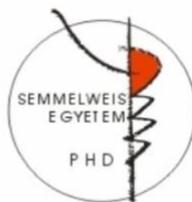


# Forensic identification of twin siblings and sex by digital palatal morphology

**Ph.D. Thesis**  
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# 1. Introduction

Forensic science constantly seeks to enhance human identification processes, and recent technological advancements, such as intraoral scanners (IOSs), offer promising possibilities. Intraoral scanners provide detailed 3D images of the oral cavity, including the palate morphology, which might be compared to existing databases or used to create individualized profiles for accurate identification.

The morphology of the palate, particularly rugae patterns, has been studied for centuries and is considered stable and identifiable over time. However, subjective assessments and a lack of objective methods have dominated the analysis of palatal rugae.

Recent data shows significant improvements in the accuracy of IOSs, which candidate them as valuable tools in disaster victim identification. We hypothesize that integrating IOSs could speed up the identification process, enhance accuracy, and improve efficiency in postmortem conditions.

Dental records, fingerprints, and DNA-based identification are increasingly used for identification purposes. However, the success of dental identification depends on the availability and quality of ante-mortem

data, as well as the digitization and standardization of information. The widespread usage of IOSs facilitates building up a digital ante-mortem dental record database. The digital data can be searched automatically, rapidly, and accurately. Therefore, scan-based identification could be a good candidate in disaster victim identification (DVI), when hundreds or thousands of corps should be identified in a short time.

Another challenge during identification is distinguishing monozygotic (MZ) twin siblings due to almost identical DNA and strong phenotype similarity. However, the proposed unique morphology of the palate might promote MZ sibling identification using IOS. Furthermore, we assumed that if a particular pattern (e.g., palatal intraoral scan) could distinguish two MZ siblings, it could be considered unique for everybody.

## **2. Objectives**

### *2.1 Application of palatal intraoral scan to identify monozygotic twins*

1. To determine the repeatability (precision) of palatal scans.
2. To compare the deviation of palatal scans between MZ siblings to the repeatability.

3. To estimate the probability of distinguishing between MZ siblings by calculating tolerance limits.
4. To estimate the probability of distinguishing between MZ and dizygotic (DZ) twins.
5. To determine the effect of age on the difference between twin siblings.

## *2.2 The discriminative potential of palatal geometry for sex discrimination and human identification*

We proposed that digital palatal geometry is a good candidate for prescreening for human identification (before verification by 3D scan superimposition), as an adjunct in forensic dental identification, and as contextual evidence for sex determination investigation.

1. To determine the contribution of the surface morphology (i.e., rugae) to the discriminative potential of the palate.
2. To determine the reliability of digital palatal geometry measurement.
3. To compare the geometry between sexes
4. To estimate the correlation between geometry parameters (height, depth, and width)
5. To determine the discriminative potential of palatal geometry for sex prediction.

6. To determine the discriminative potential of palatal geometry for identification.

### **3. Methods**

#### *3.1 Recruited twin population*

A total of 201 twin participants, including 3 triplets, were included in the study. The distribution of zygosity among the pairs was as follows: 64 MZ, 33 same-sex DZ (DZSS), and seven opposite-sex DZ (DZOS). The age range of the twins was 17 to 74 years, with a mean age of 32 and a standard deviation of 14.5 years. The first-born sibling was labeled as A, the second-born as B, and in the case of triplets, the third-born sibling was denoted as C.

##### *3.1.1 Determination of zygosity*

Based on approved ethical permission, twins were selected from the Hungarian Twin Register (HTR) database. Zygosity was determined by a standardized questionnaire with nearly 99% accuracy.

#### *3.2 Application of IOS to identify monozygotic twins*

##### *3.2.1 Study subjects*

One MZ pair was excluded from the analysis because we failed to make a proper palatal scan. Therefore, 199 twin participants were analyzed.

### *3.2.2 Data acquisition, alignment methods, and surface comparison*

The palatal area of each subject was scanned by Emerald® intraoral scanner three times. The scans were aligned by the iterative closest point algorithm, replicates within the same subject (intra-subject deviation) and between siblings within a twin pair (intra-twin deviation) in the GOM Inspect® software. The mean absolute deviation (MAD) between aligned surfaces was determined.

### *3.2.3 Statistical analysis*

Mean  $\pm$  standard error of the mean (SE) was used to indicate the data in the text and figures. Generalized linear mixed models with gamma distribution and log-link function were employed to determine the variances for ISD, as well as for the comparisons between ISD and ITD, and between mono- and dizygotic ITD. These variances were utilized to calculate one-sided tolerance intervals, ensuring 99% population coverage with 95% confidence without overlapping between the two populations.

### *3.3 The discriminative potential of palatal geometric analysis for sex discrimination and human identification*

#### *3.3.1 Study subjects*

61 MZ and 26 same-sex DZ twin pairs were selected from the recruited twin population.

#### *3.3.2 The effect of the surface morphology (rugae) on MAD, smoothing the palate*

The teeth and the marginal gingiva were digitally removed, generating a palate-only model from the included 522 scans. The palatal rugae was removed by the "Sculpt" function, to generate smoothed scans in Meshmixer software.

The superimposition was performed between the three replicates of the twin siblings on the original and smoothed scans by the GOM Inspect® software local best-fit function using an iterative closest point algorithm. The intra-twin (between siblings) MAD was calculated for the original and the smoothed scan alignment.

#### *3.3.3 Geometric measurement of the palate*

59 scans (42 MZ and 17 DZ) were analyzed after excluding 28 twin pairs of scans with inadequate imaging of the first molar. The height, depth, and width were

measured according in GOM Inspect software. The Intermolar Line (IML) determined the palatal width, while the Central Point (CP) and the Bottom Horizontal Plane (BHP) were used to define the palatal depth. The Top Horizontal Plane (THP) was constructed parallel to BHP to calculate the palatal height as the distance between the two planes.

#### *3.3.4 Statistical analysis*

The MAD was evaluated using a generalized linear mixed model with the log-link function and gamma distribution. Inter-observer variability was assessed for geometric measurements, and the male and female palatal parameters were compared using a linear mixed model. Linear discriminant analysis (LDA) was conducted to create discriminant functions for sex determination and classifications of scan pairs in identity, stranger, MZ, and DZ. Statistical analyses were performed using IBM SPSS Statistics, Version 27, with a significance level of  $p < 0.05$ .

## **4. Results**

### *4.1 Application of IOS to identify monozygotic twins*

#### *4.1.1 Repeatability (precision) of palatal intraoral scan*

The MAD of ISD was found to be  $35.3 \mu\text{m} \pm 0.78 \mu\text{m}$ , indicating good repeatability or precision. There were no

significant differences in ISD between MZ and DZ twins. The upper tolerance intervals for both twins ranged from 67  $\mu\text{m}$  to 95  $\mu\text{m}$ .

#### *4.1.2 The deviation of palatal scans between MZ siblings*

The mean ITD of the 64 MZ twins was significantly higher than the ISD values ( $411 \pm 15.2 \mu\text{m}$  vs.  $37 \pm 1.1 \mu\text{m}$ ,  $p < 0.001$ ).

#### *4.1.3 The probability of distinguishing between MZ siblings*

The calculated lower 99% tolerance limit (with 99% confidence) of the ITD of MZ twins was 147  $\mu\text{m}$ , and the upper 99% tolerance interval of ISD was 73  $\mu\text{m}$  with 99% confidence.

#### *4.1.4 The probability of distinguishing between MZ and DZ twins*

The mean ITD of MZ twins ( $406 \pm 15 \mu\text{m}$ ) was significantly lower than that of dizygotic same-sex twins (DZSS) ( $594 \mu\text{m} \pm 53 \mu\text{m}$ ,  $p < 0.01$ ) and that of dizygotic same-sex twins (DZOS) ( $853 \mu\text{m} \pm 202 \mu\text{m}$ ,  $p < 0.05$ ). No significant difference was observed between DZSS and DZOS twins. The tolerance limit was not calculated due to the substantial overlap between MZ and DZ populations.

#### *4.1.5 Effect of age on ITD*

A weak but significant correlation ( $r = 0.3$ ,  $p < 0.05$ ) was found between the ITD and the subject's age in MZ twins. From the regression equation ( $y = 384 + 3 * \text{age}$ ), the mean ITD was 430  $\mu\text{m}$  at age 17, increasing by three  $\mu\text{m}$  every year.

### *4.2 The discriminative potential of palatal geometric analysis for sex discrimination and human identification*

#### *4.2.1 The contribution of the surface morphology to the discriminative potential of the palate*

The ITD MAD of the original scan was not significantly different from the smoothed scan in either MZ ( $0.430 \pm 0.018$  mm vs.  $0.425 \pm 0.022$  mm,  $p = 0.061$ ) or DZ ( $0.621 \pm 0.058$  mm vs.  $0.586 \pm 0.053$  mm,  $p = 0.284$ ).

#### *4.2.2 Reliability of palatal geometry measurement*

The coefficient of variation was found to be 3.7% for height, 3.5% for depth, and 1.0% for width, indicating high precision in scanning repeatability. The ICC analysis showed a high level of agreement between the two observers for height, depth, and width measurements. Although there was a statistically significant difference in

height measurements between the observers, the average difference was less than 0.1 mm.

#### *4.2.3 Comparison of the geometry between sexes*

The palatal height and width were significantly higher in males than in females by 2.4 mm and 1.6 mm, respectively. However, the difference in depth was insignificant.

#### *4.2.4 Correlation between geometry parameters*

No correlation ( $r = 0.13$ ,  $p = 0.164$ ) was seen between palatal height and palatal depth nor between palatal width and either palatal height ( $r = -0.04$ ,  $p = 0.689$ ) or palatal depth ( $r = 0.13$ ,  $p = 0.163$ ). These results indicated that palatal height, depth, and width are independent variables suitable for LDA.

#### *4.2.5 Linear discriminant analysis for sex prediction*

The LDA showed significant discrimination between sexes, with height demonstrating the highest degree of discrimination ( $r=0.83$ ), followed by width ( $r=0.37$ ) and depth ( $r=0.24$ ). Two classification formulas were derived from the LDA analysis to predict the sex

$$Y_{\text{female}} = 6.45 \times \text{height} + 5.56 \times \text{width} + 4.23 \times \text{depth} - 204$$

$$Y_{\text{male}} = 7.35 \times \text{height} + 5.91 \times \text{width} + 4.33 \times \text{depth} - 233$$

The classification function correctly identified 82.2% of females and 89.3% of males in the dataset.

#### *4.2.6 The discriminant function of geometry parameters for identification*

The LDA analysis revealed significant discrimination functions, with height showing the highest potential for discrimination (0.63), followed by width (0.62) and depth (0.47). The absolute differences in geometry parameters between paired scans were calculated and used in classification equations.

$$Y_{\text{identity}} = 1.01 \times \text{sqr\_height} + 1.00 \times \text{sqr\_width} + 1.73 \times \text{sqr\_depth} - 2.46$$

$$Y_{\text{MZT}} = 2.25 \times \text{sqr\_height} + 2.15 \times \text{sqr\_width} + 2.75 \times \text{sqr\_depth} - 5.07$$

$$Y_{\text{DZT}} = 2.87 \times \text{sqr\_height} + 2.81 \times \text{sqr\_width} + 3.20 \times \text{sqr\_depth} - 7.02$$

$$Y_{\text{stranger}} = 3.59 \times \text{sqr\_height} + 3.57 \times \text{sqr\_width} + 3.65 \times \text{sqr\_depth} - 9.23$$

The LDA correctly classified scans of the same individuals with a sensitivity of 91.2% and specificity of 97.8%. For twin classification, combining MZ and DZ groups, the sensitivity was 68.5%, and the specificity was 61.9%.

## 6. Conclusions

### *6.1 Application of intraoral scanner to identify monozygotic twins*

- Monozygotic twin siblings can be distinguished with high confidence by the superimposition of intraoral scans of the palate. Therefore, identical twin discrimination implies palatal scan uniqueness for the whole human population.
- Ante- and postmortem data acquisition by IOS is quick and easy.
- The 3D superimposition is highly reliable; however, searching in big data might not be fast enough. Therefore, further data preprocessing by finding the unique feature on the palatal scan is essential.

### *6.2 The discriminative potential of palatal geometry for sex discrimination and human identification*

- Palatal geometrics possesses unique identification features.
- In addition, as the use of IOSs increases, the availability of 3D data will increase, and this technique could be a valuable triage tool by reducing the possible matches before 3D superimposition.

- Furthermore, palatal geometry could serve as additional contextual evidence to corroborate other supporting data in sex determination

## **6. Candidate's publications used for the thesis**

Simon, B., K. Aschheim, and J. Vág, The discriminative potential of palatal geometric analysis for sex discrimination and human identification. *Journal of Forensic Sciences*, 2022. 64(4).  
<https://doi.org/10.1111/1556-4029.15110>

Simon B, Lipták L, Lipták K, Tárnoki ÁD, Tárnoki DL, Melicher D, Vág J: Application of intraoral scanner to identify monozygotic twins. *BMC Oral Health* 2020, 20(1):268. <https://doi.org/10.1186/s12903-020-01261-w>