# THE RELIABILITY AND VALIDATION OF A 2D IMAGE ANALYSIS SYSTEM WHEN APPLIED TO THE MEASUREMENT OF TWIN STUDY MODEL SAMPLES

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**ABSTRACT**

This study aimed to investigate dental fluctuating asymmetry of tooth dimensions in monozygotic (MZ) co-twins as a measure of how dental development is affected by epigenetic and environmental factors. The mesiodistal (MD), buccolingual (BL) dimensions of the central incisor, lateral incisor, second premolar and first molar in each of the four quadrants, on 20 sets of study models of MZ twin pairs were determined manually and two dimensional (2D) image analysis. The extent of asymmetry was assessed by determining intra-class correlation coefficients (ICCC's) between pairs of antimeric teeth between individuals, then the fluctuating asymmetry (FA) between twin pairs determined. Intra-operator repeatability for 2D measurement was substantial or excellent. Differences were detected between left and right antimeres for all the four measurements, and these differences were not the same for both twins in a pair. 2D image analysis facilitated the additional measurements of surface area and perimeter. Asymmetry between twin pairs for each tooth type followed a pattern fitting with the morphogenetic field theory with the key teeth showing the least asymmetry (upper centrals, upper sixes, lower laterals, lower sixes) and the variable teeth showing the most asymmetry (upper laterals, upper fives and lower sixes). The MD dimension also followed this trend.

**KEYWORDS:** Twin studies, Genetic, 2D image analysis, Manual measurement, Dental development, Asymmetry.

## INTRODUCTION

### Twin Models:

Twin studies provide a useful approach to investigating the roles of genetic and environmental factors in tooth development. Such studies allow researchers to determine the heritability of different dental traits. Estimates of heritability are traditionally achieved by comparing the similarities between monozygotic (MZ) twin pairs with those between dizygotic (DZ) twin pairs[(1)]. The MZ co-twin model assumes that MZ twins share the same genotypes, so that phenotypic differences must be due to epigenetic and/or environmental factors. It also assumes that findings from MZ co-twins can be extrapolated to singletons (individual from a group) in the general population. The MZ co-twin model is a valuable approach to apply within a dental setting and it has been used previously in orthodontics in the treatment plan of a twin-pair demonstrated by Pangrazio-Kulbersh V[(2)] and in the study of dental anomalies such supernumerary teeth[(3)].

### Asymmetry:

Variation in dental crown size can be measured on contra lateral sides of the dental arch, i.e. on antimeric teeth and has been found to have a major genetic component[(4)]. Failure of antimeres to develop identically suggests underlying instability related to epigenetic and environmental effects[(5-9)]. Therefore, the study of asymmetry is a means of investigating stability of a developmental process and providing a measure of developmental interference with the "genetic blueprint" during ontogeny. There are two types of asymmetry. The first is 'directional' and always favours the same side of the individual. The second, 'fluctuating' is the random or non-directional difference between sides. Previous asymmetry studies involving twins have shown an absence of evidence of gene contribution in fluctuating asymmetry[(10-11)].

### Aims:

This study aimed to quantify dental asymmetry using an established manual methodology and 2D image analysis, as a measure of epigenetic and environmental factors affecting dimensions of permanent teeth of MZ co-twins.

### Null Hypothesis:

Reliability of the 2D image analysis is poor. MZ phenotypic differences cannot be measured using manual and 2D image analysis. There is no difference in the amount/degree asymmetry within the dentitions between pairs of MZ co-twins.

## MATERIALS AND METHODS

Two techniques of measurement were used to record the mesiodistal (MD) and buccolingual (BL) diameters of permanent teeth of MZ co-twins. These techniques were manual measurement, using electronic callipers, and 2D image analysis.

Twenty sets of dental study models of MZ twins from Adelaide, South Australia were selected for

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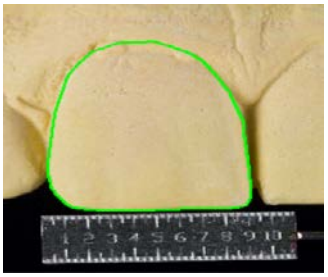
measurement. Duplicate models were stored in the University of Liverpool. Internal university ethical approval was granted (RETH000063).
The teeth types measured on each model were the central incisor, lateral incisor, second premolar and first molar in each of the four quadrants. The choice of teeth to be measured was based on concepts of variation described in the Morphogenetic Field theory[12].

**Manual Measurement:**
The methods described by Moorrees et al[13]; Hunter and Priest[14] and Lavelle[15], were applied to measure the M-D and B-L distances of the specified teeth of the study models using an electronic calliper (figure 1). Measurements were obtained in a systematic manner under standardised conditions from the upper left quadrant, and then upper right, then lower left, then lower right quadrants as reported by Brook et al.[16]


**(Figure 1)** Electronic Mitutoyo callipers used for manual measurement

**2D Image Analysis:**
The 2D image analysis system is a useful means of measuring additional variables such as perimeter and area accurately, allowing an increased discrimination between within-pair differences. It also enables permanent storage of images so that they can be reviewed later for reliability analysis and further measurement[17].
A Kodak DCS Pro SLR digital camera, which was connected to a computer (Dell XP, Pentium 4, Dell UK), was used to capture images of the study models from the labial (only for the UR1) and occlusal views. The M-D and B-L distances from each image were measured using Image Pro Plus software (Version 5, Media Cybernetics) (figure 2).



**(Figure 2)** Example of an image taken from buccal view. UR1 showing the perimeter from which the area is derived

**STATISTICAL ANALYSIS AND VALIDATION**

**Reliability:**
The results were analysed using SPSS 18.0 package. Intra-operator repeatability was determined by re-imaging and measuring the 4 selected teeth in the upper left and right quadrants of 20 study models twice using the manual and 2D image analysis method. In addition, the labial view of the UR central incisor was also taken on each of these 20 upper study models and the M-D, B-L diameters measured to validate the technique from an alternative view. This was then analysed using Fleiss intra-class correlation coefficients (ICCC)[18]. The results were then classified into the Donner and Eliasziw scale (table 1-a)[19].

**(Table 1-a)** Classification of the Value of R according to Donner and Eliasziw

| Value of R | Reliability |
|---|---|
| 0.00-0.20 | Slight |
| 0.21-0.40 | Fair |
| 0.41-0.60 | Moderate |
| 0.61-0.80 | Substantial |
| 0.81-1.00 | Excellent |

Bias calculations were then performed to assess the significance of any bias observed.

**RESULTS**
(Table 1-b) displays the results for displays the results for the intra-operator repeatability manual measurement. The ICCC results fall into the category of excellent[19].

**(Table 1-b)** Manual Intra-operator repeatability descriptive statistics for the upper right central, upper right second premolar, upper left lateral and upper left first molar teeth (occlusal views)

| Variable | UR1 Occlusal | | UR5 Occlusal | | UL2 Occlusal | | UL6 Occlusal | |
|---|---|---|---|---|---|---|---|---|
| | MD | BL | MD | BL | MD | BL | MD | BL |
| Mean Dif. | -0.02 | -0.05 | 0.00 | 0.03 | -0.01 | 0.00 | 0.00 | 0.00 |
| ICCC | 0.98 | 0.96 | 0.98 | 0.88 | 0.99 | 0.99 | 0.99 | 0.94 |

**(Table 2-a)** 2D Intra-operator repeatability descriptive statistics for the upper right central (labial and occlusal views) and second premolar (occlusal view) teeth

| Variable | UR1 Labial | UR1 Occlusal | | UR5 Occlusal | |
|---|---|---|---|---|---|
| | MD | MD | BL | MD | BL |
| Mean Dif | -0.12 | -0.16 | 0.01 | -0.03 | 0.01 |
| SD of Dif | 0.31 | 0.38 | 0.25 | 0.21 | 0.16 |
| Standard Error | 0.07 | 0.09 | 0.06 | 0.05 | 0.04 |
| ICCC | 0.71 | 0.73 | 0.9 | 0.86 | 0.93 |
| RC | 0.61 | 0.75 | 0.48 | 0.42 | 0.31 |

Mean Dif= Mean Difference, SD of Dif=Standard deviation of difference, ICCC= Intra class correlation coefficient, RC= Repeatability coefficient

**(Table 2-b)** 2D Intra-operator repeatability descriptive statistics for the upper left lateral (occlusal view) and upper left first molar (occlusal view) teeth

| Variable | UL2 Occlusal | | UL6 Occlusal | |
|---|---|---|---|---|
| | MD | BL | MD | BL |
| **Mean Dif.** | 0.02 | 0.01 | -0.04 | -0.07 |
| **SD of Dif** | 0.4 | 0.17 | 0.13 | 0.15 |
| **Standard Error** | 0.3 | 0.04 | 0.03 | 0.03 |
| **ICCC** | 0.96 | 0.64 | 0.98 | 0.98 |
| **RC** | 0.27 | 0.33 | 0.26 | 0.29 |

(Table 3) shows the correlation between measurements using the manual and 2D measurement methods. The average was taken from two repeat measurements and comparisons made. The PCC varied between 0.24 and 0.91. The UR1 had the highest overall ICCC values suggesting the least difference between the methods for this particular tooth.

**(Table 3)** Correlation data for manual and 2D measurement

| Variable | UR1 Occlusal | | UR5 Occlusal | | UL2 Occlusal | | UL6 Occlusal | |
|---|---|---|---|---|---|---|---|---|
| | MD | BL | MD | BL | MD | BL | MD | BL |
| **Mean Dif.** | -0.13 | 0.30 | 0.04 | -2.13 | 0.24 | 0.43 | -0.36 | 0.14 |
| **SD of Dif.** | 0.42 | 0.30 | 0.40 | 1.35 | 0.96 | 0.87 | 1.14 | 0.57 |
| **Standard Error** | 0.10 | 0.10 | 0.10 | 0.30 | 0.21 | 0.20 | 0.25 | 0.13 |
| **PCC** | 0.59** | 0.91** | 0.64** | 0.24 | 0.91 | 0.15 | 0.36 | 0.4 |

**= Correlation is significant at the 0.01 level (2-tailed)

## DISCUSSION

Intra-operator repeatability for manual and 2D measurement was high. For 2D measurement Fleiss' ICCC reliability results ranged from 0.639- 0.978 (table 2-a) and (table 2-b). These values indicated'substantial/good' or 'excellent' reliability according to the Donner and Eliasziw scale[19] (table 1-a). The descriptive statistics for the intra-operator repeatability measurements excluding one, (tables 1-b, 2-a, 2-b) showed no significant bias.

The reliability data for both methods (1b, 2a and 2b), shows that for 2D measurement technique was higher than those for manual measurement, as has been reported in previously[17]. The correlation data (table 3) showed that the UL6 had the lowest overall PCC value between linear measurements and the UR1 from the occlusal view had the highest. The results showed that overall the MD and BL PCC values between the two methods showed 'fair' correlation, with little difference between the two.

The extent of asymmetry was assessed using intraclass correlation coefficients between antimere pairs. Where the trend of asymmetry between tooth types fits the morphogenetic field theory[12], each variable displays a different degree of asymmetry. Twin pairs showed varied amounts of asymmetry. For manual measurement, the FA ranged from 0.03-0.28 compared with 0.01-0.16 for 2D measurement. The difference between the two methods may be due to 2D image analysis being a more accurate methodology as the measurement error is reduced.

The order of differences in FA for manual measurement showed that MD>BL and for 2D measurement BL>MD. It is important to compare the magnitude of the differences observed between the left and right sides with the differences observed in the intra-operator repeatability study, as the difference observed may be due to measurement error. The size of the asymmetries observed was less in comparison to the size of the typical measurement error in the reliability study.

## CONCLUSION

The manual and 2D image analysis measurement approaches proved reliable and the 2D system was validated for use in such studies. The current study was able to quantify dental asymmetry for tooth type and tooth dimensions between twins. The teeth that are the most variable are upper laterals, lower centrals and upper fives, with the upper centrals and lower sixes being the least variable.

## REFERENCES

1- Townsend G.C., Hughes T., Lucianio M., Bockmann M., Brook A. (2008) Genetic and environmental influences on human dental variation: a critical evaluation involving twin studies. *Arch of Oral Biol. (In press)*

2- Pangrazio-Kulbersh V., Berger JL. (1993) Treatment of identical twins with Frankel and Herbst appliances: a comparison of results. *Am J Orthod Dentofacial Orthop.* 103:131-137.

3- Townsend G, Dempsey P, Richards L. (1999) Causal components of dental variation: new approaches using twins. *Mayhall JT, Heikkinen T, eds. Dental Morphology'98. Oulu: Oulu University Press*:464-472.

4- Klingenberg CP, Barluenga M, Meyer A. (2002) Shape analysis of symmetrical structures: quantifying variation among individuals and asymmetry. *Evolution*.56:1909-20.

5- Van Valen L. A study of fluctuating asymmetry. (1962) *Evolution*.16:125-42.

6- Bailit HL, Workman PL, Niswander JD, Maclean CJ. (1970) Dental asymmetry as an indicator of genetic and environmental conditions on human populations. *Hum Biol* .42:626-38.

7- DiBennardo R. (1973) Prenatal stress, developmental 'noise' and postnatal risk. Ph.D. dissertation. New York: City University of New York.

8- Doyle WJ, Johnston O. (1977) On the meaning of increased fluctuating dental asymmetry: a cross population study. *Am J Phys Anthrop*.46:127-34.

9- Perzigian AJ. (1977)Fluctuating dental asymmetry: variation among skeletal populations. *Am J Phys Anthrop*. 47: 81-8.

10- Bailit HL, Workman PL, Niswander JD, Maclean CJ. (1970)Dental asymmetry as an indicator of genetic and environmental conditions on human populations.*Hum Biol* .42:626-38.

11- Potter RH, Nance WE. (1976) A twin study of dental dimension. I. Discordance, asymmetry and mirror imagery. *Am J Phys Anthrop.*44:391-6.
12- Butler, P.M. (1939) Studies of the mammalian dentition: differentiation on the post-canine dentition, *Proc 2001. Zool. Soc. Lond.* B 109:1
13- Moorrees C.F.A., Thomsen S.O., Jensen E., Yen P.K. (1957) Mesiodistal crown diameters of the deciduous and permanent teeth in individuals. *J.Dent.Res.* 36:39-47.
14- Hunter W.S., Priest W.R (1960) Errors and discrepancies in measurements of tooth size. *J.Dent.Res.* 39: 405-414.
15- Lavelle C.L.B. (1970) Comparison of the deciduous teeth between Caucasian, negroid and mongoloid population samples. *Dent.Pract.Rec.* 21: 121-124.
16- Brook A.H., Griffin R.C., Smith R.N., Townsend G.C., Kaur G., Davis G.R. and Fearne J. (2008) Tooth size patterns in patients with hypodontia and supernumerary teeth. *Arch Oral Biol . (In press)*
17- Brook A.H., Smith R.N., Elcock C., Al-Sharood M., Shah A., Karmo M. (1999)The measurement of tooth morphology: development and validation of a new image analysis technique. *A.Brook (ed. Current trends in dental morphology research), Sheffield Academic Press*: 380-387.
18- Fleiss J.L.(1986) The design and analysis of clinical experiments. *WiIey and Sons, Inc*. Chapter 1; Reliability of measurement: 1-32.
19- Donner A., Eliasziw M. (1987) Sample size requirements for reliability studies, *Statistics in Medicine 6*:441-448.