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**Magnitude and reproducibility of smiling
in 12-year-old Caucasian children in the
Republic of Ireland:
A comparison of Class I and Class II malocclusions**

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Thesis submitted to University College Cork in partial fulfilment of
the requirements for DCLinDent (Orthodontics)

June 2018



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ABSTRACT

Aims

- To determine if malocclusion (Class I, Class II division 1 and Class II division 2) influences the magnitude, the immediate intra-session and the short-term inter-session reproducibility of the rest position to posed smile and the rest position to maximal smile.
- To determine if gender influences the magnitude, the immediate intra-session and the short-term inter-session reproducibility of the rest position to posed smile and the rest position to maximal smile.

Materials and Methods

One hundred and ten Caucasian volunteers (55 males; 55 females) aged 12 years, with no previous history of orthodontic treatment, identifiable syndrome or facial asymmetry, were recruited. Three malocclusion categories were assessed: Class I (20 males, 20 females), Class II division 1 (20 males; 20 females) and Class II division 2 (15 males; 15 females). Three-dimensional (3D) images of three facial expressions (rest position, posed smile and maximal smile) of each subject were captured using the Di3D system. These images were repeated 15 minutes later to assess immediate intra-session reproducibility and two weeks later to assess short-term inter-session reproducibility. Twenty-six landmarks were digitally placed on all the images. Landmark identification error was assessed by re-landmarking 10 percent of the images, one month after initial landmarking.

The magnitude of movement from rest to posed smile and from rest to maximal smile averaged over all the landmarks was calculated for each session.

Results

The magnitude of mean movement averaged over all the landmarks differed significantly between rest to posed smile and rest to maximal smile ($p < 0.0001$). This difference was found in both genders ($p = 0.0012$) but was greater in males than in females ($p < 0.0001$).

Immediate intra-session reproducibility ($p = 0.1677$) was high for both rest to posed smile and rest to maximal smile. A statistically significant difference ($p < 0.0001$) of 0.27mm in short-term inter-session reproducibility was found for both rest to posed smile and rest to maximal smile. This was, however, clinically insignificant.

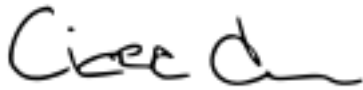
Malocclusion had no effect on magnitude of either smile ($p = 0.8138$) or immediate intra-session reproducibility ($p = 0.3878$) or short-term inter-session reproducibility ($p = 0.3396$). Similar results were found when the 10 lower-face landmarks were assessed independently.

Conclusion

Rest to posed smile and rest to maximal smile differed in terms of magnitude of movement for both genders with males displaying a greater difference. The rest to posed smile and rest to maximal smile demonstrated immediate intra-session and short-term inter-session reproducibility in males and females. Malocclusion had no effect on the magnitude or reproducibility of smiling.

DECLARATION

I hereby declare that the work described in this thesis, except where otherwise mentioned, is my own and has not been submitted previously as a requirement for a degree or diploma at this or any other institution.

A handwritten signature in black ink, appearing to read 'Ciara Ennis', written over a horizontal blue line.

Ciara Ennis

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CHAPTER ONE
INTRODUCTION

This project is focused on the magnitude and reproducibility of smiling within the malocclusion groups Class I, Class II division 1 and Class II division 2 in 12-year-old boys and girls in the Republic of Ireland (RoI).

Studies in orthodontics indicate that 36 percent of 12-year-olds within the RoI are in definite need of treatment for either aesthetic reasons or occlusal anomalies (Whelton et al., 2006). Treatment aims to improve and optimize not only dental and functional occlusion but there is also an increasing emphasis being placed on both smile aesthetics and smile reproducibility. Minimal work has been completed on the magnitude and reproducibility of smiling and there is a complete dearth of studies specifically aimed at investigating 12-year-old children with Class I and Class II malocclusions. This project, therefore, addresses this deficiency within the orthodontic literature.

Class I malocclusion is the most prevalent malocclusion. Within the RoI it has a prevalence of 46.7 percent in 12-year-olds (Whelton et al., 2006). Class II division 1 malocclusion is a common malocclusion to present for treatment with 2.5 percent of 12-year-olds having an overjet of greater than 10mm and 12.2 percent having an overjet of greater than 6mm within the RoI (Whelton et al., 2006). The specific feature of this malocclusion is that the upper incisor teeth are too prominent and there is sometimes a deep overbite. Class II division 2 malocclusion has a prevalence of approximately 10 percent in a Caucasian population (Foster and Day, 1974); there is no data for its prevalence within the RoI. The main features of this malocclusion are that the upper central incisors are retroclined and there is a deep vertical overlap of the lower teeth by the upper

teeth. The effect of prominent or retroclined upper incisors or deep overbite with regard to the magnitude and reproducibility (both intra-session and inter-session) of smiling has not been assessed in 12-year-old boys and girls in the RoI. It is important to ascertain the impact of tooth position with regard to smiling, as it is primarily what is being assessed for treatment planning and being altered by treatment.

The literature review will describe methods of assessing malocclusion before indicating classification and prevalence of the malocclusion groups related to this project. This will be followed by analysis of the smile, including its anatomy and components, the types of smile and the factors influencing smiling. Where possible, links will be drawn to highlight how these aspects affect 12-year-old children. Finally, the 2D, 3D and 4D assessment of smiling in children will be examined.

CHAPTER TWO
LITERATURE REVIEW

2.1 SEARCH METHOD

The electronic search strategy for the following literature review was conducted using the databases summarized in **Table 1**. The search was designed to identify literature related to smiling in children. The references from significant articles were analysed for any relevant studies. Literature was searched from 1900 to 2018 inclusively.

Table 1 Electronic search strategy

Database	Search Strategy/MeSH terms
PubMed	• Imaging, Three-Dimensional • Three-dimensional imaging or 3D and Orthodontics • Smiling and children • Facial expressions and children • Facial expressions and reproducibility • Smiling and reproducibility • Smiling and magnitude • Facial growth • Stereophotogrammetry and children • Facial anatomy • Facial movement • Smiles in children • Smiling in children • 2D assessment of smiling • 3D assessment • Smile line in children • Smile width in children • Smile characteristics in children
Science Direct	Same as PubMed
Embase	Same as PubMed
Google Scholar	Same as PubMed

2.2 MALOCCLUSION

Malocclusion is defined as any deviation from the ideal occlusion and is a term first coined by Edward Angle (1907). As part of this research, it was necessary to screen patients to identify their malocclusion type, decide upon eligibility and allocate patients to a suitable category. Hence, it is appropriate and relevant to describe the history and current trends of assessing malocclusion including both qualitative and quantitative methods.

2.2.1 Methods of assessing malocclusion

The methods of assessing malocclusion will be categorized according to the categories identified by Tang and Wei (1993).

Qualitative methods of recording malocclusion

The earliest methods of recording malocclusion mainly for epidemiologic studies were qualitative methods. Angle (1899) first classified molar relationship in the 19th century and this method has been widely used and accepted ever since. It must be noted that Angle produced this method of classification in order to aid prescription of treatment and not as an index of malocclusion. There are, however, several deficiencies with this method. Case (1921) deemed it to completely overlook the relationship between the face and the teeth. Its reliability was also tested by Gravely and Johnson (1974) with the finding that there were both inter- and intra-examiner errors in categorizing malocclusion type. They concluded a possible explanation for these inconsistencies might be due to asymmetry between left and right sides.

Bjoerk et al. (1964) developed a relatively complex method, which consisted of three parts: anomalies in the dentition, occlusal anomalies and deviations in space conditions. Usefully, the data obtained from this method could be analysed by computer software. Proffit and Ackermann (1973) developed a five-step procedure of assessing malocclusion which included alignment, profile, crossbite, Angle classification and bite depth; this was thought to create a systematic approach for treatment planning. They, however, provided no definitive standard for assessment. Later in the last century, Kinaan and Burke, (1981) described an index that evaluated the following five features of occlusion: overjet, overbite, posterior crossbite, buccal segment crowding or spacing and incisal segment alignment. The three studies mentioned above attempted to record elements of malocclusion that were rationally assembled together, rather than the earlier methods, which tended to indiscriminately record a few malocclusion features. It must be noted, however, that the wide variation in malocclusion assessments in epidemiological studies is due to the use of such qualitative indices. Qualitative methods are rather subjective and enable a broad range of interpretation.

Quantitative methods of measuring malocclusion

An attempt to change the way malocclusion was assessed was established with the development of quantitative methods. One of the earliest was in 1951 by Massler and Frankel, which aimed to assess the total number of rotated or displaced teeth. There was, however, a slight qualitative tendency in this index as the assessment of the tooth displacement and rotation was all or none. Summers (1971) followed on from this, 20 years later in North America, by

developing an occlusal index, which gained good popularity especially for the purpose of research. In this index nine parameters are scored as follows: overbite, overjet, molar relationship, tooth displacement, midline relation, posterior crossbite, posterior open bite, maxillary median diastema and absent upper incisors. Good reproducibility has been found for this index (Summers et al., 1971) and it allows for differing stages of dental development including the deciduous, mixed and permanent dentition.

Linder-Aronson (1974) described the Grade Index Scale (G.I.S.), developed by the Swedish Dental Society and the Swedish Medical Board, which consists of four grades of classifying malocclusion into categories ranging from ‘little need’ for orthodontic treatment to ‘urgent need’. Criteria, however, were found to be ambiguous and vague thus creating grey areas as to which category best represents an individual malocclusion (Shaw et al., 1991).

It was the above index, which was the very foundation for the creation of the Index of Orthodontic Treatment Need (IOTN), developed as a result of a government initiative in the United Kingdom. It consists of two elements – the dental health component and the aesthetic component. In the dental health component, the single worst feature is documented and placed into one of five grades with clear cut-off points, which range from ‘no need’ to ‘very great need’. The aesthetic component is based upon 10 photographs which are graded from score 1 which is the most aesthetically pleasing to score 10 which is the least aesthetically pleasing. The latter component has been criticized for suffering from subjectivity and does not include photographs of Class III incisor

relationship or anterior open bite. According to Shaw et al. (1991), the IOTN is reliable and valid. Cooper et al., (2000) found that IOTN is a reliable index over time and thus provides assurance to orthodontists that a grading noted at age 11 has a high likelihood of remaining unchanged by the age of 19.

The Peer Assessment Rating (PAR) was developed to assess the success and standard of treatment. This index provides a single summary score for the occlusion and the overall alignment, which is based upon the following features: crowding, buccal segment relationship, overjet, overbite and centerlines. According to Richmond et al. (1992), a mean percentage reduction of greater than 70 percent, is an indicator of a high standard of treatment. Shaw et al. (1991) also described the PAR index as being reliable and valid.

The Index of Complexity, Outcome and Need (ICON) was established by Daniels and Richmond (2000) and features of both IOTN and PAR are incorporated. It was intended to be a single index for assessing the start and end of treatment. ICON has been criticized for the large weighting it gives to the subjective aesthetic score and, therefore, reduces its objectivity (Savastano et al., 2003).

The Index of Orthognathic Functional Treatment Need (IOFTN) was developed to reflect the functional indications of treatment need for orthognathic patients not amenable to orthodontic treatment alone (Ireland et al., 2014). This index is, however, not applicable to the present study's cohort of patients and will not be described further.

Descriptive Classification

In 1983 The British Standards Institute (BSI) published a classification in their “Glossary of Dental Terms” which was based upon incisor relationship, with no regard for molar relationship. It is the most widely used of the descriptive classifications. It is also the classification utilized for this study to screen children for eligibility and assignment to one of the following categories: Class I, Class II division 1 and Class II division 2. The BSI definitions for these classifications are listed in **Table 2**.

Table 2 British Standards Institute Classification

Incisor relationship	Definition
Class I	The lower incisal edges occlude with or lie immediately below the cingulum plateau of the upper central incisors.
Class II division 1	The lower incisor edges lie posterior to the cingulum plateau of the upper incisors. The upper central incisors are proclined or of average inclination and there is an increase in overjet.
Class II division 2	The lower incisor edges lie posterior to the cingulum plateau of the upper incisors. The upper central incisors are retroclined. The overjet is usually minimal or may be increased.

It has been argued that this classification struggles to identify borderline cases. Williams et al. (1992) found that four clinicians demonstrated only moderate agreement using the BSI classification of incisor relationship and in 17.5 percent of the cases there was a high level of disagreement between the examiners. They advocated introducing a Class II intermediate group for cases in which the upper incisors are upright but the overjet ranges from 4 to 6 millimetres as they found

this to have good inter- and intra-examiner reliability. This suggestion, however, has not gained widespread acceptance.

The two main indices utilized within the Republic of Ireland and the United Kingdom, aside from the BSI classification, described above are: the Index of Orthodontic Treatment Need (IOTN) and the Peer Assessment Index (PAR). Indices not only offer several practical uses including the estimation of treatment need and the assessment of the standard of treatment, but also offer other advantages. According to Shaw et al. (1991) they ensure uniformity in prescribing patterns, safeguard the patient, act as an aid for patient counselling as well as monitoring and promoting standards.

2.2.2 Prevalence of malocclusion and treatment need

Malocclusion affects a large proportion of society. It is necessary to determine the prevalence or incidence of malocclusion within a population, in order to effectively help in the planning and provision of treatment at the individual or population level.

Many studies have investigated prevalence of malocclusion and the need for treatment within different population groups. This section will aim to highlight relevant aspects to the current project, which includes focus on the prevalence of the malocclusion traits: increased overjet, retroclined upper incisors and deep overbite within 12-year-old children. These features directly relate to Class II division 1 and Class II division 2 malocclusions respectively. Overjet is defined as the extent of horizontal overlap of the maxillary central incisors over the

mandibular central incisors. Retroclined upper incisors are defined as upper front teeth inclined towards the palate. Deep overbite is defined as the excessive vertical overlap of the lower teeth by the upper teeth. Studies from North America, South America, Europe, United Kingdom and RoI will be considered.

In North America, published data from the National Health Examination survey from 1966 to 1970 found that 15 percent of 12 to 17-year-olds had an overjet of 6mm or more and 10.3 percent of youths had an increased overbite (Kelly and Harvey 1977). Both malocclusion traits were statistically more common in white than in black children. In another study conducted in North America by Brunelle et al. (1996), which assessed aged 8 to 50-year-olds, 10 percent had an overjet greater than 6mm and 8 percent had a severe overbite of at least 6mm, compared to the average overbite of 2.9mm.

A South American study conducted in Colombia by Thilander et al. (2001), on a sample of almost 5000 children aged between 5 and 17 years, demonstrated that 88 percent had an anomaly ranging from mild to severe. Increased overjet was noted in 25.8 percent of children but a marked overjet of greater than 6mm was only found in 3.4 percent and was found to be more common in boys. Deep bite was found in 21.6 percent of children, was more prevalent in boys and most common in the late mixed dentition. An overbite greater than 6mm was only found in 1.8 percent of the sample. Overall 14.9 percent had a Class II division 1 malocclusion and 5.9 percent had a Class II division 2 malocclusion. The large age difference in this study could perhaps have affected the results as the assessment ranged from the deciduous dentition to the permanent dentition. It

must also be noted that due to the location of this study, the sample is unlikely to have included any Caucasian children, despite no reference to the ethnic origin of the sample within the study.

Several European studies have been conducted on the prevalence of malocclusion and this section will now focus on studies which had samples of similar age to the sample used in the current project. In Dresden, Germany, Tausche et al. (2004) aimed to estimate the prevalence of malocclusion in 1975 children aged between 6 and 8-years-old using the Index of Orthodontic Treatment Need (IOTN). Their results demonstrated that the most frequent discrepancies affecting the population sample at 27.5 percent and 46.2 percent were overjet and deep bite respectively, both of which were increased by more than 3.5mm. They also noted that with increasing age there was a decline in the percentage of patients recorded with increased overjet and deep overbite, which is in line with growth and development. The sample size in the study was large and the patients' ages were much younger than the age at which orthodontics is usually initiated, a factor, which the authors acknowledged. In a French study of children aged 9 to 12-year-olds conducted by Souames et al. (2006), 28 percent had an increased overjet and 15 percent had an increased overbite. In the increased overbite category, 45 percent had gingival contact and 10 percent had palatal or labial gingival indentation. Notably, the malocclusion status was much lower than that recorded in previous European epidemiological studies.

Within the United Kingdom, Hill (1992) studied the prevalence of malocclusion in cohorts of Glaswegian children aged 9, 12 and 15 years. He found that 13.8 percent had an overjet between 6 to 9mm, 3.7 percent had an overjet of greater

than 9mm and 3 percent had a traumatic overbite. Seventy-two percent of 9-year-olds had a malocclusion which would benefit from orthodontic treatment. Burden et al. (1994) investigated the need for orthodontic treatment within the U.K. and concluded that one third of 11 to 12 -year-olds were in need of treatment, a figure which was mirrored in the U.S., in which 29 percent of 12 to 17-year-olds were assessed to be in need of treatment (Kelly and Harvey, 1977). Overjet greater than 6mm was one of the main occlusal traits within the general population and a major influence of the finding that 33 percent of 11 to 12-year-olds were in need of orthodontic treatment (Burden et al., 1994). Chestnutt et al. (2006), in their study of the orthodontic condition of children within the UK, concluded that 35 percent of 12-year-old children had a definite need for treatment based upon dental health and/or aesthetic grounds. No information, however, on the types of malocclusion was provided in that study.

The most relevant survey to this project is the North South survey of children's oral health in Ireland (Whelton et al., 2006). Thirty-six percent of 12-year-olds were recorded as having a definite need for treatment; 2.5 percent had an overjet greater than 10mm and 12.2 percent had an overjet greater than 6mm. As incisor overjet was the only anomaly documented, no conclusions can be drawn regarding overbite.

The need for orthodontic treatment, however, varies greatly worldwide with estimates ranging from 27.5 percent to 76.5 percent (Richmond 2000). The World Health Organisation (1985) indicated that the orthodontic need in children, aged from 13 to 14-years, was between 21 percent and 64 percent. The

wide disparity could be due to the type of population, gender, age range, the criteria employed to be considered as a severe malocclusion, as well as perhaps a lack of objectivity, poor reliability and validity of the systems utilized for evaluation.

2.3 SMILE

This project focuses on three facial expressions: the rest position, posed smile and maximal smile. In order to understand the elements of these facial expressions, it is necessary to describe both smile anatomy and smile components.

2.3.1 Smile anatomy

The anatomy of a smile is an essential aspect of orthodontics, with importance placed on an in-depth knowledge of the components of the oral cavity. For the clinician to create a well-balanced and aesthetic smile, it is necessary to maintain or establish normal curvature of the lips, desirable exposure of the gingiva, an undistorted philtrum and nasolabial grooves which remain undisturbed, all of which are in harmony with the amount of tooth displayed (Matthews, 1978).

The muscles of facial expression lie within the layers of superficial fascia, providing us with the ability to express a wide range of emotions. Due to their insertions, the muscles tend to move the skin, rather than a joint, when they contract.

Many of the muscles that play a role in smiling originate in a bony structure of the head and their insertion is into the orbicularis oris, which is also referred to as the sphincter muscle of the mouth, whose fibres enclose the opening of the oral cavity. The two muscles which are the notable exceptions are the buccinator and the risorius. The buccinator muscle lies deep to the orbicularis muscle; with its origin from the oral mucosa, pterygoid raphe and mandible; it inserts into the

orbicularis oris (Rubin, 1974). The risorius, meanwhile, originates in the platysma, with its insertion into the commissure (Rubin, 1974). Both of these muscles move the mouth laterally (Cacou et al., 1996).

The muscles of facial expression all work in groups to elevate and depress the upper and lower lips (Rubin, 1974; **Figure 1**). The smile is created by contraction of the levators of the upper lip with concurrent contraction of the depressors of the lower lip and commissure (Bentsianov et al., 2004). The levator labii superioris, the levator labii superioris alaeque nasi and the zygomaticus minor/major are responsible for determining the amount of elevation of the lip that happens during smiling; these three muscles are all part of the upper oral muscle group (Hwang et al., 2009). The depressor anguli oris, which depresses the corners of the mouth, the depressor labii inferioris and mentalis, both of which protrude the lower lip; all three muscles belong to the lower oral muscle group (Vigliante, 2005). Through the combined action of all these muscles the orbicularis oris, which is innervated by Cranial Nerve VII (referred to as the facial nerve) draws the lips and commissures together, either pursing them or flattening the lips against the teeth (Vigliante, 2005).

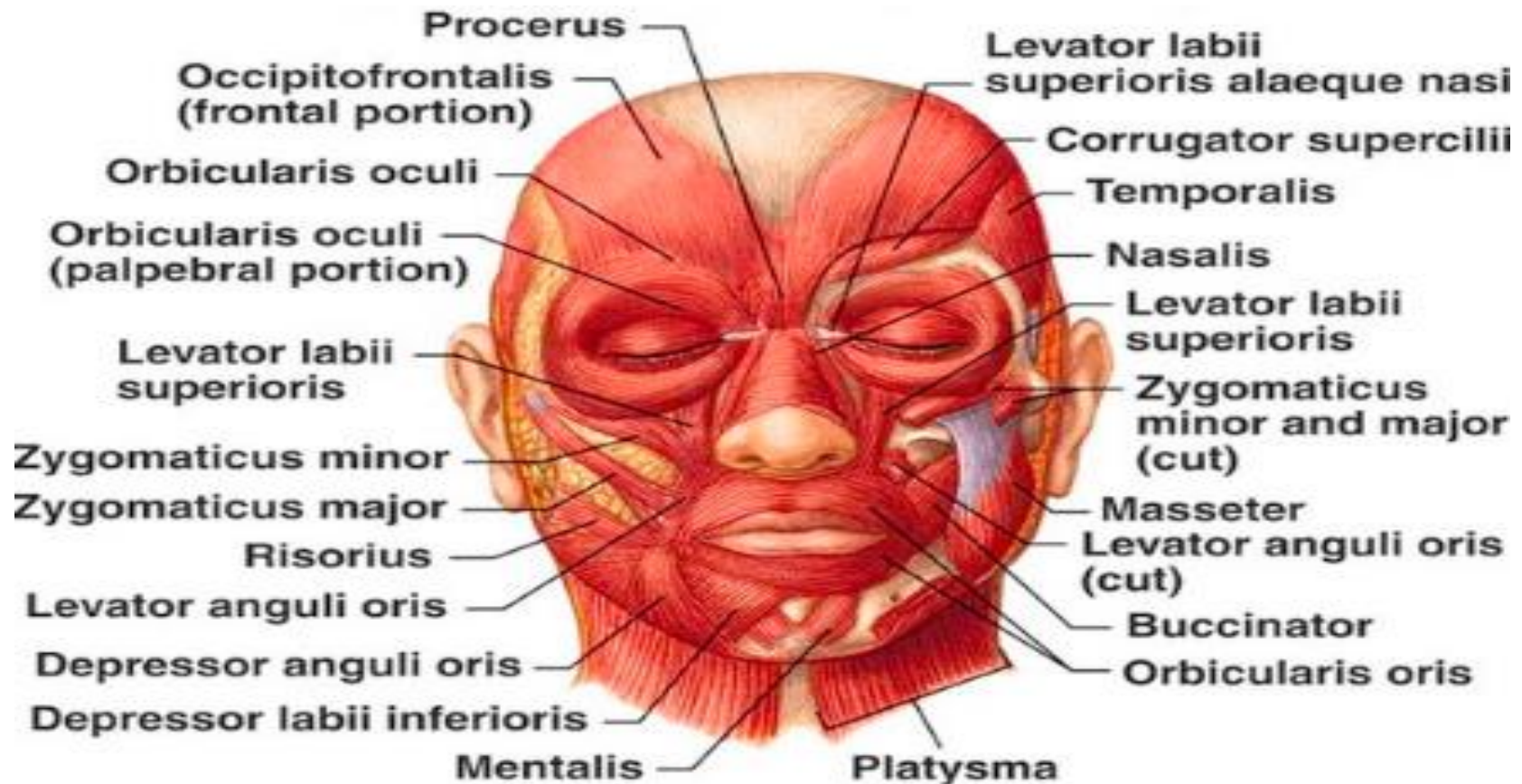


Figure 1 Facial Muscles (reproduced from Pinterest 7/05/2018) <http://digikalla.info/facial-muscles-diagram/facial-muscles-diagram-best-25-muscles-of-facial-expression-ideas-on-pinterest-drawing-ideas/>

2.3.2 Smile components

Beauty is thought to be in the eye of the beholder. An attractive smile is perceived to enhance the social acceptance of an individual and contributes to successful first impressions (Tjan et al., 1984), better calibre of employment prospects and greater financial success (Naini et al., 2006; Klages et al., 2007). The smile is a combination of many attributes, which includes both negative and positive. The lips frame the presentation zone of the smile, bordering the gingiva, the dentition and the space in the oral cavity. Yet, what is it exactly, that defines a ‘beautiful’ smile?

Hulsey (1970) described five basic components of a smile; the smile line ratio, the smile symmetry ratio, the buccal corridor ratio, the height of the upper lip and the curvature. Sabri (2005) some 35 years later aimed to quantify in greater detail what an ideal smile resembles in their paper entitled “The Eight Components of a Balanced Smile”. These comprise the lip line, smile arc, upper lip curvature, lateral negative space, smile symmetry, occlusal frontal plane, dental components and gingival components. This project is not focusing on the individual components of the smile specifically but rather the smile as a whole. Each component will, however, be described below.

Lip line

The lip line is described as the height of the upper lip in relation to the upper central incisors, with the optimal amount of exposure being when the full amount of the central incisor is displayed along with the interproximal gingiva. The lip line is described as being most attractive during smiling when the upper lip is at

the height of the gingival margin of the upper central incisor (Hulsey, 1970). In general, the lip line in females is approximately 1.5mm higher than in males; thus, during maximal smile 1 to 2mm of gingival display in females should be regarded as normal (Peck et al., 1992). The average lip length in females is 20mm and 23mm in males (Sabri, 2005). According to Peck et al. (1992), there is a significant gender difference in upper lip length with females exhibiting a shorter upper lip than males, the mean difference being 2.2mm.

Smile arc

The relationship of the curvature of the incisal edges of the upper incisors and canines to the curvature of the lower lip in the posed smile is known as the smile arc. Frush and Fisher (1958) proposed that the curvature of the incisal edges and the curvature of the upper border of the lower lip should be in harmony. Hulsey (1970) assessed 2D photographs of 40 subjects, 20 orthodontically treated and 20 with a normal occlusion. Subjects with a flatter smile arc were judged to be less attractive. Janson et al. (2011), however, concluded that the smile arc alone is not sufficient to influence the smile aesthetics.

Upper lip curvature

Upper lip curvature is evaluated from the midline to the commissure of the mouth when smiling. Hulsey (1970) found that when the corners of the smile were above the midline of the upper lip, it was rated as an aesthetically pleasing smile. If the corners of the smile were lower than the midline of the upper lip, this was still aesthetic if it contained the other desirable elements of a smile. Tjan et al. (1984) analysed 240 Korean university students with normal occlusion

and found that an upward lip curvature was relatively rare (12 percent), whilst downward (43 percent) and straight (45 percent) were much more common.

Buccal corridors

During a smile, spaces appearing bilaterally between the lip commissure and the buccal surfaces of the upper premolars and molars are known as black spaces, lateral negative spaces or buccal corridors (Ackerman and Ackerman, 2002). Ritter et al. (2006) studied 2D photographs of 60 individuals performing a forced smile. These photographs were evaluated by a panel of two orthodontists and two lay people. Their findings indicated that the mean lateral negative space for each side was 6.68mm. The lateral negative space, however, did not influence the aesthetic evaluations of the smile photographs. Both lay people and orthodontists did not consider the lateral negative space to be a vital element in their aesthetic considerations.

Smile symmetry

Smile symmetry has been defined by Janzen (1977) as being the relative positioning of the commissures of the mouth in the vertical. Symmetry can be assessed clinically by examining the parallelism of commissural and pupillary lines. Hulsey (1970) suggested that an asymmetrical smile, in which there is a large differential elevation, might be a result of muscular tonus deficiency in one side of the face.

Occlusal frontal plane

The front-occlusal plane is described as a line running from the tip of the left canine to the tip of the right canine (Sabri, 2005). A transverse cant can result because of a skeletal asymmetry of the mandible or differential eruption of the upper anterior teeth (Sarver et al., 2003b). Padwa et al., (1997) studied smiling photographs of patients with a documented occlusal cant compared to a control group. A panel made up of four untrained and five trained observers assessed the patient photographs to decide on the presence or absence of canting. The results of this study demonstrated that four degrees is the threshold for 90 percent of observers for recognizing an occlusal cant.

Dental components

An aesthetic smile is dependent upon the quality and attractiveness of the dental components and how these harmonise with the other afore mentioned elements. Dental components include the size, shape, colour, alignment, crown angulation, midline and arch symmetry (Sabri, 2005). The dental midline should coincide with two important anatomical landmarks, which are, the base of the philtrum and nasion. Johnston et al. (1999) investigated the influence of dental to facial midline discrepancies on dental attractiveness ratings. It was 56 percent probable that a layperson would record a less favourable attractiveness score when there was a 2mm discrepancy between facial and dental midlines.

Gingival components

The gingival components, which impact on smile attractiveness, include the gingival height, colour, texture and contour (Sabri, 2005). These form the

gingival scaffold of the smile (Ackerman and Ackerman, 2002). The preservation of papilla in the gingival embrasure of the aesthetic zone is an important consideration in both restorative and orthodontic treatment. Open gingival embrasures can have a big impact on the quality of a patient's smile. Kokich et al. (1999) found that orthodontists rated an open gingival embrasure of 2mm as less attractive than a perceived ideal smile with a regular open embrasure. General dentists and the general population, however, noted that gingival embrasures became unattractive at 3mm.

In conclusion, an aesthetically pleasing smile should possess the following desirable elements: an upper lip that is coincident with gingival margins, with a straight or upward curvature, the upper incisal levels should be parallel with the border of the lower lip, little or no buccal corridors, an occlusal frontal plane and commissural line that parallels the pupillary line with finally dental and gingival components which harmoniously integrate (Sabri et al., 2005).

2.3.3 Types of smile

This study assessed rest position, posed smile and maximal smile in 12-year-old children. The third type of smile, the natural smile was not assessed, but will be considered below for completeness.

Our smile is a valuable aspect of our social interaction and nonverbal communication, projecting positive sentiments such as joy, cheerfulness and comedy and as best described by Darwin (1998), it would appear that we all smile in the same language. By responding with or without a smile, children

communicate to others feelings of happiness or sadness, confidence or uncertainty (Källestål et al., 2000).

2.3.4 Natural smile

The social smile or unposed smile, which can also be referred to, as the Duchenne smile is one that is induced by happiness or laughter and is involuntary (Grover et al., 2015). In contrast to the posed smile, social smiles are not sustained.

Matthews (1978) described how the natural smile begins at the corner of the mouth extending laterally and as the smile continues to expand the corners of the mouth move upwards, exposing the teeth. Tarantili et al., (2005) studied the natural smile in dynamic motion in 15 subjects (6 males; 9 females) aged 10 to 14 years using video. They identified that the social smile has three stages known as the smile cycle: the “initial attack” which consisted of a period from rest position to the full smile, the “sustaining period” in which some subjects exhibited a decline in their smile and finally a “fade-out” in which the rest position is reassumed. The smile extended over the whole face, including contraction of the orbicularis oculi and wrinkling around the orbital region, hence confirming the smile resulted from emotional enjoyment. They concluded that the dynamics of the natural smile raises a convincing argument regarding the validity of utilizing photographs for both diagnosis and treatment planning. Philips (1999) explained, in his classification of smile patterns, that the natural smile is the third stage of four in the smile cycle. The majority of people tend to smile to three-quarters of their full or expanded smile during the natural smile.

It is virtually impossible to repeat the social smile exactly during one photographic session and these odds decrease over a longer period of time (Ackerman and Ackerman, 2002). These authors also demonstrated, that if several consecutive photographs of the natural smile are taken, variations in the smile are obvious. They hypothesized, that in children, this phenomenon is a direct result of late maturation of the social or natural smile. As a result of the difficulties associated with eliciting a natural smile, it was not assessed within this study and instead only posed smile and maximal smile were analysed.

2.3.5 Posed smile

The posed smile is voluntary in nature and is not provoked by any particular emotion. It is the expression usually made when being introduced to someone or posing for a photograph. It is static and hence easily sustainable making it reliably repeatable in nature (Ackerman et al., 1998). Studies, therefore, predominately refer to the posed smile as it can be used as a stable reference point (Sabri, 2005). It is also important in orthodontic diagnosis and treatment planning (Grover et al., 2015).

In previous studies, patients were asked to repeat the phrase “Chelsea eats cheesecake by the Chesapeake”, to relax and then smile in order to evoke the posed smile (Sarver and Ackerman, 2003b). The posed smile may also be elicited by requesting the patient to bite their teeth gently together and say “cheese” and is the method that has been used in a number of studies, including this study (Zachrisson, 1998; Johnston et al., 2003; Campbell et al., 2012; Darby et al., 2015).

2.3.6 Maximal smile

During a maximal smile, the mouth corners turn up to extensively expose the closed front teeth in a broad smile (Tidd and Lockard, 1978). As the description would imply, the maximal smile is performed with the facial muscles at maximum stretch. The maximum smile may be produced by requesting, the patient to bite their teeth lightly together and smile maximally (Johnston et al., 2003; Campbell et al., 2012; Darby et al., 2015). These instructions were adopted in the present study too.

Males smile less expansively and less often than their female counterparts (Otto 1998). They described that females demonstrate more accuracy in the reproduction of a maximal smile. Houstis and Kiliaridis (2009) proposed that children and females use the expression more frequently than males and subsequently have ‘wider’ smiles. As boys mature into adulthood, the maximal smile demonstrates a greater vertical component, which could be due to expression of the male characteristic or the ‘aggressive demeanor’ (Björkqvist, 1994).

2.4 FACTORS INFLUENCING SMILING: AGE, ETHNICITY AND GENDER

This study's sample consisted of 12-year-old boys and girls of Caucasian origin. It is therefore, necessary to ascertain if age, ethnicity and gender have an impact on smiling.

2.4.1 Age

Dimensions of the lips

With the passage of time, soft tissue and skeletal changes occur, the latter having a direct effect on the overlying soft tissues, the connected muscles and their function. Mamandras (1988) studied the effect of growth on the dimensions of lips in untreated male and female subjects from 8 to 18 years. In males, lip thickness achieved its maximum thickness at age 16 and thereafter, began to thin. In females, however, lip thickness reached its maximum by age 14 remaining the same until 16 years and then, similar to males, begins to thin. Hashim et al. (1997) used serial lateral radiographs to study vertical and horizontal linear growth of the upper and lower lips of 27 children aged 3 to 18 years. A 35 percent increase in the length of the upper lip was found in males and a 24 percent increase in females. Bernal de Jaramillo et al. (2015) found that the length and thickness of the lower lip were significantly greater in children in the mixed dentition and in the late mixed dentition stages compared to the deciduous dentition stage. These findings could have an impact on the shift of the smile line as the patient ages from that, of a high smile, to a medium smile.

Upper lip length

By age 7, 88 percent of the growth in the upper lip length is complete in males and 95 percent in females. This would suggest that a short upper lip at age 7 will be present at age 18 and is a significant factor to take into account during treatment planning (Nanda et al., 1990). That study also noted that the average upper lip length increase during growth in males is more than twice that of females (Nanda et al., 1990).

Profile

Facial growth and profile changes were studied by Formby et al. (1994). Male profiles tended to straighten with age with both lips becoming more retrusive; females, however, neither experienced a straightening of the profile nor did the lips become as retrusive as their male counterparts. Hard and soft tissue growth in both males and females continued until between the ages of 25 and 42 years.

Smile line

Aging has an effect, not just on teeth, but also on the periodontium and soft tissues. Higher smile lines that display the entire upper incisors are associated with youth (Van Der Geld et al., 2007). Lip coverage of the maxillary incisors tends to increase with age (Vig and Brundo, 1978), with high smile lines reducing with advancing age (Peck et al., 1992). The high smile line has been found to be predominant in adolescence with no subject in the 15 to 19-year-old category found to demonstrate a low smile line (Desai et al., 2009).

Incisor display

Choi et al. (1995) expanded on Vig and Brundo's (1978) research by measuring the amount of tooth exposure during smiling. Subjects aged between 20 and 30 years showed on average 2mm of mandibular central incisors during smiling and subjects over 60 years displayed 4mm. With advancing age, both lower incisor and gingival exposure increased on smiling (Dong et al., 1999). In relation to the upper incisors, Desai et al. (2009) found that with advancing age, there is an average of 1.57 to 2.03mm less display on smiling.

2.4.2 Ethnicity

McAlister et al. (1998) studied the effect of the lip levator musculature, in a mixed ethnic group, which included 30 Caucasian and 24 Asian undergraduate dental students. Females had higher smile lines than males and the former also had thicker zygomaticus major muscles. With regard to smile height, no statistical difference was found between the two groups in relation to muscle thickness. The thickness of the levator labii superioris or zygomaticus major muscles was not found to influence the height of the smile line.

At rest, Vig and Brundo (1978) found Afro-Caribbeans and Asians to exhibit less of the upper and more of the lower incisors than Caucasians. They hypothesized that these differences would likely also be observed during smiling.

Studies of facial movement differences between ethnicities have found that Europeans generally exhibit larger facial movements than Asians (Tzou et al., 2004). The eyebrow, nose and mouth regions particularly tend to show statistically significantly larger excursions. The eye region is the exception, however, where Asians exhibit a larger excursion of the eyelids. The difference

in facial movement between ethnicities increases with advancing age. This is thought to be due to thinner epidermis in Caucasians (Lee et al., 2002). With age there is a loss of elasticity, stiffening of collagen fibers (Medina, 1997) and the result of long-term exposure to gravity, culminates in larger excursion of landmarks during facial movements.

Whilst all of these studies had adult cohorts, it is important to recognize the impact of ethnicity on facial movements. Although there appears to be no literature to analyse, ethnicity is also likely to demonstrate a difference during smiling in children.

2.4.3 Gender

Extent and direction of smile

Weeden et al. (2001) used 3D techniques in their investigation of the influence of gender during facial movement and found that males had greater movement than females. Maulik and Nanda (2007) showed that females have higher posterior and anterior smile heights, less buccal corridor and a more parallel smile arc than males. Houstis and Kiliardis (2009) found that males have a larger vertical component to their smile whilst females have a more horizontal capacity. Bernal de Jaramillo et al. (2015) found no sexual dimorphism in terms of smile type or smile arc in a group of boys and girls aged 3 to 12 years with a Class I malocclusion.

2.5 THREE-DIMENSIONAL FACIAL IMAGING

Technological advancement of three-dimensional (3D) facial imaging has provided a tool for accurate representations of facial soft tissues (Linney et al., 1989; Kau et al., 2005). It overcomes the significant disadvantage of two-dimensional (2D) imaging methods, which is the evaluation of 3D objects by degrading them to 2D (Ayoub et al., 1998).

The use of 3D facial imaging in orthodontics extends to: the assessment of facial aesthetics and asymmetry, treatment planning, pre- and post-orthodontic assessment of dentoskeletal relationships and evaluation of growth changes (Hajeer et al., 2004). The image can also be used as a communication tool during the consent process by providing a graphic representation of the problem and the treatment required (Johal et al., 2018).

In this project, assessment of smiling was undertaken using 3D facial imaging. In this section, general 3D concepts and systems will be considered briefly before giving an overview of stereophotogrammetry, which was used in the present study.

2.5.1 General 3D concepts

3D images consist of three axes: x-axis (the transverse dimension), y-axis (the vertical dimension) and the z-axis (the anteroposterior dimension). These Cartesian coordinates define a space known as the 3D space in which multi-dimensional data are represented (Udupa et al., 1999).

The 3D image generation involves several steps (Hajeer et al., 2004):

- ‘Modelling’: the object is viewed as a polygonal mesh. A surface is added to the object by placing a layer of pixels known as ‘image’ or ‘texture mapping’;
- Shading and lighting are added;
- ‘Rendering’: the computer converts the anatomical data into a life-like 3D image.

There are a number of 3D facial imaging techniques available (**Table 3**) including 3D cephalometry, 3D CT scanning, 3D laser scanning, Moiré topography, structured light techniques, 3D Facial Morphometry, 3D ultrasonography and stereophotogrammetry (Hajeer et al., 2004). Stereophotogrammetry, is the only technique, which will be described in detail, as it is the technique used in the present study.

Table 3 3D facial imaging techniques

3D method (radiographic based methods)	Limitations
3D Cephalometry	Time consuming, radiation exposure, poor soft tissue definition.
3D CT scanning	High dose radiation, limited resolution of facial soft tissues.
3D Laser scanning	Slow capture time, eye safety protection required, inability to capture soft tissue surface texture.
3D method (vision-based scanning methods)	Limitations
Moiré topography	Does not capture normal facial texture, landmark identification difficult.
Structured light techniques	Face needs to be illuminated: increased capture time, possibility of head movement.
3D Facial Morphometry	Not a true imaging system, no life-like models produced.
3D Ultrasonography	Time consuming, co-operative patients required.
Stereophotogrammetry: described below	

2.5.2 Stereophotogrammetry 3D imaging technique

This technique is capable of accurately reproducing the surface geometry of the face by means of two cameras configured as a stereopair followed by mapping realistic colour and texture data onto the geometric shape resulting in a lifelike rendering (Hajeer et al., 2004; Heike et al., 2010).

These systems offer a number of advantages over previous systems: minimal invasiveness, absence of harmful radiation, ease of use (Kau et al., 2005), speed

of image capture (Lane, 2008) and the 360-degree surface coverage (Heike et al., 2010). The safety and speed of the data acquisition is particularly helpful when working with children (Farkas, 1996).

The Di3D is a stereophotogrammetry system that was used in this project. The system captures two stereo pairs of images (four cameras) and specialist software is used to create a 3D surface using triangulation (www.DI4D.com). The 3D life-like model of the patient's head can be viewed in high definition and measured in three dimensions (Ayoub et al., 1998; Hajeer et al., 2004) on the Di3D viewing software.

In photogrammetric methods, specification of landmarks is required in order to enable the process of measuring the face. This, however, is a challenging undertaking as the face has only some obvious and well-defined landmarks, which are easy to recognize (Tarantili et al., 2005).

Winder et al. (2008) assessed the performance of Di3D surface image system. They found that the system is capable of measuring the same object to a high degree of repeatability, with no evidence of non-uniformity, or non-linear distortion. The mean error was 0.166mm which was considered to be acceptable.

2.6 ASSESSMENT OF SMILING IN CHILDREN

2.6.1 2D, 3D and 4D assessment of smiling in children

This study assessed smiling in 12-year-old Caucasian children using stereophotogrammetry. This section will evaluate studies of relevance. There are 24 studies employing 2D (5 studies), 3D (13 studies) and 4D (6 studies) techniques illustrated in **Table 4** and **Table 5**. Methods utilized include standard two-dimensional photographs, three-dimensional computerised mesh diagram analysis, optical surface scanning, stereophotogrammetry, video and laser scanning. Details of each regarding ethnic origin, sample size, age, malocclusion, expression (s) recorded, numbers of landmarks used and a brief conclusion are also presented in **Table 4** and **Table 5**.

Several general observations can be seen regarding these studies

- Only three studies evaluated 12-year-olds specifically (Kau et al., 2008, McNamara et al., 2008, Ferrario et al., 1999).
- Sample sizes amongst these studies varied from 2 subjects to 918 subjects.
- Malocclusion was only specified in six studies and in four of these, all of the subjects were classified as having a Class I malocclusion.
- Two studies compared children with Class III malocclusion to children with Class I malocclusion (Johal et al., 2018; Krneta et al., 2014).
- 15 of the 3D and 4D studies utilized landmarks.

Table 4 Studies employing 2D photographs for smile assessment in children

Author/Year	Technique	Facial expression Reproducibility assessed (Y/N)	Sample details M:F, Mean Age (SD/Range), Ethnicity Malocclusion	Landmarks	Conclusions/Findings
Lukez et al. (2015)	Facial photographs	Posed smile N	155 patients: 55 M: 100 F 21 years (12-39) Caucasian (Croatian)	None	Subjects not as focused on details of smile as on distinctive malposition of teeth.
Verdecchia et al. (2011)	Facial photographs	Posed smile N	20 patients: 10 M: 10 F 9 years Caucasian	None	Well-aligned teeth viewed more favourably by peers.
Schabel et al. (2009)	Facial photographs	Posed smile N	48 patients: 12-20 years Caucasian (Italian)	None	No objective measure of the smile could predict attractive or unattractive smiles.
Ackerman et al. (1998)	Facial photographs	Posed smile Y: intra-session reproducibility	5 patients: 11.1 years Caucasian	None	Four of five children produced nearly identical unstrained posed smiles consistently.

Peck et al. (1992)	Facial photographs	Rest position Maximal smile N	115 patients: 15.5 years (14.5 years median age) Caucasian	None	Gingival smiles related to: anterior vertical maxillary excess and muscular ability to raise upper lip significantly higher than average when smiling.
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Table 5 3D and 4D techniques for smile assessment in children

Author/Year	Technique	Facial expression Reproducibility assessed (Y/N)	Sample details M:F, Age (SD/Range), Ethnicity Malocclusion	Landmarks	Conclusions/Findings
Johal et al. (2018)	Laser scanner	Rest Position N	25 patients: 11-13 years Caucasian Class III 25 patients: 11-13 years Caucasian Class I	10	Significant detectable differences found in surface facial features of developing Class III subjects.
Toth et al. (2016)	3D scan and lateral cephs	Rest position Posed smile N	110 F 14.05 (1.65) (12-18 years) White (American)	6	Interlabial gap increased as smile index decreased.

Bernal De Jaramillo et al. (2015)	Video	Rest position Posed smile Unforced smile N	122 patients: 3-12 years Class I South American (Colombian)	None	High smile line predominant in children in deciduous and early mixed dentition. Medium smile found in mixed and late mixed dentition. No sexual dimorphism with regard to smile arc or smile type.
Krneta et al. (2014)	Laser scanner	Rest position N	48 patients: 21 M: 27 F 7.1 years (0.8) Caucasian Class III 91 patients (control): 52 M: 39 F 7.3 years (0.7) Caucasian Class I	8	Class III subjects show clinically relevant facial and jaw characteristics in pre-pubertal growth period.
Bugaighis et al. (2013)	3D scan	Rest position N	80 patients: 39 M: 41 F 8-12 years Caucasian Class I	39	Shape analysis confirmed similarities between males and females for shape and form.

Verze et al. (2011)	Laser scanner	Rest position 6 facial expressions (brows lift, frowning, eyes closure, grimace, smile, lip purse) Y: intra-session reproducibility	12 patients: 6 M: 6 F 7-11 years Caucasian	18	Movements characterized by similar displacements in same facial area in all subjects.
Djordjevic et al. (2011)	Laser scanner	Rest position Y: inter-session reproducibility	60 patients: 30 M: 30 F 11.5 years (10-13) Caucasian	21	Facial growth of healthy individuals during adolescence is symmetric.
Sforza et al. (2010)		Rest position N	918 patients: 532 M: 386 F 4-73 years Caucasian	50	Mouth width, width of philtrum, total lip height and lip volumes significantly larger in males than females.
Houstis and Kiliaridis (2009)	Video	Rest position, lip pucker, posed smile N	40 patients: 20 M: 20 F 10.6 years (6.9-12.3 years) Caucasian	7	Vertical characteristics in facial expressions not established in children.
Kau et al. (2008)	Laser scanner	Rest position (over a 2 year period) Y: inter-session reproducibility	59 patients: 33 M: 26 F 12.14 years Caucasian	5	Surface changes greater in boys than girls, boys exhibit more changes later. Eyes deepen and cheeks become flatter.

McNamara et al. (2008)	Video	Posed smile N	60 patients: 27 M: 30 F 12.5 years North American white descent		Vertical lip thickness was most influential variable in smile aesthetics.
Kau et al. (2006)	Laser scanner	Rest position N	72 patients: 42 M: 30 F: 11.8 years Caucasian	None	Difference between average male and female face was 0.460 +/- 0.353mm.
Miyakawa et al. (2006)	Video	Rest position Posed smile Lip pursing Y: intra-session reproducibility	18 patients: 7 M: 11 F 5.5 years East Asian (Japanese)	11	Lip pursing and smiling are facial expressions with high degrees of reproducibility.
Kau et al. (2005)	Laser scanner	Rest position (over a 6 month period) Y: inter-session reproducibility	2 patients: 2 F 11.6 years British Caucasian Class I	5	Changes in height and weight correlated with changes in facial morphology.

Tarantili et al. (2005)	Video	Rest position Spontaneous smile N	15 patients: 6 M: 9 F 10.5 years 7-14 years Caucasian (Greek)	4	Upper lip elevated 28 percent relative to rest position and the mouth increased in width by 27 percent. Corners of the mouth moved laterally and superiorly at an angle of 47 degrees.
Ackerman et al. (2004)	Video	Maximum smile Posed smile N	50 patients: 27 M: 23 F 12.5 years (10.6-14.6 years) Class I White	None	Lineaments of anterior tooth display at speech and posed social smile should not be recorded independently but evaluated as part of a dynamic range.
Nute et al. (2000)	Optical surface scanner	Rest position N	132 patients: 72 M: 60 F 5-10 years British Caucasians Skeletal I	15	Mid-face prominence and width changed little with age, whilst the prominence and width of lower face increased more.

Ferrario et al. (1999)	3D computerised mesh diagram analysis	Rest position N	534 patients: 263 M: 271F 6-15 years Caucasian (Italian)	22	Male faces on average had a larger forehead, longer and more vertical nose, more inferior and posterior gonion, more inferior and prominent lips, a larger mouth than female faces of corresponding age.
Burke, (1971)	Stereophotogr- ammetry	Rest position N	48 patients: 24 M: 24 F 7.65-19.67 years	8	Appeared to be a slight tendency for left side of maxilla to be larger.

Expressions recorded in 2D

Rest position, posed smile and maximal smile have been assessed using 2D photographs (Peck et al., 1992; Ackerman et al., 1998)

Expressions recorded in 3D and 4D

The most common expression recorded in 17 of the 3D and 4D studies was the rest position (Johal et al., 2018; Toth et al., 2016; Bernal De Jaramillo et al., 2015; Krneta et al., 2014; Bugaighis et al., 2013; Verze et al., 2011; Djordjevic et al., 2011; Sforza et al., 2010; Houstis and Kiliaridis, 2009; Kau et al., 2008; Kau et al., 2006; Miyakawa et al., 2006; Kau et al., 2005; Tarantili et al., 2005; Nute et al., 2000, Ferrario et al., 1999; Burke, 1971). Posed smile was assessed in four studies (Bernal De Jaramillo et al., 2015; Verze et al. 2011; Houstis and Kiliaridis, 2009; Miyakawa et al. 2006). Unforced or spontaneous smile was assessed in two studies (Bernal De Jaramillo et al. 2015; Tarantili et al., 2005). None of the studies assessed all three positions together.

2.6.2 Magnitude of smiling in children

The study reported here assessed the magnitude of smiling in both male and female children. Magnitude is defined by the Oxford English Dictionary as “the great size or extent of something”. This applied to smiling includes both the inter-commissural width, also referred to as the smile width and the inter-labial gap. Inter-commissural width is described by Tjan et al., (1984) as the distance from external corner to external corner. They also outlined the description of

inter-labial gap as the distance between the lowest portion of the upper lip tubercle and the deepest point of the midline at the top margin of the lower lip.

2D assessment of the magnitude of smiling

One study (Schabel et al., 2009) assessed posed smiles from 2D photographs in subjects aged 12 to 20 years with a Class I malocclusion. These were rated by a panel of orthodontists and the subjects' parents. Those rated with an attractive smile had a smile width of 59.4mm and an interlabial gap of 11.7mm. Those rated as possessing an unattractive smile had a smile width of 58.3mm but had a larger interlabial gap of 12.2mm. The latter factor was one of the main contributors for an "extremely unattractive smile" and was due to excessive height of the smile or a deficient smile width. The nature of this study, however, was rather subjective.

3D and 4D assessment of the magnitude of smiling

Ackerman et al., (2004) used 12-frame image sequences from digital video streams of Class I subjects with a mean age of 12.5 years to analyse posed smiles. They found that the inter-labial gap was 8.66mm for boys and 8.11mm for girls and the inter-commissural width was 50.33mm for boys and 48.28mm for girls.

Bernal De Jaramillo et al., (2015) used video to assess the posed unforced smile in children aged 3 to 12 years with a Class I malocclusion. They found that the inter-commissural width increased incrementally from 48.99mm in the deciduous

dentition to 58.95mm in the late mixed dentition. Similarly, the inter-labial gap also increased with age but not as dramatically, measuring 7.70mm at the deciduous stage and 9.31mm in the late mixed dentition. Magnitude also demonstrated sexual dimorphism with lower values in females than in males across all age groups. Females repeatedly demonstrated a smaller inter-labial gap and inter-commissural width than their male counterparts (Ackerman et al., 2004; Bernal De Jaramillo et al., 2015).

2.6.3 Intra- and inter-session reproducibility of facial expressions in 2D, 3D and 4D

The present study assessed both intra- and inter-session reproducibility of rest position, posed and maximal smile. The term ‘reproducibility’ as defined by the Oxford English Dictionary is: “The extent to which consistent results are obtained when an experiment is repeated”. To fully understand what equates to being reproducible, it is necessary to ascertain the measurement of change which can be accepted before recordings can no longer be termed reproducible. Trotman et al., (1996) reported that the range of coordinates for 3D landmarks assumed to be stable was $0.6 \pm 0.6\text{mm}$. Whilst intra-session and inter-session reproducibility of rest, posed and maximal smile have been evaluated in adults (Trotman et al., 1996; Johnston et al., 2003; Campbell et al., 2012; Darby et al., 2015), few studies have been conducted in 12-year-olds.

Intra-session reproducibility

Two studies assessed intra-session reproducibility; one in 2D and one in 4D.

Ackerman et al. (1998) analysed reproducibility of the posed smile in 11-year-olds, within the same session by capturing two photographs of each subject and then comparing the results. They found that four out of five of the children demonstrated a remarkable ability to produce nearly identical unstrained smiles.

Miyakawa et al., (2006) used video to record intra-session reliability of the rest position, lip pursing and posed smile in 5-year-olds over a period of 10 seconds of recording. This was then repeated twice within the same session. They indicated that the 'mandibular rest position' or 'centric occlusion' is an excellent facial expression to instruct the subject to assume as a reference expression for measurement of soft tissue movements. Posed smile was found to be the second most reproducible position but a head restraint was used to prevent any unwanted movements. This created an unnatural environment and perhaps the child did not produce a relaxed expression.

Inter-session reproducibility

Four studies compared inter-session reproducibility, all of which utilised 3D techniques. Kau et al., (2005) recorded 3D scans over two time periods, six months apart in 11-year-olds whilst Kau et al., (2008) repeated 3D scans five times over a two year period in 12-year-olds, each scan was six months apart. Both of these studies only assessed the rest position.

Using a head and face colour 3D scanner to assess subjects aged 7 to 11 years compared to an adult cohort, Verze et al., (2011) recorded facial scans several times over a few days. A number of facial expressions including rest position, brows lift, frowning, eyes closure, grimace, smile and lip purse were recorded. They found differences between the left and ride side of the face in children, which was not evident in the adult cohort of patients; differences were particularly evident in the middle and lower parts of the face. Adults were much more repetitive and symmetric in the direction of their movements than children. Djordjevic et al., (2011) repeated laser 3D scans of the rest position, after 2.5 years and 4.5 years in children with a mean initial age of 11.5 years. Facial growth was symmetric during adolescence in healthy individuals.

CHAPTER THREE

AIMS AND NULL HYPOTHESES

3.1 AIMS

1. To determine if different types of malocclusion; Class I, Class II division 1 and Class II division 2 influence the magnitude of the rest position to posed smile and the rest position to maximal smile.
2. To determine if different types of malocclusion; Class I, Class II division 1 and Class II division 2 influence the immediate intra-session reproducibility or the short-term inter-session reproducibility of the rest position to posed smile and the rest position to maximal smile
3. To determine if gender influences the magnitude of the rest position to posed smile and the rest position to maximal smile.
4. To determine if gender influences the immediate intra-session reproducibility or short-term inter-session reproducibility of the rest position to posed smile and the rest position to maximal smile.

3.2 NULL HYPOTHESES

1. Malocclusion has no effect on the magnitude of the rest position to posed smile and the rest position to maximal smile.
2. Malocclusion has no effect on the reproducibility of the rest position to posed smile and the rest position to maximal smile.
3. Gender has no effect on the magnitude of the rest position to posed smile and the rest position to maximal smile.
4. Gender has no effect on the reproducibility of the rest position to posed smile and the rest position to maximal smile.

CHAPTER FOUR
MATERIALS AND METHODS

4.1 STUDY DESIGN

Ethical approval was obtained from the Clinical Research Ethics Committee of the Cork Teaching Hospitals, which included the protocol submission form, consent form and detailed protocol including instruments involved (**Appendix A** and **B**).

4.1.1 Recruitment

Volunteers were recruited from orthodontic and paediatric dentistry clinics in Cork University Dental School and Hospital (CUDSH) and from the orthodontic treatment waiting list. Volunteers were also recruited from dental clinics in the North Cork area of HSE South, most notably Blackrock Hall Primary Care Centre and the Cope Foundation Clinic. Children of staff members within these clinics were also asked to volunteer.

4.1.2 Inclusion Criteria

The inclusion criteria were as follows:

- Caucasian males and females
- Aged 12 years
- Willing to attend for 3D images on two occasions
- No history of orthodontic treatment
- Malocclusion groups (as defined by the British Standards Institute 1983):
 - Class I
 - Class II division 1
 - Class II division 2

4.1.3 Exclusion criteria

The following criteria excluded a subject from the study:

- Non-Caucasian
- History of congenital orofacial clefting
- Subjects with suspected or identifiable syndromes, facial deformity, muscular disorders or palsy, trauma, burns, paralysis, scars, skin disease, surgery of the facial region or gross facial asymmetry
- Previous orthodontic treatment
- Those with a malocclusion other than Class I, Class II division 1 or Class II division 2 incisor relationship.

4.1.4 Method

For those who met the above inclusion criteria, written information leaflets were provided which detailed the purpose of the study and the procedure involved.

Written informed consent was obtained from the parent or guardian of each volunteer. Personal contact details were obtained from each parent or guardian in order to arrange a suitable time for the 3D scans. Each parent or guardian received a text reminder two days before and a phone call one day before their appointment to allow for any change required and to attempt to increase the attendance rate. The date of birth, malocclusion category and overjet were also recorded at recruitment. The overjet was again carefully recorded on the day of the 3D scan, using a stainless-steel orthodontic ruler held parallel to the floor, with the subject seated upright and the teeth in maximum intercuspation. Each subject was paid €10 for each visit.

4.1.5 Sample size calculation

The research question, the study design and the difference to be detected were considered to calculate the sample size. A previous study has used a sample of 15 patients to detect a meaningful difference of 1.5mm between mean movements of landmarks between the test and control groups (Johnston et al., 2003). A sample size of 30 (15 males and 15 females) in each group was, therefore, determined to detect differences of the order of 1.5mm between similar expressions at $p < 0.05$. Where possible, to allow for attrition, 20 subjects would be recruited to each group.

4.2 EQUIPMENT

4.2.1 Imaging technique

A stereophotogrammetric camera system (Di3D) was used for the imaging in this study (Ayoub et al., 2003). This facial image capture system utilizes standard digital still cameras and normal photographic flash illumination to create instant simultaneous accurate ultra-high resolution, stereo pairs of images of a subject. Dimensional imaging software processes the images, creating a map image, which combined with the original image forms a high definition 3D surface image. This system is capable of measuring the same object to a high degree of reproducibility (Winder et al., 2008).

The stereophotogrammetry system (www.di4d.com/systems/Di3d-system) in CUDSH is set up in a designated 3D imaging room with four cameras (two pairs) connected to a computer.

The imaging system consists of (**Figure 2**):

- Four 10-megapixel cameras (Canon 1000D Digital cameras 50mm lenses)
- Two Esprit 500DX digital flashes
- Two pairs of cameras mounted 85 cm apart and converging at 97 cm from the subject being imaged
- Supporting stand for the cameras
- Dell computer using imaging software (Di3DCapture™)



Figure 2 Di3D system

4.2.2 Calibration

To ensure consistent imaging quality and prior to image capturing, the system was calibrated using a target (a number of circles of known sizes located at known distances apart on a white card) (**Figure 3**). This determined the image centres and orientation of each camera to the other and the location of the focal length along with the intrinsic camera parameters. The calibration process was performed at the beginning of each week.

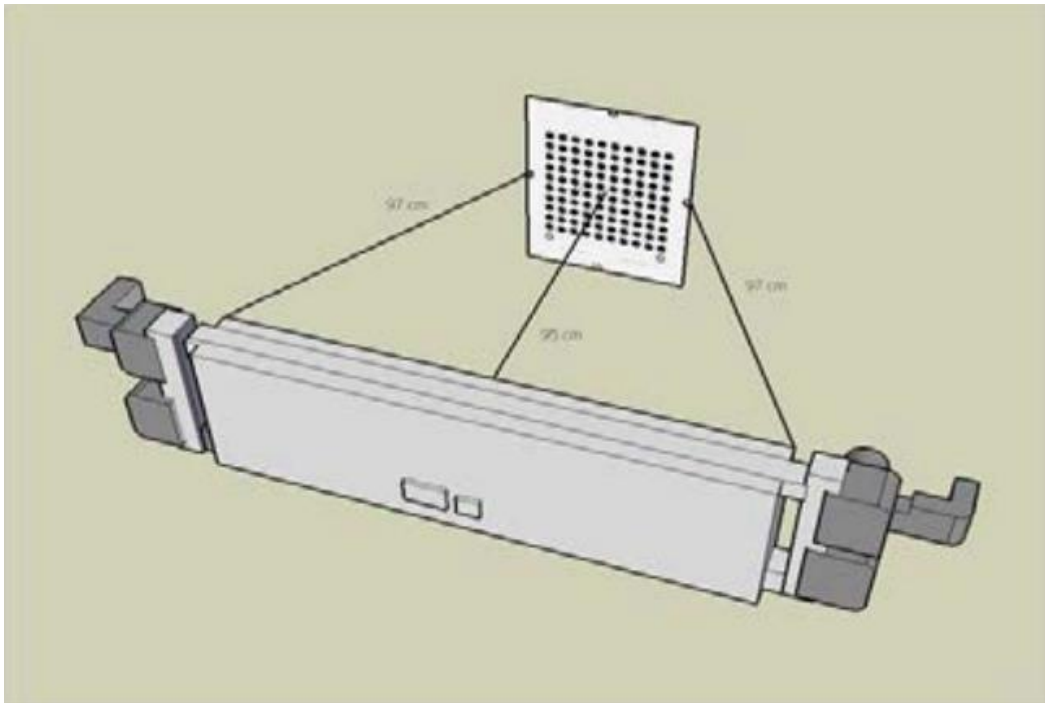


Figure 3 Illustration of the target placement to a pre-set distance from the camera for accurate calibration

4.2.3 Image capture

All 3D images were taken by one experienced operator. The subjects were seated on a chair directly in front of the camera system at a distance of 95 cm. Each subject sat in an upright and comfortable position with the head in the

natural position. Subjects were asked to look straight into a mirror and to remain still. A trial image was taken to familiarise the subject with the equipment.

Verbal explanations of the three facial expressions: rest position, posed smile and maximal smile were given. The positions were performed, as per Zachrisson's (1998) instructions, to elicit the correct movement and to maximise the likelihood of reproducibility:

- Rest position: say 'Mississippi', then swallow and say 'N'
- Posed smile: Bite teeth gently together and say 'Cheese'
- Maximal smile: Bite teeth gently together and smile maximally.

Subjects practiced the positions twice before images were taken to ensure the same lip position was achieved. Following image capture, the computer software created the 3D models in approximately 120 seconds, allowing for immediate detection of any errors within the system or image blurring. Once viewed and checked, images were saved for future processing.

4.3 DATA COLLECTION

A total of 12 images were captured per subject (**Figures 4, 5, 6**).

Session 1: First Capture:

- Three facial expressions (rest, posed smile and maximal smile) were recorded.

Session 1: Second Capture:

- Following a rest period of 15 minutes, images of the three facial expressions were recorded.

Session 2: First Capture:

- Two weeks later at the same time morning or afternoon, the three facial expressions were recorded.

Session 2: Second Capture:

- Following a rest period of 15 minutes, images of the three facial expressions were recorded.

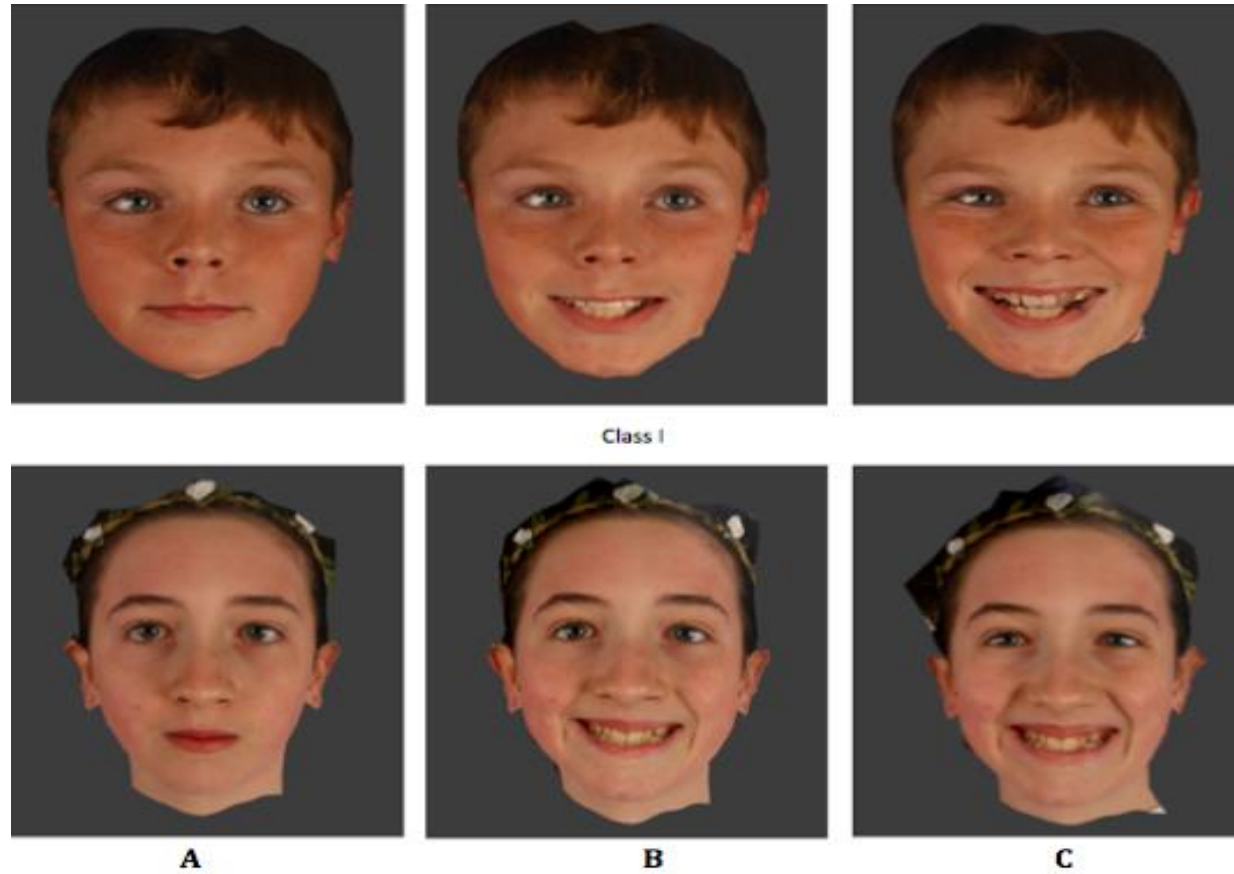


Figure 4 Facial expressions recorded on a male and female subject with a Class I malocclusion
A. Rest position **B.** Posed Smile **C.** Maximal Smile

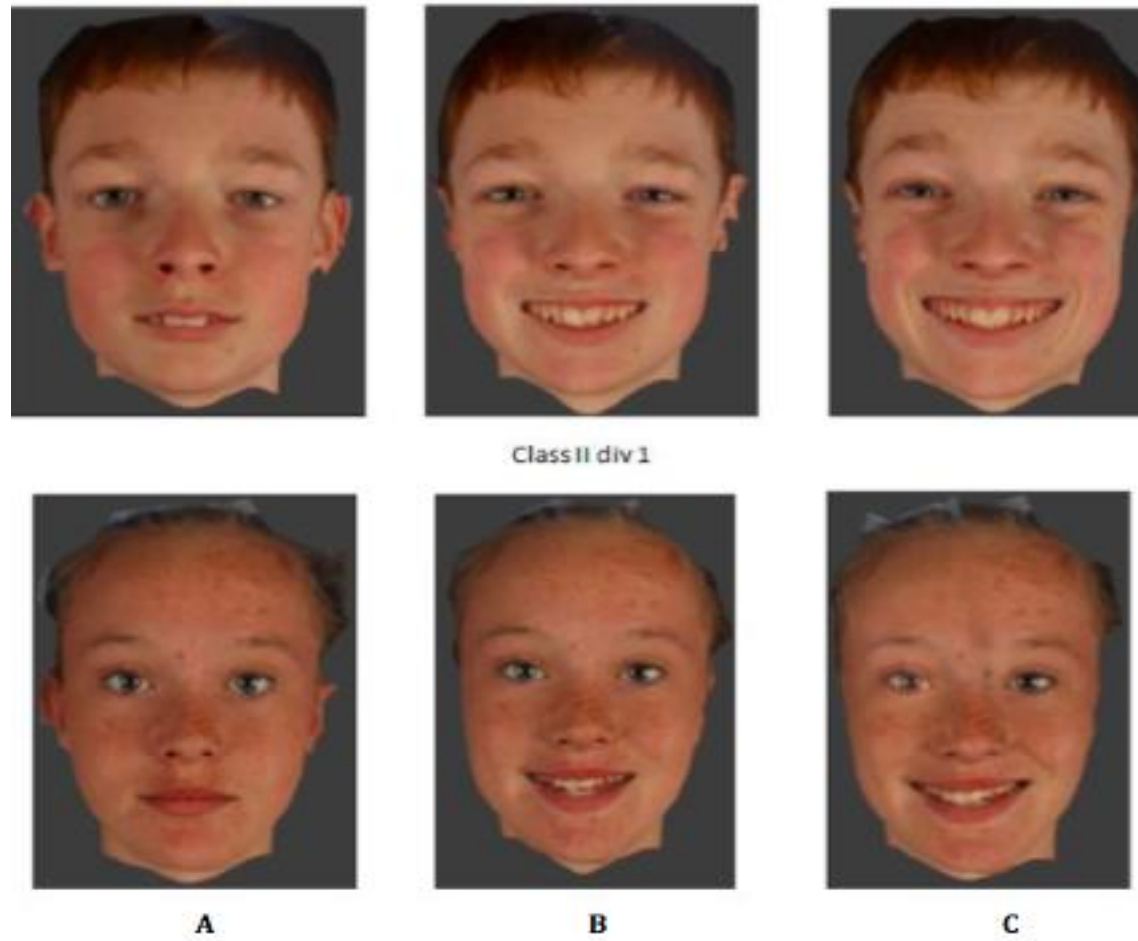


Figure 5 Facial expressions recorded on a male and a female subject with a Class II division 1 malocclusion
A. Rest position **B.** Posed Smile **C.** Maximal Smile

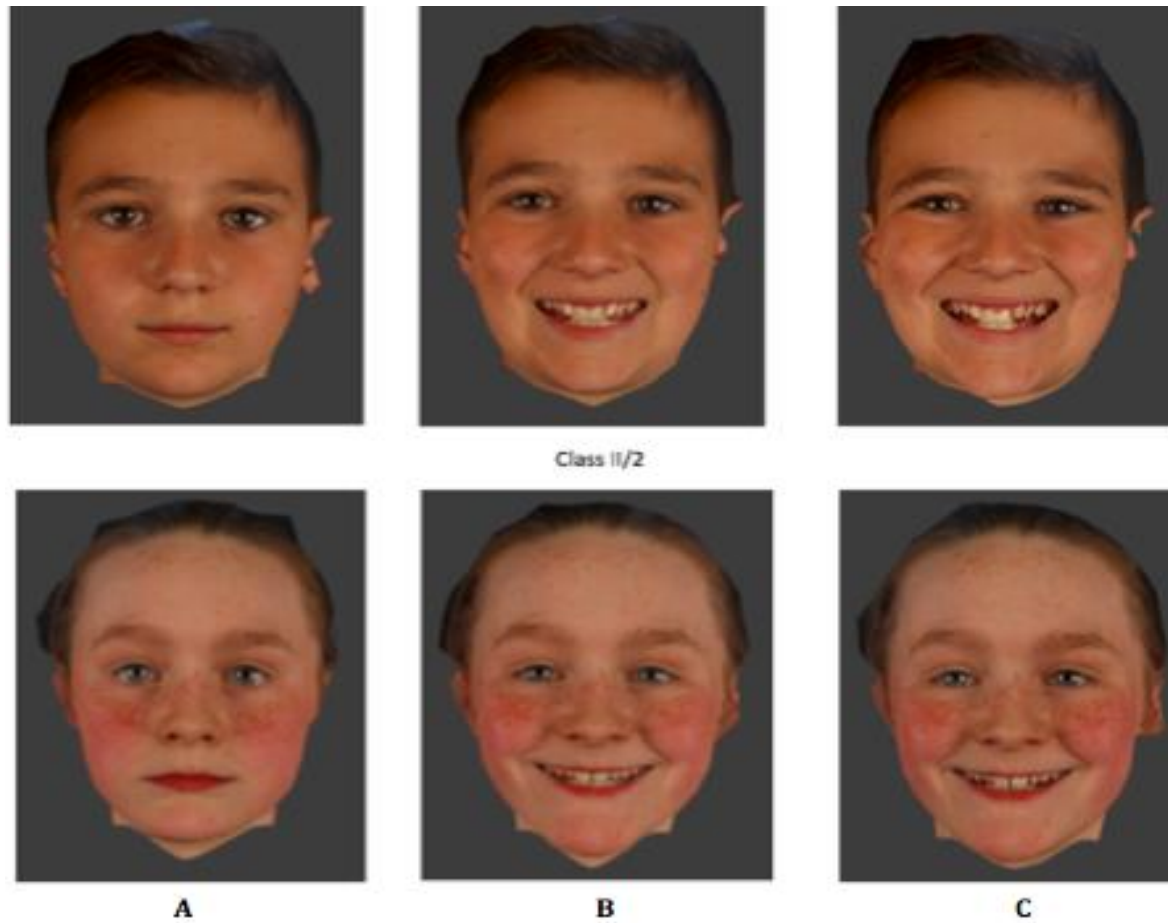


Figure 6 Facial expressions recorded on a male and a female subject with a Class II division 2 malocclusion
A. Rest position **B.** Posed Smile **C.** Maximal Smile

4.4 MEASUREMENTS

4.4.1 Image processing

Following calibration, the centre of what is termed the “world axis” is set as the calibration target centre. When an image is captured, the centre of the computer screen displays this “world axis” and the image appears relative to this “world axis”.

The images were then scaled to the same size and translated, using the best fit algorithm known as the Iterative Closest Points (ICP) technique (Hajeer et al., 2004). For intra-session and inter-session comparison, the images were superimposed on an area of the face, which is unlikely to change position, for example the forehead (Rana et al., 2011). The images were superimposed in all three planes of space.

4.4.2 Landmarks

A single experienced operator recorded the 3D coordinates for the 26 landmarks placed on each image (Farkas et al., 1980). Three coordinates (X, Y and Z) determined the position of each landmark. A random sample of 10 percent of the images were re-landmarked one month later by the same operator to assess intra-observer reproducibility by comparing the 3D coordinate values with the originals (Johnston et al 2002). The facial landmarks used in this study were those used in previous smile reproducibility studies which are described below (Johnston et al., 2003; Campbell et al., 2012; Darby et al., 2015); these were also suggested by Farkas (1994). These landmarks are listed in **Table 6** and indicated

on a full-size image and on a subject from each malocclusion group in **Figures 7, 8, 9, 10.**

Table 6 3D landmarks and definitions

Landmark	Definition
Glabella (g)	Most prominent midline between the eyebrows
Soft tissue nasion (n)	Point in the midline of both the nasal root and nasofrontal suture
Exocanthion right (exR)	Outer most point on commissure of eye fissure on the right
Exocanthion left (exL)	Outer most point on commissure of eye fissure on the left
Mid pupil right (mpR)	Centre point of the right pupil
Mid pupil left (mpL)	Centre point of the left pupil
Orbitale right (oR)	Lowest point on the lower margin of the right orbit
Orbitale left (oL)	Lowest point on the lower margin of the left orbit
Endocanthion right (enR)	Inner most point on the commissure of eye fissure on the right
Endocanthion left (enL)	Inner most point on the commissure of eye fissure on the left
Pronasale (prn)	Most protruded point of the apex nasi
Subnasale (sn)	Midpoint of the angle where lower nasal septum and lips meet
Alar crest point right (acR)	Most lateral point in the curved base line of the ala on the right
Alar crest point left (acL)	Most lateral point in the curved base line of the ala on the left
Alare right (alR)	Most lateral point on the alar contour on the right
Alare left (alL)	Most lateral point on the alar contour on the left

Cheilion right (chR)	Outermost point of lip commissure on the right
Cheilion left (chL)	Outermost point of lip commissure on the left
Christa philtri right (cphR)	Point on elevated margin of the philtrum just above vermillion line on the right
Christa philtri left (cphL)	Point on elevated margin of the philtrum just above vermillion line on the left
Labiale superius (ls)	Midpoint on the upper vermillion border
Labiale inferius (li)	Lower border of lower lip
Lower lip right (llR)	Midway between cheilion right and labiale inferius
Lower lip left (llL)	Midway between cheilion left and labiale inferius
Sublabiale (sl)	Lower border of the lower lip or the upper border of the chin
Pogonion (pg)	Most anterior midpoint on the chin

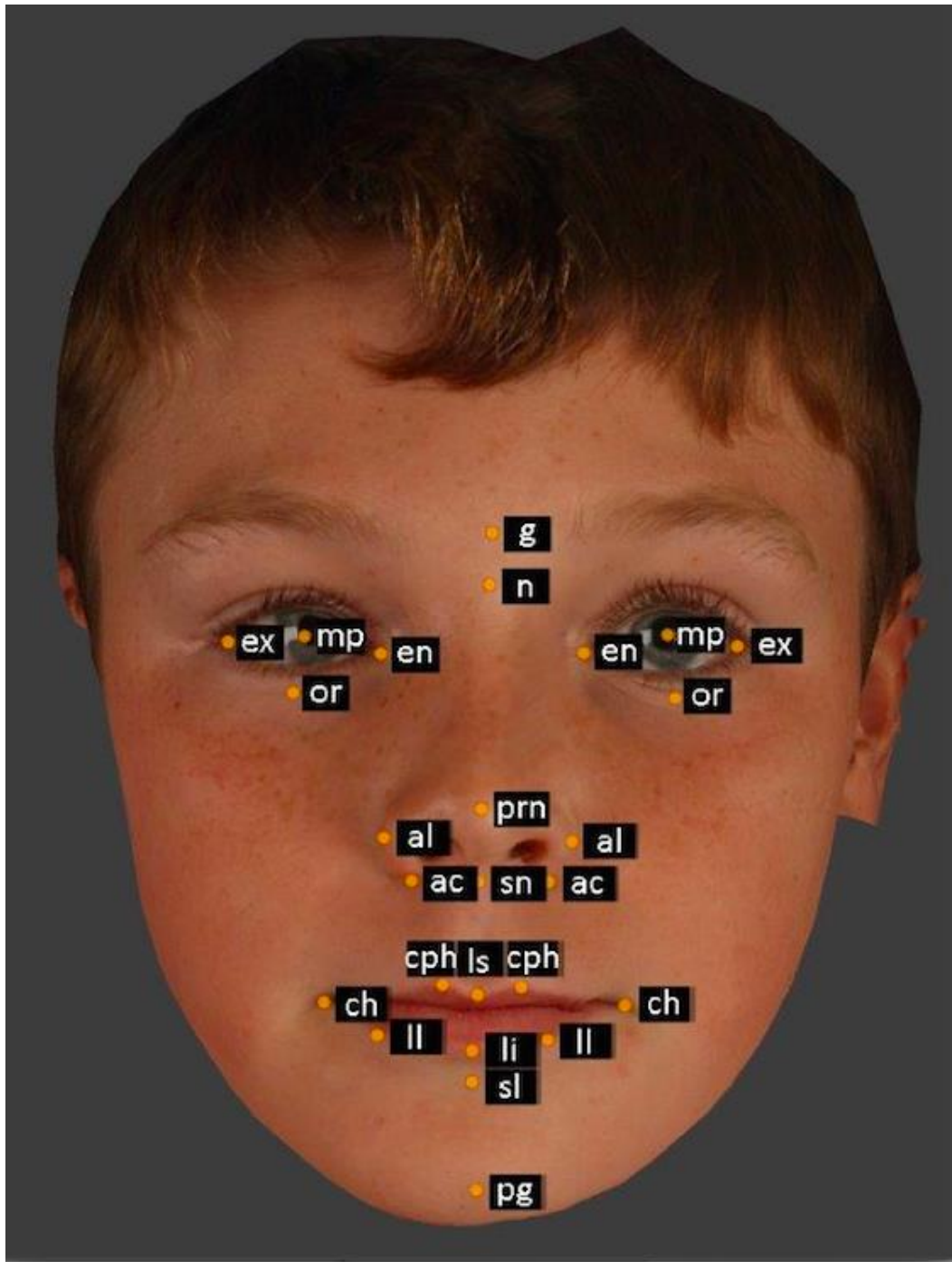


Figure 7 Facial Landmarks shown on an image of a male subject

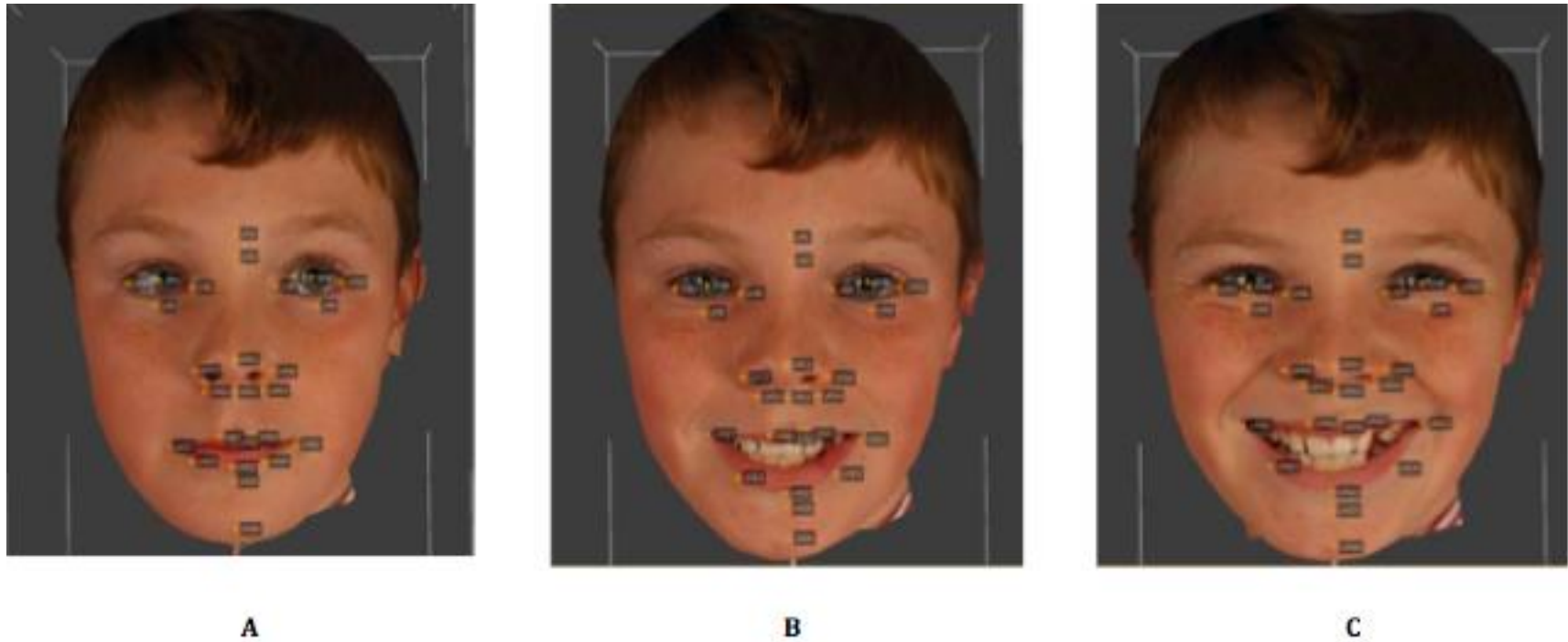
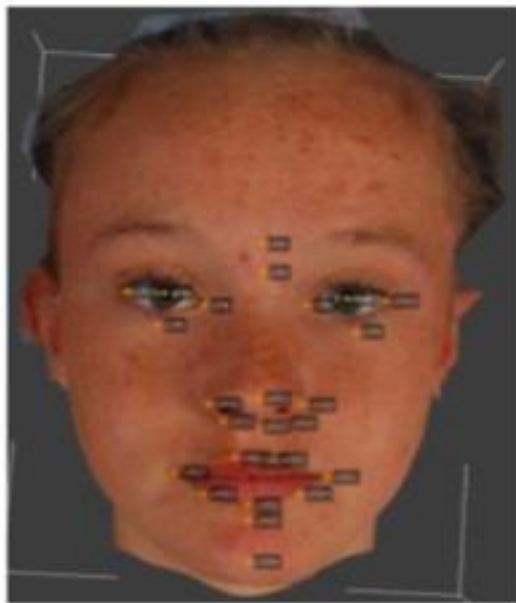


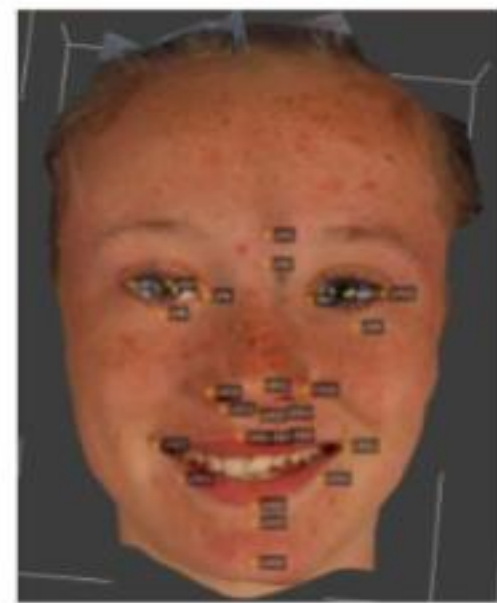
Figure 8 Facial Landmarks shown on an image of a male subject with a Class I malocclusion
A. Rest position **B.** Posed Smile **C.** Maximal Smile



A



B



C

Figure 9 Facial Landmarks shown on an image of a female subject with a Class II division 1 malocclusion
A. Rest position **B.** Posed Smile **C.** Maximal Smile

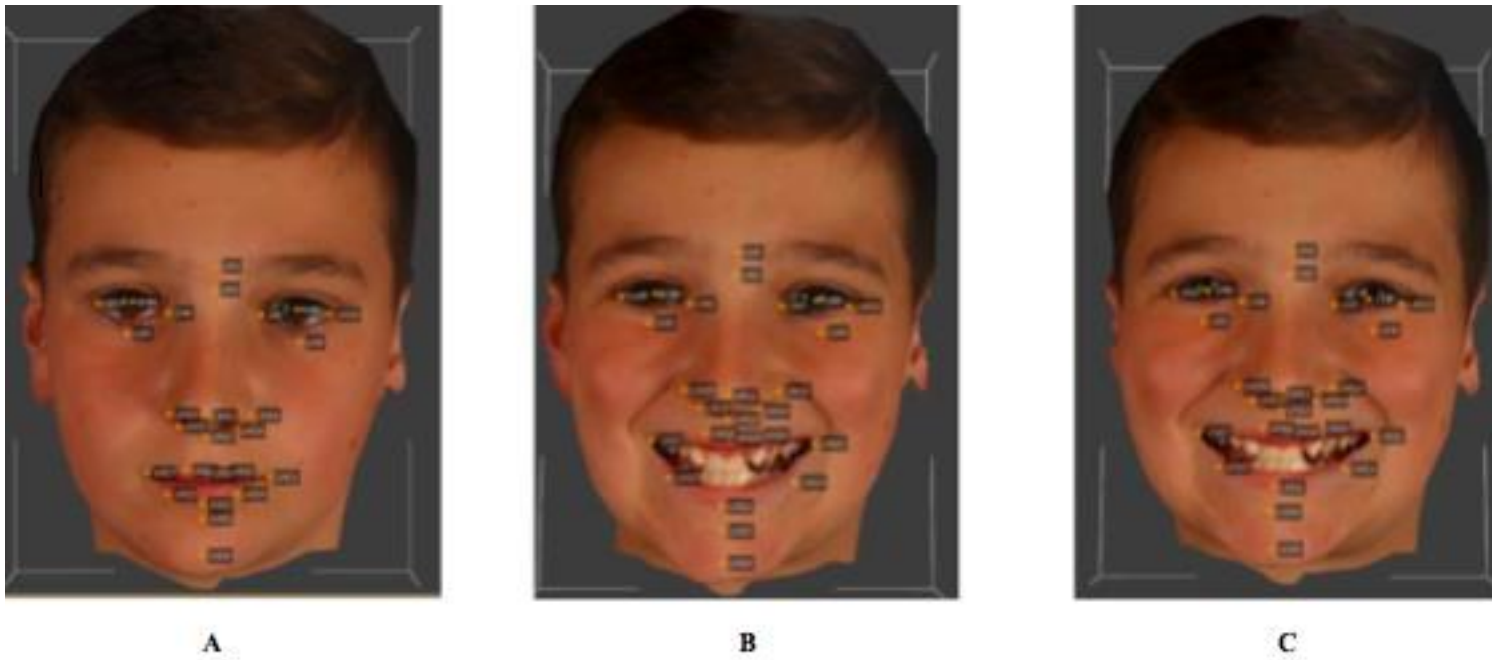


Figure 10 Facial Landmarks shown on an image of a male subject with a Class II division 2 malocclusion
A. Rest position **B.** Posed Smile **C.** Maximal Smile

4.5 STATISTICAL ANALYSIS

The movement data were analysed using a linear mixed-effects model. An analysis was performed averaged over all the landmarks and also averaged over the 10 lower-face landmarks only. Malocclusion, Capture (A, B), Session (1,2) and Expression (rest to posed smile, rest to maximal smile) were all included as fixed factors. The level of significance used was 5 percent and all statistical analyses were performed in SAS® (Version 9.4).

CHAPTER FIVE

RESULTS

5.1 SUBJECT DETAILS

Sample characteristics regarding the 110 volunteers (55 males, 55 females) including malocclusion category, mean age and mean overjet in millimetres are illustrated in **Table 7**.

Table 7 Sample characteristics: Malocclusion category, mean age and mean overjet

Malocclusion		Number of subjects	Mean Age in years (SD)	Mean Overjet mm (SD)
Class I	Males	20	12.4 ($\pm$ 0.3)	2.9 ($\pm$ 0.4)
	Females	20	12.3 ($\pm$ 0.3)	2.6 ($\pm$ 0.5)
	Total	40	12.4 ($\pm$ 0.3)	2.8 ($\pm$ 0.5)
Class II division 1	Males	20	12.5 ($\pm$ 0.3)	9.5 ($\pm$ 2.5)
	Females	20	12.4 ($\pm$ 0.3)	9.1 ($\pm$ 1.8)
	Total	40	12.5 ($\pm$ 0.3)	9.3 ($\pm$ 2.2)
Class II division 2	Males	15	12.3 ($\pm$ 0.3)	3.4 ($\pm$ 1.8)
	Females	15	12.4 ($\pm$ 0.3)	2.3 ($\pm$ 0.8)
	Total	30	12.4 ($\pm$ 0.3)	2.9 ($\pm$ 1.2)

5.2 SUMMARY STATISTICS FOR MAGNITUDE AND REPRODUCIBILITY OF SMILING

The formal analysis of the mean movements (averaged over all the landmarks) for rest to posed smile and rest to maximal smile is illustrated in **Table 8**.

Table 8 Formal analysis of mean movements (averaged over all landmarks)

Effect	NUM DF	Den DF	F Value	p-value
Malocclusion	2	106	0.47	0.06294
Session	1	106	26.84	<0.0001
A/B	1	106	1.93	0.1677
Smile	1	106	134.69	<0.0001
Gender	1	106	0.02	0.8972
Malocclusion*Session	2	106	1.09	0.3396
Malocclusion*A/B	2	106	0.96	0.3878
Malocclusion*Smile	2	106	0.21	0.8138
Session*A/B	1	106	2.63	0.1081
Smile*Gender	1	106	11.1	0.0012

Key: A/B = Capture A, Capture B; Session = Session 1, Session 2;

NUM DF = Numerator degrees of freedom; Den DF = Denominator degrees of freedom

5.3 MAGNITUDE OF SMILING

A difference was found in the magnitude (mean landmark movement across all landmarks) between the rest position to posed smile and the rest position to maximal smile ($p < 0.0001$). This difference was found to be gender specific with a greater mean difference between the rest to maximal smile in males ($5.26\text{mm} \pm 1.53\text{mm}$) than in females ($4.90\text{mm} \pm 1.17\text{mm}$). There was also a greater mean difference seen between the rest to posed smile and rest to maximal smile in males than in females (shown in **Figure 11**).

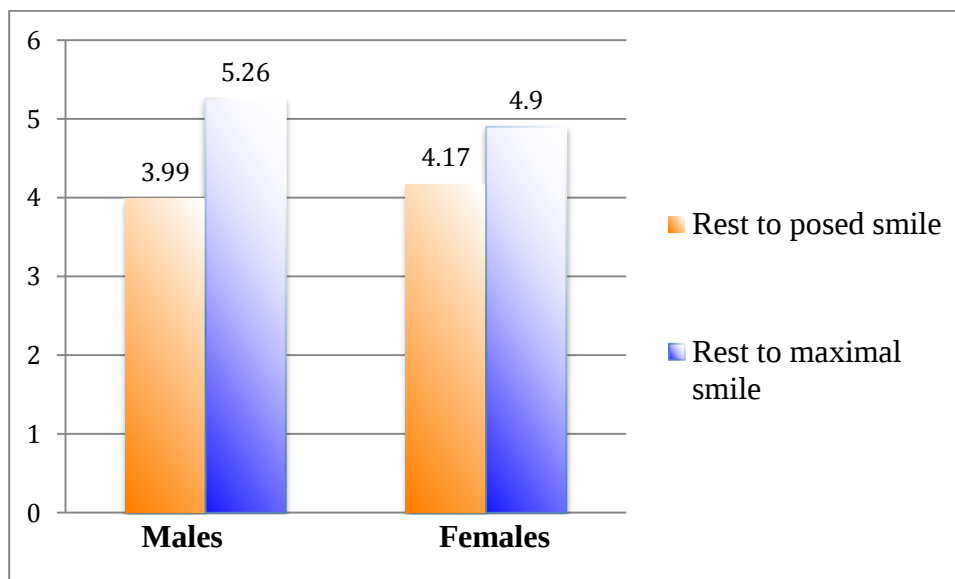


Figure 11 Difference in mean movement for each expression for males and females

5.4 IMMEDIATE INTRA-SESSION REPRODUCIBILITY

The mean movements, averaged over all the landmarks for rest to posed smile and rest to maximal smile for males, females and the combined sample for immediate intra-session reproducibility are shown in **Table 9**. Statistical analysis showed there was no statistical difference ($p = 0.1677$) as illustrated in **Table 8** between Capture A and Capture B within a session, which demonstrates immediate reproducibility of rest to posed smile and rest to maximal smile for both males and females within a session.

Table 9 Immediate intra-session reproducibility (Capture A versus Capture B) of mean (SD) movement for rest to posed smile (mm) and rest to maximal smile (mm) for males and females

Expression	Capture	Males Mean (SD)	Females Mean (SD)	Total (Males & Females) Mean (SD)
Rest to Posed Smile	A	3.93 ($\pm$ 1.36)	4.15 ($\pm$ 1.12)	4.04 ($\pm$ 1.25)
	B	4.04 ($\pm$ 1.37)	4.19 ($\pm$ 1.16)	4.12 ($\pm$ 1.27)
Rest to Maximal Smile	A	5.25 ($\pm$ 1.54)	4.88 ($\pm$ 1.10)	5.06 ($\pm$ 1.35)
	B	5.27 ($\pm$ 1.53)	4.92 ($\pm$ 1.25)	5.10 ($\pm$ 1.41)

5.5 SHORT-TERM INTER-SESSION REPRODUCIBILITY

The mean movements, averaged over all the landmarks for rest to posed smile and rest to maximal smile for males, females and the combined sample for short-term inter-session reproducibility at 2 weeks are shown in **Table 10**. Statistical analysis showed there was a statistical difference of 0.27mm found between session 1 (4.44mm) and session 2 (4.72mm) for the total sample, which although statistically significant ($p < 0.0001$) as shown in **Table 8**, is unlikely to be of any clinical significance.

Table 10 Short-term inter-session reproducibility (Session 1 versus Session 2) of mean (SD) movement for rest to posed smile (mm) and rest to maximal smile (mm) for males and females

Expression	Session	Males Mean (SD)	Females Mean (SD)	Total (Males & Females) Mean (SD)
Rest to Posed Smile	1	3.83 ($\pm$ 1.44)	4.02 ($\pm$ 1.15)	3.93 ($\pm$ 1.31)
	2	4.13 ($\pm$ 1.26)	4.32 ($\pm$ 1.10)	4.23 ($\pm$ 1.18)
Rest to Maximal Smile	1	5.17 ($\pm$ 1.65)	4.73 ($\pm$ 1.20)	3.93 ($\pm$ 1.31)
	2	5.34 ($\pm$ 1.44)	5.06 ($\pm$ 1.13)	4.23 ($\pm$ 1.18)
Total	1			4.44 ($\pm$ 1.47)
	2			4.72 ($\pm$ 1.33)

5.6 MALOCCLUSION

The effect of malocclusion on Capture A and Capture B (immediate intra-session reproducibility), Session 1 and Session 2 (short-term inter-session reproducibility) and in males, females and for the combined sample is illustrated in the following Tables (**Table 11**, **Table 12**, **Table 13**, **Table 14**, **Table 15** and **Table 16**)

Magnitude of smiling

Malocclusion had no statistically significant effect on the magnitude of movement ($p = 0.8138$) for rest to posed smile or rest to maximal smile. For Class I the difference between rest to posed smile and rest to maximal smile was 1.04mm. This was similar for Class II division 1 (1.03mm) and slightly smaller for Class II division 2 (0.91mm).

Immediate intra-session reproducibility

Malocclusion was found to have no statistically significant effect on immediate intra-session reproducibility of either rest to posed smile or rest to maximal smile between Capture A and Capture B ($p = 0.3878$). For example, the immediate intra-session reproducibility for the rest to posed smile the difference in Class I females between Capture A (4.04mm) and Capture B (4.02mm) was 0.02mm. The same difference was recorded between captures for Class II division 1 (0.2mm) and a slightly larger difference for Class II division 2 (0.3mm).

Short-term reproducibility

Malocclusion was found to have no statistically significant effect on short-term reproducibility of either rest to posed smile or rest to maximal smile between Session 1 and Session 2 ($p = 0.3396$). For example, the short-term inter-session reproducibility for the rest to maximal smile in Class I males between Session 1 (5.50mm) and Session 2 (5.43mm) was 0.07mm, in Class II division 1 the difference was larger at 0.31mm and in Class II division 2 the difference also 0.31mm.

Table 11 Summary statistics for movements: Rest to posed smile in males (mm): All landmarks

Rest to Posed Smile									
Malocclusion	Session	n	A		B		All		
			Mean	SD	Mean	SD	Mean	SD	
I	1	20	4.04	1.49	3.99	1.53	4.01	1.49	
	2	20	4.36	1.13	4.54	1.27	4.45	1.19	
	Total	40	4.20	1.31	4.54	1.27	4.45	1.19	
II/1	1	20	3.66	1.44	4.07	1.21	3.86	1.33	
	2	20	4.03	1.22	4.13	1.17	4.08	1.18	
	Total	40	3.85	1.33	4.1	1.17	3.97	1.25	
II/2	1	15	3.52	1.49	3.58	1.62	3.55	1.53	
	2	15	3.86	1.42	3.78	1.45	3.82	1.41	
	Total	30	3.69	1.44	3.68	1.52	3.69	1.47	
Total	1	55	3.76	1.46	3.91	1.44	3.83	1.44	
	2	55	4.11	1.24	4.18	1.3	4.14	1.26	
	Total	110	3.93	1.36	4.04	1.37	3.99	1.36	

Table 12 Summary statistics for movements: Rest to maximal smile in males (mm): All landmarks

Rest to Maximal Smile									
Malocclusion	Session	n	A		B		All		
			Mean	SD	Mean	SD	Mean	SD	
I	1	20	5.64	1.86	5.36	1.64	5.5	1.74	
	2	20	5.32	1.54	5.54	1.68	5.43	1.59	
	Total	40	5.48	1.69	5.45	1.64	5.46	1.66	
II/1	1	20	5.18	1.83	5.04	1.85	5.11	1.82	
	2	20	5.33	1.54	5.5	1.54	5.42	1.52	
	Total	40	5.26	1.67	5.27	1.70	5.26	1.68	
II/2	1	15	4.79	0.86	4.86	1.22	4.83	1.05	
	2	15	5.07	1.18	5.20	1.00	5.14	1.08	
	Total	30	4.93	1.04	5.03	1.11	4.98	1.07	
Total	1	55	5.24	1.65	5.11	1.61	5.17	1.62	
	2	55	5.25	1.43	5.43	1.46	5.34	1.44	
	Total	110	5.25	1.54	5.27	1.53	5.26	1.53	

Table 13 Summary statistics for movements: Rest to posed smile in females (mm): All landmarks

Rest to Posed Smile								
Malocclusion	Session	n	A		B		All	
			Mean	SD	Mean	SD	Mean	SD
I	1	20	4.00	1.16	3.97	1.26	3.98	1.19
	2	20	4.08	1.36	4.08	1.26	4.08	1.29
	Total	40	4.04	1.25	4.02	1.24	4.03	1.24
II/1	1	20	4.03	1.05	4.12	1.32	4.07	1.18
	2	20	5.51	0.99	4.45	1.04	4.48	1.00
	Total	40	4.27	1.04	4.29	1.18	4.28	1.10
II/2	1	15	3.93	1.13	4.05	1.11	3.99	1.10
	2	15	4.37	0.96	4.51	0.86	4.44	0.90
	Total	30	4.15	1.06	4.28	1.00	4.22	1.02
Total	1	55	3.99	1.09	4.04	1.22	4.02	1.15
	2	55	4.32	1.13	4.33	1.08	4.32	1.10
	Total	110	4.15	1.12	4.19	1.16	4.17	1.13

Table 14 Summary statistics for movements: Rest to maximal smile in females (mm): All landmarks

Rest to Maximal Smile								
Malocclusion	Session	n	A		B		All	
			Mean	SD	Mean	SD	Mean	SD
I	1	20	4.76	1.14	4.80	1.18	4.78	1.14
	2	20	4.98	1.20	4.98	1.17	4.98	1.17
	Total	40	4.87	1.16	4.89	1.16	4.88	1.15
II/1	1	20	4.82	0.9	4.68	1.31	4.75	1.11
	2	20	5.18	0.98	5.43	0.95	5.31	0.96
	Total	40	5.00	0.95	5.06	1.19	5.03	1.07
II/2	1	15	5.65	1.41	4.64	1.47	4.64	1.42
	2	15	4.79	1.08	4.92	1.44	4.86	1.25
	Total	30	4.72	1.23	4.78	1.44	4.75	1.33
Total	1	55	4.75	1.12	4.71	1.29	4.73	1.20
	2	55	5.00	2.08	5.13	1.18	5.06	1.13
	Total	110	4.88	1.10	4.92	1.25	4.90	1.17

Table 15 Summary statistics for movements: Rest to posed smile in males and females (mm): All landmarks

Rest to Posed Smile									
Malocclusion	Session	n	A		B		All		
			Mean	SD	Mean	SD	Mean	SD	
I	1	20	4.02	1.32	3.98	1.38	4.00	1.34	
	2	20	4.22	1.24	4.31	1.27	4.26	1.25	
	Total	40	4.12	1.27	4.14	1.33	4.13	1.30	
II/1	1	20	3.84	1.26	4.09	1.25	3.97	1.25	
	2	20	4.27	1.12	4.29	1.10	4.28	1.10	
	Total	40	4.06	1.20	4.19	1.17	4.12	1.19	
II/2	1	15	3.73	1.31	3.81	1.39	3.77	1.34	
	2	15	4.12	1.22	4.15	1.23	4.13	1.22	
	Total	30	3.92	1.27	3.98	1.31	3.95	1.29	
Total	1	55	3.88	1.29	3.97	1.33	3.93	1.31	
	2	55	4.21	1.18	4.26	1.19	4.23	1.18	
	Total	110	4.04	1.25	4.12	1.27	4.08	1.25	

Table 16 Summary statistics for movements: Rest to maximal smile in males and females (mm): All landmarks

Rest to Maximal Smile									
Malocclusion	Session	n	A		B		All		
			Mean	SD	Mean	SD	Mean	SD	
I	1	20	5.20	1.59	5.08	1.44	5.14	1.51	
	2	20	5.15	1.37	5.26	1.46	5.20	1.41	
	Total	40	5.17	1.47	5.17	1.44	5.17	1.45	
II/1	1	20	5.00	1.44	4.86	1.6	4.93	1.51	
	2	20	5.26	1.27	5.47	1.27	5.36	1.27	
	Total	40	5.13	1.36	5.17	1.46	5.15	1.41	
II/2	1	15	4.72	1.16	4.75	1.33	4.73	1.24	
	2	15	4.93	1.12	5.06	1.23	5.00	1.17	
	Total	30	4.82	1.14	4.91	1.28	4.86	1.21	
Total	1	55	5.00	1.43	4.91	1.46	4.95	1.44	
	2	55	5.13	1.27	5.28	1.33	5.20	1.30	
	Total	110	5.06	1.35	5.10	1.41	5.08	1.38	

5.7 LOWER-FACE LANDMARKS

In order to ensure that results across all landmarks were not diluting potential movements in the lower face for the expressions, rest to posed smile and rest to maximal smile, a separate statistical analysis was performed. This was averaged over the 10 lower-face landmarks (Chellion Right, Chellion Left, Christa Philtri Right, Christa Philtri Left, Labiale Superiorus, Labiale Inferioris, Lower Lip Right, Lower Lip Left, Sublabiale, Pogonion). The results of this formal analysis of mean movements are illustrated in **Table 17**.

This analysis confirms the findings of the analysis for overall facial landmarks. The magnitude of the mean movement was found to be significant between expressions ($p < 0.0001$) and was also found to be gender specific with a greater difference in males. No difference was found in immediate intra-session reproducibility ($p = 0.2058$). Although a statistically significant difference of 0.33mm was shown in short-term inter-session reproducibility across both expressions and genders, this was, however, clinically insignificant. Finally, malocclusion was found to have no effect on the magnitude or the reproducibility of smiling.

Table 17 Formal analysis of mean movements (Averaged over 10 lower-face landmarks)

Effect	NUM DF	Den DF	F Value	p-value
Malocclusion	2	106	0.61	0.5452
Session	1	106	8.88	0.0036
A/B	1	106	1.62	0.2058
Smile	1	106	116.92	<0.0001
Gender	1	106	0.02	0.8936
Malocclusion*Session	2	106	0.96	0.3866
Malocclusion*A/B	2	106	0.77	0.4637
Malocclusion*Smile	2	106	0.31	0.7349
Session*A/B	1	106	3.10	0.0813
Smile*Gender	1	106	10.25	0.0018

Key: A/B = Capture A, Capture B; Session = Session 1, Session 2;

NUM DF = Numerator degrees of freedom; Den DF = Denominator degrees of freedom

CHAPTER SIX

DISCUSSION

This would appear to be the first study to assess magnitude and reproducibility of smiling in 12-year-old children. It is also the first study to compare Class I, Class II division 1 and Class II division 2 malocclusions in males and females and their association with magnitude and reproducibility of the rest to posed smile and rest to maximal smile in that age group.

6.1 STUDY DESIGN

This was a prospective study assessing the effect of malocclusion and gender on the magnitude and reproducibility of smiling. The method of evaluation was by stereophotogrammetry: a 3D imaging technique using the DI3D system. Previous smile studies have also utilised 3D imaging to assess rest position, posed smile and maximal smile (Johnston et al., 2003; Campbell et al., 2012; Darby et al., 2015). They, however, differed from this study as they assessed adult samples and did not make comparisons between gender and Class I, Class II division 1 and Class II division 2 malocclusions.

6.2 SAMPLE SIZE

A previous study that assessed facial expressions had a sample size of 30 adult subjects with 15 in each group (Johnston et al., 2003). The power of the present study, which was based on that study, was determined to be 80 percent to detect differences of 1.5mm between similar expressions. In a previous study assessing smiling in female adults, 40 patients were recruited with 20 in each group, to allow for potential drop outs (Campbell et al., 2011). A similar study based on

children had a sample size initially of 60 subjects, but following drop outs, the final sample consisted of 19 males and 20 females (Djordjevic et al., 2011). Johal et al. (2018) recruited 50 patients, with 25 in each group, but the numbers of males and females within each group was not specified.

6.3 SUBJECT DETAILS

An equal number of male and female subjects were recruited. Previous adult and child smile studies also recruited equal numbers of male and female subjects (Campbell et al., 2012; Verze et al., 2011; Houstis and Kiliaridis, 2009; Johnston et al, 2003; Burke, 1971).

6.3.1 Sample Age

The groups were matched for age in an attempt to reduce confounding factors. The age chosen was 12 years, as it is the age in which most orthodontic treatment usually commences to coincide with the establishment of the permanent dentition (Tulloch et al., 1984; Burden 1995).

Although, still classified as children, subjects of this age were able to follow direction to produce the rest position, the posed smile and the maximal smile. Duffy et al. (2000) found that older children, much like the sample age within this study, can follow instructions to keep a relaxed and neutral face with the mouth shut and lips gently touching. It has also been suggested that asking the subject to swallow may help them to relax (Plooi et al., 2009). Younger children, however, are more likely to require distraction devices to focus their

attention in the preferred direction, which would include: a children's video or bubbles or toys (Plooij et al., 2009). These, however, were not required in the sample group used in this study.

6.3.2 Malocclusion

This study consisted of three groups of malocclusion: Class I (20 males, 20 females, Class II division 1 (20 males, 20 females) and Class II division 2 (15 males, 15 females) malocclusions. The malocclusion was assessed based upon the incisor classification as defined by the British Standards Institute (Institute., 1983). The Class II division 2 malocclusion category had a smaller sample size than the other two categories due to the difficulties in recruiting 12-year-old children displaying this malocclusion. The prevalence of Class II division 2 is estimated to be as low as 10 percent in a Caucasian population (Foster and Day, 1974). These data, however, are over 40 years old and prevalence figures appear to be lower within the RoI, but no exact data are available.

Previous studies, assessing smiling in children, compared Class III malocclusion to Class I malocclusion (Johal et al., 2018; Krneta et al., 2014). Campbell et al. (2012) in a study involving female adults, compared a Class II division 1 malocclusion group, to a Class I malocclusion group. There appears to have been no study involving children comparing Class I malocclusion to Class II malocclusion in relation to smile assessment.

6.4 EXPRESSIONS RECORDED

The rest to posed smile and rest to maximal smile were recorded to assess both intra-session and inter-session magnitude and reproducibility of smiling. **Table 4** and **Table 5** outline previous studies which have assessed smiling in children. Peck et al. (1992) and Ackerman et al. (2004) assessed rest and maximal smile, Bernal De Jaramillo et al. (2015) assessed rest, posed and unforced smile; Miyakawa et al. (2006); Houstis and Kiliaridis (2009) and Verze et al. (2011) assessed rest position and posed smile while Tarantili et al. (2005) assessed rest position and spontaneous smile. None of the studies assessed all three expressions together.

6.4.1 Method of reporting expressions recorded

Previous adult studies assessing smiling have recorded the rest position, posed smile and maximal smile (Johnston et al., 2003; Campbell et al., 2012; Darby et al., 2015). These studies reported results for individual expressions; what should be reported, however, is the mean magnitude of movement for the rest to posed smile and rest to maximal smile. To avoid confusion we have been explicit in our terminology regarding the expressions recorded: rest to posed smile and rest to maximal smile.

6.5 STANDARDISATION OF IMAGE CAPTURE

To standardize the interval between Capture A and Capture B, in order to accurately assess intra-session reproducibility, a 15-minute interval was chosen.

This was similar to a previous study involving adults assessing the reproducibility of smiling (Campbell et al., 2012). A two-week interval was chosen between the first and second session in order to assess inter-session reproducibility. All subjects returned at this time period. This is unlike previous studies involving adults where the interval ranged from 1 to 4 weeks and from 4 to 6 weeks respectively (Johnston et al., 2003; Campbell et al., 2012). The interval in previous studies involving children ranged from: a few days, 6 months, 2 years and 4.5 years (Kau et al., 2005; Kau et al., 2008; Verze et al., 2011; Djordjevic et al., 2011).

In order to standardise how the subjects performed each expression, the subjects were provided with verbal instructions as per Zachrisson's (1998) instructions. These instructions have been previously validated in a number of studies involving adults (Johnston et al., 2003; Campbell et al., 2012; Darby et al., 2015).

6.6 LANDMARK IDENTIFICATION ERROR

The landmarks used in this study have all been previously validated (Hajeer et al., 2002). The landmarks in this study were placed on the 3D image following capture; this increases the potential for error in landmark identification. Ten percent of the images across all landmarks, time points and positions were re-landmarked one month after initial imaging and landmarking. This process was conducted by the 3D imaging assistant. The intra-observer landmark identification error was found to be 0.54mm.

This is comparable to the intra-observer landmark error of 0.58mm recorded previously in a similar 3D study of smiling (Campbell et al., 2012). It is much less than earlier studies, which reported intra-observer reproducibility of 1mm and 2mm respectively (Ras et al., 1996; Ferrario et al., 1997). Gwilliam et al., (2006) previously found that familiarity with 3D facial scans and associated software programs improves the intra-observer reproducibility. This could explain the high reproducibility in the present study, as the 3D research assistant has eight years experience with the system.

6.7 3D SYSTEM

The validity and accuracy of the three-dimensional imaging system (Di3D) used to capture facial images in this study has previously been reported (Khambay et al., 2008). The Di3D system error was found to be within 0.2mm, which is within clinically acceptable limits. The reproducibility error of the Di3D system was also found to be low at 0.13mm (Khambay et al., 2008). This demonstrates that the imaging system is stable over a period of time, which provides confidence to use the system in reproducibility studies.

6.8 LANDMARK AND ANALYSES

Twenty six landmarks were placed on each 3D image, similar to previous studies (Campbell et al., 2012; Darby et al., 2015). Analysis was performed by averaging the mean movement across all 26 landmarks, for each expression. A separate analysis was performed for the 10 lower-face landmarks. As the latter

results supported the findings of the overall analysis, discussion will focus solely on the results of the former.

6.9 MAGNITUDE OF SMILING

Weeden et al. (2001) demonstrated in an adult sample (25 males, 25 females) that males had larger movements than females during facial movements. Johnston et al. (2003) also found a greater variation in relation to magnitude between maximal smiles in males than in females. Ju et al. (2016) found no significant differences between 16 males and 16 females, aged 18 to 35 years, in the difference of magnitude for the maximal smile. In the study by Campbell et al. (2012) mean movement was affected by malocclusion, with greater movement in the Class I malocclusion group than in the Class II division 1 malocclusion group for both the natural smile (Class 1: 3.12mm; Class II division 1: 2.60mm) and the maximal smile (Class I: 4.35mm; Class II division 1: 3.85mm); gender was not assessed in terms of magnitude as the sample only consisted of females. In our study, the mean movement recorded for the rest to posed smile in Class I malocclusion females was 4.03mm and in Class II division 1 malocclusion females was 4.88mm; both measures are larger than those found in the study by Campbell et al. (2012). A possible factor influencing this difference may be that with the process of aging, the skin becomes thinner and less flexible. Pawlaczyk et al. (2013) found that the elasticity modulus of skin is higher in children than in adults.

The magnitude of movement between smiles was also found to be greater in our study in males than in females. Average upper lip length increase during growth

in males is more than twice that of females (Nanda et al., 1990), which could be a possible explanation for this difference found in magnitude of movement.

The measurement of magnitude has differed between studies so it is difficult to draw accurate conclusions. Previous smile studies in children have measured the size of the smile between points (McNamara et al., 2008; Schabel et al., 2009; Bernal De Jaramillo et al., 2015), whereas in our study the magnitude was measured as the mean movement averaged across all landmarks from rest position to posed smile and rest position to maximal smile. Direct comparison is, therefore, not possible.

6.10 IMMEDIATE INTRA-SESSION REPRODUCIBILITY

Two previous studies involving children assessed intra-session reproducibility of smiling. Ackerman et al. (1998) used photographs to assess the reproducibility of the posed smile within the same session. That study had a small sample size of only five subjects and they were not provided with any instruction in how to elicit the posed smile; rather, they were simply asked to just smile and not laugh. Miyakawa et al. (2006) used video to assess facial expressions, including posed smile, twice on the same day in 17 subjects (7 males, 11 females). In that study they had to use a head restraint as the software program they used could not correct for horizontal positional errors. This may, however, have provided a false environment for the children, in which they may not have been completely relaxed. In both the former and the latter study, subjects were aged 11 years and 5 years respectively. Although malocclusion was not stated in either study, in both studies the posed smile was found to be highly reproducible.

Johnston et al. (2003) assessed the reproducibility of facial expressions in adults using stereophotogrammetry and found the rest position, posed smile and the maximal smile all to be reproducible within sessions. An adult study, which compared females with a Class II division 1 malocclusion to females with a Class I malocclusion, found high intra-session reproducibility for both groups for all three expressions: rest position, natural smile and maximal smile (Campbell et al., 2012). None of these studies, matched gender for their sample groups and none of the studies involving children assessed the intra-session reproducibility of smiling amongst different malocclusion groups. Nonetheless, findings of previous studies support those of the present study.

6.11 SHORT-TERM INTER-SESSION REPRODUCIBILITY

Four studies assessed the inter-session reproducibility of smiling in children as discussed previously in **Section 2.6.3**. Only one of these studies by Verze et al., (2011) is relevant to our study as they repeated 3D scans of 20 adult subjects (10 males, 10 females) and 12 children aged 7 to 11 years (6 males, 6 females) over a number of days. As a means of landmarking, they placed reflective markers directly onto the face, prior to carrying out the head and face colour 3D scans. They, however, failed to address the limitation of this technique when reporting on the reproducibility between sessions. Similarly, Johnston et al. (2003) also had to reposition landmarks directly onto the face between visits and acknowledged that they were unable to directly compare results from Day 1 and Day 2 as a result of this limitation.

In contrast, Campbell et al. (2012) placed landmarks on the images following capture, which reduced placement error potential. They found the rest position, natural smile and maximal smile all to be reproducible over an average period of 42 days. This was also the case in a 3D capture study by Tanikawa et al., (2017) who found that the overall inter-session reliability of the rest position and maximal smile was in almost perfect agreement, and the posed smile, had moderate agreement. The sample in that study consisted of 12 adult subjects (6 males and 6 females) with a one-week interval between sessions. In our study the statistical analysis provided a doubt regarding the short-term reproducibility of smiling due to the difference of 0.27mm. Previous studies by Strauss et al. (1997) reported that repeated recordings would have to vary by less than 1mm to be described as reproducible. Popat et al. (2008) considered that repeat recordings should vary by less than 0.5mm to be considered reproducible. Under these two definitions of reproducibility, our finding of 0.27mm averaged across all landmarks can be considered to be reproducible. Therefore, the more recent studies (Campbell et al. 2012 and Tanikawa et al. 2017) support our finding that inter-session smiling is reproducible.

6.12 MALOCCLUSION AND SMILING

To our knowledge, there are no previous studies assessing the effect of malocclusion on smiling in children. The only study to which tenuous comparisons can be drawn, is by Campbell et al. (2012), which assessed the effect of overjet on smiling. They found that the control group (Class I malocclusion adult females) displayed greater mean movement for rest to natural

and maximal smile than the test group (Class II division 1 malocclusion adult females). Prior to undertaking this study, it was thought that malocclusion may demonstrate a difference in smiling, but tooth position (proclined or retroclined upper incisors) was found to have no effect on the magnitude of smiling or on the intra-session or inter-session reproducibility of smiling.

The age cohort that we assessed, perhaps, contributed to the lack of any difference, as conceivably they had not yet developed any self-consciousness and were happy and confident to smile. Barbosa et al. (2008) reported that at the age of 12 years, children begin to develop the understanding of well-being and view their health as a multi-dimensional concept. How quickly these concepts settle varies greatly and depends upon their exposure to different experiences. Hetherington et al. (1999) found that children enter a period of adolescence from 11 to 14 years, which is characterized by increased centrality of peer influence and preoccupation with others' views of self. It is possible that our patient cohort had not yet entered this period or that this period had not established completely. This is in contrast to the adult sample in the study by Campbell et al. (2012), which may have caused them to smile in a learned manner masking their malocclusion.

6.13 STRENGTHS OF THE STUDY

There are several strengths of this study. These are:

- Prospective study utilising Di3D system assessing a new dimension on the effect of malocclusion on smiling in children.
- Malocclusion groups were matched for age and gender.

- Sample sizes were comparable to similar previous studies.
- Capture environment and interval between captures was standardised.
- Landmark error study was performed.

6.14 LIMITATIONS OF THE STUDY

- Class III malocclusion was not included, as it was thought due to the prevalence of 4 percent in European populations (Hardy et al. 2012), recruitment would have proved unsuccessful.
- Subjects were allocated due to their respective groups only by incisor relationship, disregarding underlying skeletal patterns, which perhaps could have influenced results.
- Subjects were reimbursed in a monetary fashion for their time, which perhaps could have influenced their cooperation with smiling and following directions.

6.15 CLINICAL IMPLICATIONS

The clinical implications of this study include the findings that whether teeth are proclined (Class II division 1 malocclusion) or retroclined (Class II division 2 malocclusion), it does not inhibit the magnitude or reproducibility of the smile. Some studies indicate that malocclusion is negatively correlated with psychosocial effects particularly extreme overjet and overbite (Helm et al., 1985). These perceived self-esteem issues did not inhibit smiling in our study. Therefore, the smile both rest to posed and rest to maximal, can be recorded with

confidence in it's magnitude and reproducibility in children of this age group and these two expressions are a valid means of assessing the smile prior to orthodontic treatment.

6.16 SUGGESTIONS FOR FUTURE RESEARCH

A further study could be conducted with a similar design but also include both male and female Class III malocclusion groups. The magnitude and reproducibility of smiling could also be assessed in 4D which provides a more realistic insight into the animation and motion of the smile. Finally, this sample of 12-year-old children could be used as a control group for comparison to a cleft lip and palate sample of the same age, to assess the magnitude and reproducibility of smiling following lip revision and to separate treatment effects from normal variation in the magnitude and reproducibility of smiling.

CHAPTER SEVEN

CONCLUSIONS

7.1 CONCLUSIONS

The aims and null hypothesis were previously outlined in **Chapter 3**.

Aim 1: To determine if different types of malocclusion; Class I, Class II division 1 and Class II division 2, influence the magnitude of the rest position to posed smile and the rest position to maximal smile.

Conclusion:

- Malocclusion had no impact on the magnitude of the rest position to posed smile and the rest position to maximal smile.

Null Hypothesis 1: Malocclusion has no effect on the magnitude of the rest position to posed smile and the rest position to maximal smile.

Null hypothesis was accepted.

Aim 2: To determine if different types of malocclusion; Class I, Class II division 1 and Class II division 2, influence the immediate intra-session reproducibility or the short-term inter-session reproducibility of the rest position to posed smile and the rest position to maximal smile.

Conclusion:

- Malocclusion had no effect on the immediate intra-session or the short-term inter-session reproducibility of the rest position to posed smile and the rest position to maximal smile.

Null Hypothesis 2: Malocclusion has no effect on the reproducibility of the rest position to posed smile and the rest position to maximal smile.

Null hypothesis was accepted.

Aim 3: To determine if gender influences the magnitude of the rest position to posed smile and the rest position to maximal smile.

Conclusion:

- There was a statistical difference found between the groups and between rest to posed smile and rest to maximal smile with males demonstrating a greater difference between smiles.

Null Hypothesis 3: Gender has no effect on the magnitude of the rest position to posed smile and the rest position to maximal smile.

Null hypothesis was rejected.

Aim 4: To determine if gender influences the immediate intra-session reproducibility or short-term inter-session reproducibility of the rest position to posed smile and the rest position to maximal smile.

Conclusion:

Gender had no effect on the immediate intra-session or the short-term inter-session reproducibility of the rest position to posed smile and the rest position to maximal smile.

Null Hypothesis 4: Gender has no effect on the reproducibility smiling.

Null hypothesis was accepted.

CHAPTER EIGHT

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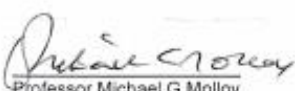
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CHAPTER NINE

APPENDICES

9.1 APPENDIX A

 UCC Tel: + 353-21-490 1901 Fax: + 353-21-490 1919	COISTE EITICE UM THAIGHDE CLINICIÚIL Clinical Research Ethics Committee Lancaster Hall, 6 Little Hanover Street, Cork, Ireland.
Coláiste na hOllscoile Corcaigh, Éire University College Cork, Ireland	
Our ref: ECM 4 (u) 01/03/16	
29th February 2016 Professor Declan Millett Professor of Orthodontics Cork University Dental School and Hospital Wilton Cork Re: The reproducibility and magnitude of smiling – a comparison of Class 1, Class II and Division 1 and Class II Division 2 malocclusions. Dear Professor Millett Expedited approval is granted to carry out the above study at: ➤ Cork University Dental Hospital. The Chairman approved the following: ➤ Signed Application Form ➤ CV for Chief Investigator ➤ Study Protocol Version 1 dated February 2016 ➤ Consent Form Version 1 dated February 2016. We note that the co-investigators involved in this study will be: ➤ Niamh Kelly, 3D Imaging Assistant, Dr John Bowe, Dr Jacqueline Clune, Dr Ciara Ennis and Dr Raphy Paul, Postgraduate Students. Yours sincerely  Professor Michael G Molloy Chairman Clinical Research Ethics Committee of the Cork Teaching Hospitals	
The Clinical Research Ethics Committee of the Cork Teaching Hospitals, UCC, is a recognised Ethics Committee under Regulation 7 of the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations 2004, and is authorised by the Department of Health and Children to carry out the ethical review of clinical trials of investigational medicinal products. The Committee is fully compliant with the Regulations as they relate to Ethics Committees and the conditions and principles of Good Clinical Practice.	
Ollscoil na hÉireann, Corcaigh - National University of Ireland, Cork.	

9.2 APPENDIX B



Tel: + 353-21-490 1901
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COISTE EITICE UM THAIGHDE CLINICIÚIL
Clinical Research Ethics Committee

Lancaster Hall,
6 Little Hanover Street,
Cork,
Ireland.

Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

Our ref: ECM 4 (u) 01/03/16 & ECM 3 (ff) 06/12/16

1st December 2016

Professor Declan Millett
Professor of Orthodontics
Cork University Dental School and Hospital
Wilton
Cork

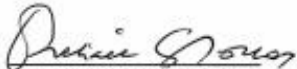
Re: The reproducibility and magnitude of smiling – a comparison of Class 1, Class II and Division 1 and Class II Division 2 malocclusions.

Dear Professor Millett

The Chairman approved the following:

- Cover Letter dated 15th November 2016
- Amendment Application Form signed 14th November 2016
- Revised Study Protocol Version 2 dated November 2016.

Yours sincerely



Professor Michael G Molloy
Chairman
Clinical Research Ethics Committee
of the Cork Teaching Hospitals

The Clinical Research Ethics Committee of the Cork Teaching Hospitals, UCC, is a recognised Ethics Committee under Regulation 7 of the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations 2004, and is authorised by the Department of Health and Children to carry out the ethical review of clinical trials of investigational medicinal products. The Committee is fully compliant with the Regulations as they relate to Ethics Committees and the conditions and principles of Good Clinical Practice.

Ollscoil na hÉireann, Corcaigh - National University of Ireland, Cork.