson and Harvati, 2006)
c) *the examination of morphological (dis)similarity to assess patterns of ontogeny and growth* (O'Higgins and Jones, 1998; Collard and O'Higgins, 2001; O'Higgins and Collard, 2002; Viðarsdo´ttir *et al.*, 2002; Bookstein *et al.*, 2003; Cobb and O'Higgins, 2004; Mitteroecker *et al.*, 2004)
d) *studies of population admixture* (Martinez-Abadı´as *et al.*, 2006)

This study focused on the application of the GMM to classify sternum based on age or age group. Geometric Morphometrics (GMM) was used to assess the sternum shape (manubrium specifically) and it was determined whether GMM is successful in classifying the sternum or not.

CHAPTER 2

OBJECTIVES OF THE STUDY

2.1 General Objective

2.1.1 To perform geometric morphometrics assessments on the sternum by age

2.2 Specific Objectives

2.2.1 To perform geometric morphometric assessments on the sternum from 7 age groups (Group A, B, C, D, E, F & G).

2.2.2 To determine whether the geometric morphometrics assessment can be utilized for classification of sternum by age.

2.2.3 To classify the sternum based on age or age groups

2.3 Significance of the Study

2.3.1 Able to classify the sternum based on age or age groups with the application of geometric morphometrics assessments of the sternum.

2.3.2 Can be useful for the estimation of age in cases involving unknown body or decomposed body.

2.3.3 Can be helpful in estimation and confirmation of the age in living persons especially those involved in criminal cases.

CHAPTER 3

LITERATURE REVIEW

3.1 Geometric Morphometrics

Morphometrics is the study of shape variation and its covariation with other variables (Bookstein, 1991; Dryden & Mardia, 1998 in Adams *et al.*, 2004). Historically, the methods involved in the analysis of collections of distances or angles, but recent theoretical, computational, and other advances have shifted the focus of morphometric procedures to the Cartesian coordinates of anatomical points that might be used to define the more traditional measurements (Slice, 2007). A review of the field of morphometrics called this new approach 'geometric morphometrics' and suggested that this paradigm shift signaled a "revolution in morphometrics" (Rohlf & Marcus, 1993 in Adams *et al.*, 2004).

Based on Webster and Sheets (2010), there are 3 general styles of morphometrics that are often recognized, distinguished by the nature of data being analyzed. The styles include traditional morphometrics, landmark-based geometric morphometrics, and outline-based geometric morphometrics.

a) *Traditional Morphometrics*

This style involves summarizing morphology in terms of length measurements, ratios, or angles, that can be investigated individually (univariate analyses) or several at a time (bivariate and multivariate analyses).

b) *Landmark-Based Geometric Morphometrics*

This style involves summarizing shape in terms of a landmark configuration (a constellation of discrete anatomical loci, each described by 2- or 3-dimensional Cartesian coordinates), and is inherently multidimensional.

c) *Outline-Based Geometric Morphometrics*

This style involves summarizing the shape of open or closed curves (perimeters), typically without fixed landmarks.

Nontraditional morphometrics or GMM have found wide application in the biological sciences, especially in anthropology, a field with a strong history of measurement of biological form (Richtsmeier, Deleon & Lele, 2002). Based on Perez, Bernal & Gonzalez (2006), the most widely used GMM are landmark-based approaches that use sets of two- or three-dimensional coordinates of biological landmarks (Bookstein, 1996;1998). Walker (2010) stated that landmark-based geometric morphometrics have been increasingly exploited during the past decade to explore systematic, developmental, ecological, or pathological differences among individuals or populations. However, based on previous research, from biological viewpoint, the use of landmarks may not be sufficient because they cannot describe some biological forms, patterns (Oxnard, 1978 in on Perez, Bernal & Gonzalez, 2006) and salient features of morphology are overlooked when landmark data are utilized exclusively (Read & Lestrel, 1986 in Richtsmeier, Deleon, & Lele, 2002). Further information may be obtained by increasing the number of point coordinates.

According to Slice (2007), Geometric Morphometric (GMM) methods focus on the retention of geometric information throughout a study and provide efficient, statistically powerful analyses that can readily relate abstract, multivariate results to the physical structure of the original specimens. The basis of this method is the identification and quantification of landmarks, defined as ''a point of correspondence on an object that matches between and within populations'' (Dryden and Mardia, 1998 in Cramon-Taubadel *et al.*, 2007). This method is one common approach to shape analysis (Adams & Otarala-Castillo, 2013) that utilizes the coordinates of landmarks to record the relative positions of morphological points, boundary curves and surfaces as the basis of shape quantification and typically accomplished through a series of steps that can be called the Procrustes paradigm (Adams, Rohlf & Slice, 2013).

Among several geometric approaches to morphometrics, the Procrustes method is the most widespread and best understood in its mathematical and statistical properties (Bookstein, 1996; Small, 1996; Dryden & Mardia, 1998 in Mitteroecker & Gunz, 2009). Other frequently used morphometric methods are Euclidian distance matrix analysis (Lele and Richtsmeier 1991, 2001), elliptic Fourier analysis (Lestrel 1982), and non-label based approaches like voxel-based morphometry (e.g., Ashburner and Friston 2000), which is mainly applied in brain imaging. Procrustes method scales all configurations to unit size, superimposes them by their center of gravity (centroids), and rotates them to an orientation of optimal fit to a consensus configuration (Dryden & Maria, 1998 in Braga & Treil, 2007). Adams, Rohlf, and Slice (2013) described procrustes paradigm as a methodological approach to shape analysis arising from the intersection of the statistical shape theory and analytical procedures for obtaining shape variables from landmark data. They explained that in a typical morphometric analysis, the Procrustes paradigm is implemented as a series of operations.

First, from each specimen, a set of two- or three-dimensional landmark coordinates are obtained, which record the relative positions anatomically-definable locations. Landmarks are points of correspondence on each specimen that match between and within populations or, equivalently biologically homologous anatomical loci recognizable on all specimens in the study (Bookstein, 1991; Dryden & Mardia, 1998 in Webster & Sheets, 2010). According to Webster & Sheets (2010), several factors should be considered when selecting a landmark. The factors include that the landmark must be a homologous anatomical locus recognizable on each specimen in the study, landmark configurations should be selected to offer an adequate summary of morphology and should be reliably digitizable. Ideas for landmark selection sometimes can also be obtained from the previous literature.

Mitteroecker & Gunz (2009) stated that two-dimensional (2D) landmark coordinates are usually captured using a digitizing tablet or by measuring an image on the computer, while the three-dimensional (3D) landmark data can be captured directly using a coordinate digitizer such as Microscribe or Polhemus, or may be measured on surface scans or volumetric scans. Volumetric data are based on image-slices from computed tomographic (CT) or magnet resonance imaging (MRI) scanners—or their high-resolution versions μCT and μMRI (Sensen & Hallgrimsson, 2009). These slices contain gray-values that correspond to tissue densities and are concatenated to obtain a 3D representation of an object.

Webster and Sheets (2010) further explained that 2D landmark coordinates can be extracted from a digital image of a specimen using free software such as tpsDig, ImageJ, or ScionImage, while the technique (and cost) for extracting 3D landmark coordinates will vary depending on the size of the specimen. Reflex measurement microscopes can be incorporated for small specimens, while 3D scanners, Microscribe digitizers, and MRI or CT scans (for large specimens) are potential options. Furthermore, they also added that great attention should be paid to maximizing the quality of the specimen and/or photograph prior to digitizing the landmarks because reliable landmark data are unlikely to be obtained from a poor-contrast, out-of-focus photograph of a specimen.

Bookstein (1991) in MacLeod (2011) identified three classes of biological landmarks: discrete juxtapositions of structures or tissues (Type 1), maxima of curvature (Type 2), or extrema (Type 3). This classification focuses attention on the amount of information necessary to identify to relocate the landmark (when process of landmarking is repeated for a few times). Zelditch, Swiderski, & Sheets (2012) explained about the different types of landmark in their book.

a) Type 1 landmark can be defined in terms of local informations, such as a landmark located at the junction of three or two bones and a muscle (i.e. anatomical features that meet at a point).
b) Type 2 landmark is defined by a relatively local property, such as the maximum or minimum of curvature of a small bulge or at the endpoint of a structure.
c) Type 3 landmark is defined in terms of extremal points, such as the landmark on the rostrum *furthest away from* the foramen magnum.

Next, a generalized Procrustes analysis (GPA: Gower 1975; Rohlf and Slice 1990) is employed to superimpose the configurations of landmarks in all specimens to a common coordinate system, and to generate a set of shape variables. This least-squares procedure translates all specimens to the origin, scales them to unit centroid size, and rotates them to minimize the total sums-of-squares deviations of the landmark coordinates from all specimens to the average configuration. This least-squares oriented approach involved three steps and the coordinates of the resulting centered, scaled, and rotated landmarks are called Procrustes shape coordinates (Mitteroecker & Gunz, 2009).

The first step is translation of the landmark configurations of all objects so that they share the same centroid (the coordinate-wise average of the landmarks of one form).

Next is the scaling of the landmark configurations so that they all have the same centroid size. Centroid size is a measure of scale for landmark configurations, which has been shown to be approximately uncorrelate with shape for small isotropic landmark variation (Bookstein, 199; Dryden & Mardia, 1998). As a convention, centroid size is set to one for all landmark configurations. Finally, when superimposing two landmark configurations, one of the two centered and scaled configurations is rotated around its centroid until the sum of the squared Euclidian distances between the homologous landmarks is minimal. The specialized software available for the analysis of geometric morphometric data will typically handle superimposition internally (Webster & Sheets, 2010). For more than two forms, this algorithm has been extended to Generalized Procrustes Analysis (GPA), where the rotation step is an iterative algorithm (Gower 1975; Rohlf and Slice 1990).

The final step in Procrustes paradigm is to utilize the multivariate statistical methods to test the biological hypotheses before finally utilizing the graphical method to visualize patterns of shape variation and facilitate descriptions of shape changes. Based on walker (2010), principal components analysis (PCA) is one of the several statistical methods that is nothing more than sophisticated, exploratory methods to summarize patterns of shape variation that are too difficult to recognize from superimposed figures or a series of TPSs (Thin-plate Splines). PCA results from superimposition and TPS decomposition methods have been compared with real data sets (Walker, 1996), but their performance with simulated data sets has not been evaluated.

3.2 Sternum and Rib Cage

The sternum is a flat bone, slightly convex anteriorly and concave posteriorly. It connects to the ribs via costal cartilage to form the anterior section of the thoracic skeleton (Weaver *et al.*, 2014) and comprises of three distinct parts. The parts include a manubrium superiorly, a mesosternum or the main body in the middle, and a xiphoid process placed inferiorly (Chandrakanth *et al.*, 2012). Restrepo and his associates (2009) described each part of the sternum in their article.

a) Manubrium is the broadest portion of the sternum, and the farthest cephalad. It has a superior central notch (suprasternal or jugular notch) and two lateral fossae (clavicular notches) that articulate with the clavicles. The manubrium also articulates with the first and second ribs and the body of the sternum.

b) Mesosternum or the body of the sternum is flat, with an irregular anterior surface. Superiorly, it articulates with the manubrium at the manubriosternal joint (also called the sternal angle or symphysis). Inferiorly, it articulates with the xiphoid process. The lateral borders of the sternum articulate with the second through seventh ribs.

c) The xiphoid process is a thin and elongated bone that is subject to many variations. It is cartilaginous early in life and may become completely ossified and fused to the sternal body in old age (Goodman, Teplick, & Kay, 1983; Gray, Williams, & Bannister, 1995; Stark & Jaramillo, 1986)

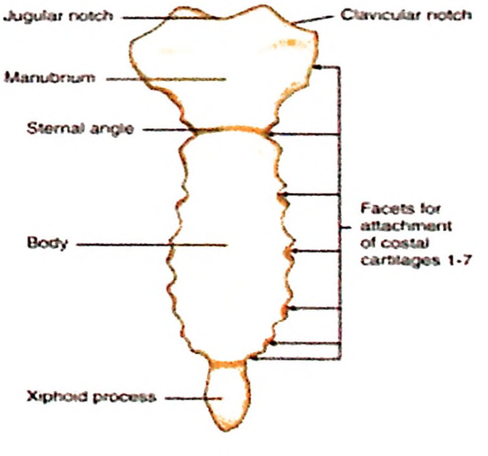

Figure 3.1: Anatomy of Sternum (Retrieved from Google Images)

Figure 3.1 shows the anatomy of sternum, which illustrates the main parts of sternum. Other than being one of the anatomic parts of the thorax which influences the global stiffness of the rib cage, sternum can also be used as an osseous age estimator as surface area and volume measurements of the ossified sternum correlate well with age for the pediatric population (Riach, 1967; Sandoz *et al.*, 2013). In an article by Tayal and his associates (2013), sternum was regarded as the bone that has the potential to show the effects of age because it has been established to be a perfect bone to show the advancement of age by Howell (1962).

However, in the same article by Tayal and his associates (2013), it was also stated that data on the sternum morphology is still insufficient thus the signs of ageing displayed in the joints of the sternum cannot be read with confidence and 100% accuracy. Age estimation from the sternum is unreliable due to the variability in sternum ossification patterns and fusion based on previous studies by Dwight (1881), Scheur & Black (2000), and Weaver (2014). Other than functioning as an age estimator, in the past, sternum has been utilized by researchers for estimation of sex and stature (Hunnargi *et al.*, 2009; Menezes *et al.*, 2009; Macaluso, 2010; Ramadan *et al.*, 2010; Singh, Pathak & Chavali, 2011; Menezes *et* al., 2011; Franklin *et al.*, 2012; Marinho *et al.*, 2012 in Chandrakanth *et al.*, 2012).

According to Chandrakanth and his associates (2012), the adult sternum is a highly variable structure due to its distinct embryological development. They further explained that there are two types of studies that have been conducted for estimation of age from sternum, which are based on the direct inspection or visual examination of the fusion on the bone and based on the radiographic analysis of the fusion on the bone. Radiological examination of sternum especially fusion of sternal segments and fusion of xiphoid process with sternum offer considerable help in age estimation of the living (Umap, Bardale, & Shriramwar, 2013).

A study conducted by Wadhawan, Muraru, & Murali (2009) found that fusion of manubriomesosternal joint (manubrium and mesosternum) and xiphiod process with mesosternum can help in estimating the approximate age of the individual. In another study that was conducted by Tailor and his associates (2013), they confirmed the fusion of the sternum and confirmed by using the radiological aspect (type of radiology imaging used was not mentioned in the article). Based on the result, the gross finding shows early fusion as compared to radiological findings. Moreover, they also showed that the sternal segments fused in between ages of 21-30 years old, xiphoid process shows fusion at the age of 40 years old and manubrium fused at late advanced stages.

There are no articles or previous studies regarding the application of geometric morphometrics methods (GMM) on the assessment of sternum. However, a study by Gayzik and his associates (2008) had applied GMM as a method for quantifying age-related shape change in the complete human rib cage. The article also stated that Slice and Stitzel (2004) identified the benefit of applying GMM in the development of computational human body models for injury biomechanics research, and had demonstrated the feasibility of applying this approach to anthropological studies of the rib cage (Slice, 1998; Slice and Stitzel, 2005). Sternal positioning within the thoracic skeleton has also been quantified and the sternum is known to move inferiorly from childhood into adulthood (Openshaw *et al.*, 1984; Grivas *et al.*, 1991; Galan *et al.*, 1992 in Weaver *et al.*, 2014). However, based on weaver and his associates (2014), while sternal length, area, volume, shape, and positioning changes with age and sex have been analyzed in a variety of studies, a complete quantification of 3D sternum morphology across ages and sexes is lacking in the literature.

3.3 Computed Tomography (CT) Scans

Imaging techniques plays a significant role in determining the age and sex of an individual. Recent advances in age estimation have been facilitated by the development of cross-sectional imaging, including CT, which is increasingly adopted in forensic medicine (Grabherr *et al.*, 2009; Bassed, 2012 in Oldrini *et al.*, 2016). According to an article review by Aggrawal (2009), newer imaging methods that have been recently applied for the age estimation include imaging by ultrasound (Castriota-Scanderbeg *et al.*, 1998; Bilgili *et al.*, 2003; Schulz *et al.*, 2008), echocardiography (Belozerova, 2006) and computed tomography (Schulze *et al.*, 2006; Yang *et al.*, 2006). Multiplanar and 3D reconstructed CT images are useful in the evaluation of the human skeleton, especially in complex parts such as the skull base, shoulder, and sternum.

Dkhar (2014) revealed that Forensic Radiology of the study of the sternum was first performed by Wenzel in 1788. He described the difference in the ratio between the length of manubrium and that of mesosternum in both sexes. Based on previous studies that were stated in an article by Restrepo and his associates (2009), CT is the modality of choice to evaluate anatomic detail as well as pathologic conditions of the sternum, sternoclavicular joints, and adjacent soft tissues. They further explained that magnetic resonance imaging (MRI) is of great value as a secondary modality as it can help clarify CT findings and can provide additional information about the bone marrow and soft tissues adjacent to the sternum (Aslam, 2002). The significant advantages of CT have been discussed in previous studies. Hatfield and his associates (1984) listed several significant advantages that offered by the CT scan in their article, which include:

a) Compared to plain radiography, CT provides far superior images (Levinsohn, Bunnell, & Yuan, 1979) because of better contrast resolution and transverse tomographic display.
b) Compared to conventional tomography, CT requires less cumulative radiation exposure (Maue-Dickson, Trefler, & Dickson, 1979)

Furthermore, CT enables the visualization of the image in Bone-Window and Volume Render Technique (VRT) format, in which one can have 3D view and therefore the image can be rotated. Thus, the status between xiphoid and sternum can be visualized from all sides i.e. anteriorly, laterally and posteriorly. As all work is done at work station the image can be preserved digitally and can be retrieved at any time and can be transferred to anywhere if required (Umap, Bardale, & Shriramwar, 2013).

3.4 Software

3.4.1 Picture Archiving and Communication Systems (PACS)

PACS is a computerized means of replacing the roles of conventional radiological film and when it is installed throughout the hospital, a filmless clinical environment results. The images are acquired, stored, transmitted, and displayed digitally (Strickland, 2000).

3.4.2 Medical Imaging Interaction Toolkit (MITK)

MITK is an open source toolkit for the development of interactive medical image analysis software. MITK is based on the open-source Insight Toolkit (ITK) and Visualization Toolkit (VTK) and extends them with features required for interactive systems. Based on Dolatabadi, Dargazany, & Nouri (2014), MITK is a C++ library for integrated medical image processing and analyzing developed by the Medical Image

Processing Group (guided by Dr. Tian), Key Laboratory of Complex Systems and Intelligence Science, Institute of Automation, the Chinese Academy of Sciences.

They also described the function of ITK and VTK in their article. According to them, ITK is an open-source software system to support the Visible Human Project. Basically it provides developers such tools for image analysis Application domain of ITK is restricted to registration and segmentation in two, three and more dimensions. VTK is a freely available software system for 3D computer graphics, image processing, and visualization used widely around the world. This supports a lot of visualization algorithms such as scalar, vector, tensor, texture and volumetric methods; and advanced modelling techniques such as mesh smoothing and cutting.

3.4.3 MeshLab

MeshLab is an open source, extensible, mesh processing system that has been developed at the Visual Computing Lab of the Institute of Information Science and Technology (in Italian, *Istituto di Scienza e Tecnologie dell'Informazione*) of the Italian National Research Council (CNR) with the help of tens of students (Cignoni *et al.*, 2008). MeshLab presents itself as a mesh viewer application, where a 3D object, stored in a variety of formats can be loaded and interactively inspected in an easy way, by simply dragging and clicking on the mesh itself.

3.4.4 Institute for Data Analysis and Visualization (IDAV) Landmark Software

This Landmark software was developed by the scientists at the Institute for Data Analysis and Visualization (IDAV) and the University of California, Davis. Working together with collaborators at the American Museum of Natural History, the team was able to build an easy to use tool for landmark editing and placement on geometric surfaces typically obtained via laser range scans (Wiley *et al.*, 2005). The main purpose of Landmark is to easily place landmark points and semi-landmark points accurately and with high repeatability on complex surfaces for the purpose of registration, alignment, morphing, and computation of hypothetical ancestors in evolutionary trees.

3.4.5 Notepad++

Notepad++ is a source code editor which supports several programming languages under Windows environment. It's also a lightweight replacement of Notepad software available in Windows ® Operating System by Microsoft ®. Notepad++ was utilized to prepare the source code that consist of the information from the *.dta format files that were saved in the Landmark software previously.

3.4.6 MorphoJ

MorphoJ is an integrated program package for doing geometric morphometrics analysis for two- and three-dimensional landmark data. The goal of the program is to provide a platform for the most important types of analyses in geometric morphometrics in a user-friendly package. MorphoJ is written in pure Java and should therefore run on any computer for which a Java virtual machine is available, including the Microsoft Windows, Apple Macintosh (from OS X version 10.4, "Tiger", onward), and various Linux and Unix platforms.

CHAPTER 4

METHODOLOGY

This project was conducted by employing several applications and software which were utilized in the following order:

a) Picture Archiving and Communication Systems (PACS)
b) Medical Imaging Interaction Toolkit (MITK)
c) MeshLab
d) Institute for Data Analysis and Visualization (IDAV) Landmark Software
e) Notepad++
f) MorphoJ

4.1 Picture Archiving and Communication Systems (PACS)

The process of data collection was carried out with the aid of Radiology Department PACS in Hospital Universiti Sains Malaysia (HUSM), Kubang Kerian, Kelantan. The first step in data collection is to choose the patient data with the appropriate sample requirement. This project required 35 male and 35 female patient data (a total of 70 samples). The samples were randomly selected from the PACS system with due regard to the sample requirements. Ethical approval has been obtained prior to research. The code for the research ethic is USM/JEPeM/1403106. The copy of the ethical approval is attached in the appendices section.

During searching and selecting the samples (patient data), it is important to choose the imaging modality as CT-scan and study description as thorax before hitting the search button. A list of patient data will be displayed and only patients who were referred for thoracic CT examination for various clinical reasons were chosen. Patients having thoracic skeletal deformity or having trauma were excluded. The registration ID of the selected patients were noted for further use. The samples were divided into seven age groups as shown in Table 4.1, which are:

Table 4.1: Distribution of the Samples

	Male Patient Data	**Female Patient Data**
Group A (0-10 years old)	5	5
Group B (11-20 years old)	5	5
Group C (21-30 years old)	5	5
Group D (31-40 years old)	5	5
Group E (41-50 years old)	5	5
Group F (51-60 years old)	5	5
Group G (61 years old and above)	5	5

A routine thoracic CT protocol of the selected samples was followed and axial plane images were obtained and transferred from the PACS to CT-scan room personal computer (PC) workstation for post processing. This was conducted by inserting the patient's registration ID in the search inquiry and the thorax CT-scan image (where the sternum can be found) of the patient will be chosen as the samples. Once the CT-scan image of the patients were completely transferred to the PC, the next step which was the image segmentation of the sternum can be done. However, before proceeding with the segmentation process, the image of the sternum must first be obtained from the selected thorax CT-scan image. The image other than sternum must be cropped out and removed so that only sternum image was left.

The desired sternum image was then segmented to form the multi-planar reformatting (MPR) of the sagittal and coronal plane images performed and three dimensional images of the sternum were obtained so that the anatomical details and ossification centre could be visualized in a better way. The bony anatomy of the sternum was segmented from the CT-scans of each sample using automated operations (thresholding and region growing) and manual editing. The segmentations included the whole parts of sternum consisting of manubrium, mesosternum, and xiphoid process. The images of the sternum were cut and segmented horizontally with slice thickness of 1mm as recommended by Schulz and his associates (2005) in order to achieve the best possible results and ensure maximum accuracy in age estimation practice. After completion of the segmentation process, the images of the sternum were saved and were transferred back to the PACS.

4.2 Medical Imaging Interaction Toolkit (MITK)

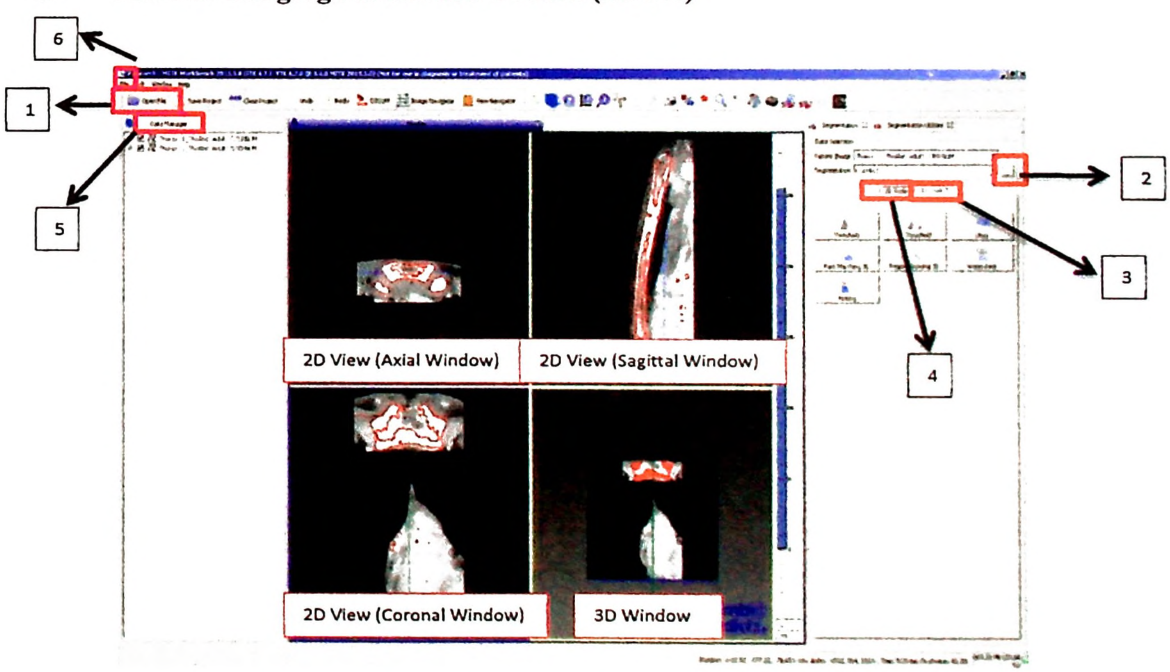

Figure 4.1: Components of MITK

In this project, MITK was employed to clean the three dimensional sternum images from unwanted object or disturbances (e.g. non-sternum bony structure or attached cartilage). As shown in figure 4.1, the four window view is the heart of the MITK image viewing. The standard layout is three 2D windows and one 3D window. The different planes form a crosshair that can be seen in the 3D window. In the 3D window, the object (sternum 3D image) can be rotated by pressing the left mouse button and moving the mouse, zoom either with the right mouse button as in 2D or with the mouse wheel, and pan the object by moving the mouse while the mouse wheel is pressed. The sternum images in the PACS was retrieved and saved to the external hard disk to be utilized for further process using this software. The processes involved are as follows (based on Figure 4.1):

1. Image file of the sternum was loaded into MITK software by clicking button 1 ("Open File") and the location of the saved file previously was browsed.
2. Once the image was loaded to the window, the button 2 was clicked. A small window will be displayed and needed to be filled in with the file name (the name was determined by the researcher and also included the threshold level that needed for the process). Threshold level was inserted by clicking the button 3 and selecting "threshold". Determination of threshold levels to enhance the bone density was also determined by the researchers itself and the default threshold level is 255. However, the researcher should adjust the threshold level according to the quality of the sternum 3D model after applying certain threshold level.
3. After defining the required threshold level, the arrow of the mouse was placed in the axial window and the mouse wheel was scrolled to navigate through the slices of the sternum 3D model. The disturbances or unwanted object in this case is the white material or bony structure that attached to the bony structure of the sternum.