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ESTUDO TRIDIMENSIONAL DA UNICIDADE DA DENTIÇÃO HUMANA APLICADO À ANÁLISE DE MARCAS DE MORDIDA

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Tese apresentada ao Programa de Pós-graduação em Odontologia, área de concentração em Estomatologia, da Escola de Ciências da Vida, da Pontifícia Universidade Católica do Paraná, como requisito parcial para a obtenção do título de Doutor em Odontologia.

Orientador: Prof. Paulo Henrique Couto Souza
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Resumo

A unicidade da dentição humana (UDH) é um dos tópicos mais polêmicos em Ciências Forenses. A UDH é comumente referida nos campos da identificação humana e análise de marcas de mordida. Em identificações humanas, a UDH é dificilmente contestada legalmente. Contudo, quando considerada para análise de marcas de mordida, a UDH é fonte de incerteza desde 2009. A UDH consiste em um mecanismo fundamental que garante que duas pessoas não apresentam as mesmas características dentais. A falta deste mecanismo representa um problema de grandes proporções para o sistema jurídico. No contexto das identificações humanas, os familiares da suposta vítima poderiam questionar a confiabilidade do laudo pericial, alegando que o corpo examinado não pertence a um membro da família supostamente desaparecido. Este fato é ainda exacerbado considerando os fatos psicológicos que impactam os familiares em um potencial cenário de luto. No contexto de análise de marcas de mordida, o suposto suspeito poderia alegar que as impressões dentais encontradas na cena do crime não pertencem a ele, sugerindo que características dentais iguais poderiam ser encontradas em outras pessoas.

Muitos estudos investigaram a UDH, mas poucos obtiveram êxito em prová-la. Os poucos estudos que provaram a existência de UDH apresentaram importantes limitações metodológicas. Em específico, a imaginologia 3D, pouco viável em décadas passadas, restringiu a análise de morfologia dental por meio de fotografias e escaneamento 2D. Além disso, o conhecimento estatístico aplicado aos modelos de pesquisa experimentais foi refutado quando reproduzido recentemente. A amostragem populacional também revela influência limitante nos estudos passados, sendo geralmente reduzida e dificilmente representativa para um cenário aberto. Alem de limitada pelo tamanho, a amostragem foi também prejudicada em relação ao material coletado. Alguns estudos objetivaram testar a UDH comparando a morfologia dental entre modelos odontológicos e marcas de mordida, enquanto outros compararam apenas marcas de mordidas entre si. Atualmente, sabe-se que provar a UDH é o primeiro passo em direção a validar as análises periciais em marcas de mordida. Após isto, faz-se necessário verificar se a UDH é transferida para o substrato mordido. Assim, a UDH deve ser essencialmente investigada comparando apenas modelos odontológicos entre si.

Considerando essas limitações metodológicas, tem-se na a utilização de imaginologia para escaneamento e comparação morfológica 3D uma ferramenta alternativa para otimizar as investigações em UDH. Esta ferramenta foi utilizada nesta pesquisa por trabalhar em um panorama mais realístico e por providenciar resultados mais precisos comparados aos estudos anteriores. Em paralelo, a amostragem também foi aprimorada. Estratificação populacional foi realizada, selecionando indivíduos com características dentais semelhantes, permitindo investigar a UDH em amostras de menor tamanho, mas mantendo a confiabilidade dos resultados.

A combinação de tecnologia e amostragem reestruturadas resultou no aprimoramento das investigações acerca da UDH. Dentições únicas foram observadas entre indivíduos estratificados pela similaridade no alinhamento (pacientes ortodônticos) e morfologia (gêmeos) dental. Contudo, para tornar estes resultados aplicáveis às perícias em marcas de mordida, foi realizada uma análise acerca da influência da quantidade de material dental sobre a diferenciação entre indivíduos. Tendo a influência confirmada, a investigação da UDH foi reconduzida, utilizando apenas a borda incisal dos dentes anteriores (porção dental geralmente registrada em marcas de mordida). Neste contexto, a dentição humana não pôde ser considerada única.

Estes resultados indicam que as perícias em marcas de mordida devem ser realizadas dependendo de cada caso, sendo especialmente evitadas quando os suspeitos apresentam similaridade dental. Estes achados devem ser interpretados como passos iniciais no campo das perícias em marcas de mordida. Estudos futuros devem verificar se a UDH é transferida para a pele humana; e avaliar a capacidade dos Odontolegistas frente à análise de evidências. Faz-se importante notar que o campo de identificações humanas pode também se beneficiar do presente estudo, uma vez que a UDH é útil para respaldar a identificação e diferenciação entre vítimas perante a justiça.

Palavras-chave: Unicidade; Dentição humana; Morfologia; Marcas de mordida; Imaginologia.

Abstract

The uniqueness of the human dentition (UHD) is one of the most polemic topics in forensic sciences. It is commonly assumed in forensic odontology fields of dental human identification and bitemark (BM) analysis. Uniqueness in dental human identifications is hardly contested by the legal system. Yet in BM analysis, uncertainty was raised in 2009 and remained up to now.

UHD is the basic mechanism that guarantees that two persons in the world will not have the same dental features. The lack of this mechanism represents a problem of large proportions for the legal system. In the dental human identification context, the relatives of deceased victims would doubt the reliability of the forensic report claiming that the identified body did not belong to the identified missing person. This fact is even worse considering the psychological impact of death involved in the dental human identification environment. In the BM analysis context, the identified perpetrator would claim that the dental impression found in a crime scene does not belong to him, suggesting that equal dental features could be found in an open population.

Several researches investigated the UHD, but few studies succeeded in proving it. All the researches that proved the UHD presented important limitations, mostly related to contemporary aspects. Specifically, the lack of 3D technology was common in the past decades, making necessary to restrict the registration of dental morphology with 2D imaging devices, such as photographic cameras and flatbed scanners. Moreover, the lack of proper statistical inferences was detected in experiments in the past. These experiments were reproduced within a more contemporary statistical scope resulting with totally different outcomes. Sampling also figured as the main limitations found in studies proving the UHD. Small random populations not representative for a worldwide scenario were used, and sample stratification was barely attempted. Additionally, the sample was not only limited in size but also related to the used material. Some studies aimed to search for uniqueness comparing dental casts with bite impressions, or even comparing two bite impressions. Currently, it is known that a first step towards the researches in BM analysis is to find out if dentitions are unique. The second step is to verify if the uniqueness is transferred to the impression material. This is the reason why uniqueness should be investigated comparing pair-wise dental casts.

In face of the limitations in the past, contemporary technology such as 3D laser scanners, advanced software packages for 3D modeling and automated image superimposition revealed to be alternative tools for the optimization of comparisons between human dentitions. These tools not only work on a more realistic panorama but also provide more accurate results compared to the methodologies in the past. In parallel, sampling was also improved. Stratification of populations enabled the search for uniqueness in smaller scenarios, reducing the labor work but maintaining the reliability of outcomes.

The combination of enhanced technology and sampling culminated in the improvement on the investigations about the UHD. Unique dentitions were found in populations of individuals with known similar dental arrangement – orthodontically treated patients; and morphology – twins when analyzing the complete dental crown morphology of the anterior teeth. However, the uniqueness observed was not completely corresponding to the level of BM. A test on the tooth quantity was performed to verify its influence within the morphological differentiation between dentitions. Once the influence was confirmed, the analysis of the UHD was performed considering only the incisal edges of the anterior teeth. As consequence, the UHD was not observed in the same populations.

The outcomes shown in the present research showed that BM cases should be performed preferably based on case selection, and especially avoided when the suspects present similar morphological dental traits. Moreover, these findings should be interpreted as initial steps towards testing the reliability of the BM evidence. Further studies must be performed to verify if the UHDs is transferred to the human skin, or foodstuff. Additionally, an important step to be executed is the technical assessment of forensic odontologists on the analysis and interpretation of BM evidences. Finally, it is worth to highlight that the research field of dental human identifications also benefited from the present outcomes, once most of the data from the UHD may be extrapolated and interpreted for identification purposes.

Keywords: Uniqueness; Human dentition; Morphology; Bitemarks; Imaging.

Abreviaturas

2D – Bidimensional

3D – Tridimensional

3D-DMI – Imagem tridimensional de modelo odontológico

3D-ROMI – Imagem tridimensional de modelo de objeto retangular

ABFO – American Board of Forensic Odontology

AM – Ante-Mortem

AVL – Comprimento médio residual

BM – Bitemark (marca de mordida)

CC – Cloud Compare® software

CEP – Comitê de Ética em Pesquisa

DCF – Arquivo digital de modelo

DNA – Ácido desoxirribonucleico

GS – Geomagic Studio® software

INTERPOL – International Criminal Police Organization

KUL – Katholieke Universiteit Leuven

MS – Maestro 3D Ortho Viewer® software

N/M – Não mencionado

NAS – National Academy of Science

PM – Post-Mortem

PUCPR – Pontifícia Universidade Católica do Paraná

RMSS – Raiz média quadrática

UHD – Unicidade da dentição humana

Sumário

Introdução Geral

Capítulo 1

Capítulo baseado no artigo científico:

Ademir Franco. Unique or not unique?! That is the question! – Opinion article on a bitemark scope. RBOL 2015;2(2):126-13

A etimologia do termo “unicidade” deriva de “único”, um adjetivo/substantivo do Latim utilizado para descrever algo sem outro igual (Wordsmyth 2016) ou algo diferente de qualquer outro em comparação (Abdulkader et al. 2004; Dictionary-Oxford 2015; Dictionary-Thesaurus 2015; Dictionary-Cambridge 2015). Similarmente, este termo é também utilizado em lógica matemática para indicar que existe “um e apenas um” objeto com uma determinada característica (Wackerly 2012).

Nas ciências forenses, os procedimentos que relacionam vítimas e suspeitos às cenas de crimes dependem principalmente na comparação de evidências. Neste contexto, a unicidade garante que duas ou mais vítimas ou suspeitos não apresentem as mesmas características (evidências) (Lucas & Henneberg 2015). Por outro lado, a unicidade também permite que uma marca deixada na cena de um crime possa ser rastreada exclusivamente para “um e apenas um” objeto de origem por meio da convergência de uma ou muitas características convergentes (Kaye 2009). A importância forense da unicidade é atualmente mais evidente para a Papiloscopia, e para a análise dental e genética forense (Page et al. 2011). Contudo, é também essencial para a Rugoscopia (Dawasaz & Dinkar 2013), a Queiloscopia (Raghu et al. 2013), a Grafoscopia (Faigman 2008) e a Balística (Nichols 2007). A unicidade é uma variável categórica nominal, o que implica que algo não pode ser classificado como muito ou pouco único, mas sim único ou não (Wordsmyth 2016). Já para classificar algo como único, a probabilidade de encontrar sua duplicata deve ser nula (Page et al. 2011).

Apesar de dicotômica (existente ou ausente), a probabilidade de se encontrar algo único pode ser quantificada. Isto se dá não apenas pelo avanço científico em estatística aplicada (Kaye 2003), mas também pela necessidade em se obter maior certeza quanto às populações e características dentais examinadas, assim como os potenciais erros inerentes à investigação da unicidade. Apesar de ser amplamente aceita perante a lei, a unicidade não é completamente aceita pela comunidade científica quando relacionada à Papiloscopia (Kaye 2003). Este criticismo científico varia desde aspectos biológicos a fundamentos estatísticos, como o incerto mecanismo biológico que controla a manifestação de padrões datiloscópicos diferentes em cada pessoa

(Page et al. 2011), e a falta de padrões para a análise estatística em pesquisas científicas (Kaye 2003), respectivamente.

Com o objetivo de minimizar a probabilidade de correspondência entre impressões digitais de diferentes pessoas, um número mínimo de pontos de concordância foi estabelecido para a análise comparativa. Na maioria dos sistemas legais, esta análise baseia-se na concordância de, pelo menos, 12 pontos correspondentes (Acharya & Taylor 2003). Contudo, a literature não relata consenso exato na probabilidade de falsas concordâncias. Estimativas quantificadas apontam uma variância de falsas concordâncias de 1 em 1.2×10^{-80} a 1 em 1.4×10^{-2} (Pankanti et al. 2002). No mesmo sentido, a análise forense de DNA é aceita com alta confiabilidade quando solicitada pela justiça, tornando-se padrão ouro para a análise retrospectiva de casos não solucionados no passado (Committee on Identifying the Needs of the Forensic Sciences Community 2009).

Apesar disto, criticismo neste campo também é relatado, principalmente relacionado à total independência de alelos considerada em pesquisas científicas, a qual pode gerar resultados menos confiáveis para a probabilidade de falsas concordâncias (Page et al. 2011). Um número mínimo de pontos concordantes também foi estabelecido para a análise comparativa de DNA forense. Este pontos são representados pelos locos genéticos, que em geral devem atingir 13 correspondências (Nelson & Cox 2013; Butler 2014). As diferentes abordagens estatísticas usadas para testar a probabilidade de correspondências entre locos genéticos, assim como sua variabilidade, é discutida entre cientistas forenses. Valores quantificados reportados para falsas correspondências são de 1 em 1.32×10^{-12} em Hispânicos, e 1 em 5.59×10^{-14} em Negros, considerando 5 locos, independentemente (Risch & Devlin 1992).

A Odontologia Forense também encontra dificuldades para a quantificação de valores concretos para a unicidade da dentição humana (Franco et al. 2015). Neste campo forense, a unicidade desempenha papel essencial em duas principais áreas: identificações humanas e análise de marcas de mordida (BM). Assim como a Papiloscopia e a genética forense, a identificação humana é amplamente aceita perante a lei (INTERPOL 2014). Nesta área, a unicidade é usada para indicar que duas vítimas não apresentam as mesmas características dentais. Estas características podem ser ralacionadas à morfologia dental (Silva et al. 2015; da Silva et al. 2008) (ex: tamanho, forma, angulação e posição dos dentes), tratamentos odontológicos (Zondag & Phillips 2009; Phillips & Stuhlinger 2009b; Phillips & Stuhlinger 2009a; R. Silva et al. 2014) (e.g. restaurações e próteses dentais), patologias (Waleed et al. 2015) (e.g. dentes cariados e lesões periapicais) e a combinação destas. Diferentemente da análise de impressões digitais e genética Forense, não um número mínimo de pontos coincidentes a serem estabelecidos para a identificação dentária (Acharya & Taylor 2003; Silva et al. 2009). Mais especificamente, o processo de identificação pode se basear na análise qualitativa de até uma única característica dental. Provar a unicidade da dentição humana (UHD) neste contexto garante que os dados ante-mortem (AM) de uma única pessoa irão convergir com os dados post-mortem (PM) de uma vítima (Senn & Weems 2013; Senn & Stimson 2010). As consequências legais de não se provar a UHD é dar aos familiares da vítima a possibilidade de reivindicar que o corpo identificado pertence à outra pessoa.

Análises estatísticas e probabilidades concretas para se assumir a UHD não foram estabelecidas para a identificação humana. Contudo, esta ciência é considerada confiável na prática Forense. Isto se dá, primeiramente, pela possibilidade de múltiplas combinações de características dentais. Estudos apontam que 2.5 bilhões de combinações são possíveis considerando os 32 dentes permanentes (Pereira & Santos 2013). Além disso, a identificação humana é considerada confiável devido à unicidade potencialmente conferida às características dentais terapêuticas e patológicas. Por outro lado, a análise de marcas de mordida permanece sob os holofotes do criticismo há aproximadamente uma década. Em 2009, a Academia Nacional Norte Americana de Ciência liberou um relatório compilado com limitações metodológicas que careciam de melhorias. As principais questões a serem respondidas por meio das melhorias científicas foram: 1) A dentição humana é única? 2) Caso seja, essa unicidade é transferida para a pele humana? e 3) Caso seja, as marcas de mordida têm sido devidamente interpretada pelos peritos forenses? (Committee on Identifying the Needs of the Forensic Sciences Community 2009). Claramente, a UHD compreende a base fundamental das limitações inerentes à análise de marcas de mordida.

A comprovação acerca da UHD aplicada à marcas de mordida deve levar em consideração principalmente dois fatores (Franco et al. 2015): primeiramente, o número reduzido de possíveis combinações de caracteres dentais – que são, em sua maioria de origem morfológica (e.g. anatomia da coroa dental, tamanho, angulação e posição) (Franco et al. 2015); em segundo, a quantidade reduzida de partes dentais envolvidas nas BM, a qual se restringe (principalmente) à borda incisal dos 6 dentes anteriores (dentição anterior) de cada arcada (Franco 2015; Dorion 2011). A dentição considerada no presente estudo compreende a combinação dos dentes anteriores, incluindo sua morfologia individual e sua posição na arcada. Neste contexto, faz-se importante saber que as BM podem ser encontradas não somente na pele humana, mas também em material inanimada, como alimentos e objetos (Dorion 2011; Bernitz et al. 2000; Bernitz & Kloppers 2002; Webster 1982). Nos inanimados, a quantidade de material dental envolvida pode ser diferente daquela envolvida na pela humana, contribuindo ou dificultando com a análise forense.

Provar a UHD envolvida nas BM garante que apenas um único suspeito possa ser relacionado ao crime. Por outro lado, não provar a UHD fornece ao suspeito a possibilidade de reivindicar inocência, sugerindo que outra pessoa cometeu o crime. A literatura científica corrobora com o fato de que todos as aspectos envolvidos em BM devem ser investigados sistematicamente antes de sua aplicação prática (Bush et al., 2011a; Pretty, 2006; Pretty & Sweet, 2001). Casos de condenações de inocentes baseados na análise de BM emergiram gradualmente nos últimos anos, ilustrando e confirmado este cenário problemático (The Innocence Project 2007; Balko 2015). Com base nestas informações, a aceitação legal (The Innocence Project 2016) da existência (ou não) da UHD deve ser investigada em relação aos padrões de Daubert para a prova técnica pericial (regra 104): *“A UHD foi testada em condições realísticas? A UHD foi sujeitada à revisão por pares e publicação? Qual é o potencial de erro para se testar a UHD? Existem padrões para o controle de testes envolvendo a UHD? A UHD tem sido aceita na comunidade científica?”*. A presente pesquisa surge neste contexto para respaldar cientificamente as deficiências encontradas nos estudos relacionados à UHD. Em específico, esta pesquisa visa responder tecnicamente questões acerca da prova ou inexistência da UHD no contexto de BM em pele humana.

No início dos anos 80, a investigação científica da UHD no contexto de BM era realizada bidimensionalmente (2D) utilizando o contorno das bordas incisais de impressões dentais registradas em cera (Sognnaes et al. 1982; Rawson et al. 1984). Nesta época, a UHD foi comprovada, e de acordo com alguns autores reivindicada “sem qualquer sombra de dúvidas” (Rawson et al. 1984). Contudo, a análise 2D realizada para a investigar estruturas tridimensionais (3D) não era realística, resultando em uma prova não confiável da UHD. Adicionalmente, a informação morfológica extraída de registros dentais em cera apresentava distorções inerentes por se tratar de um mecanismo indireto de coleta de dados – diferente da análise de dentições propriamente ditas, por exemplo.

Aproximadamente 25 depois, os estudos em 2D ainda eram conduzidos. Desta vez o contorno da borda incisal dos dentes anteriores eram registrados por meio do escaneamento de modelos de gesso (Kieser et al. 2007). Em paralelo, a geometria morfométrica era utilizada, permitindo a quantificação das informações morfológicas extraídas de formas geométricas (Kieser et al. 2007) por meio da comparação da Posição especial de landmarks homólogos registrados como coordenadas Cartesianas em cada modelo de gesso (Webster & Sheets 2010). Apesar disto, a limitação de se analisar estruturas 3D em 2D permanecia. Além disto, está técnica era baseada na comparação de landmarks posicionados manualmente, dependendo da performance de cada examinador.

Atualmente, a imaginologia 3D se tornou parte dos mecanismos de registro de estruturas bucais. A imaginologia 3D é utilizada em diversos ramos da Odontologia, como Ortodontia, Cirurgia Bucomaxilofacial, Estomatologia e Radiologia, para o diagnóstico, plano de tratamento, e acompanhamento clínico de pacientes (Baltacıoĝlu et al. 2016; Santos et al. 2016; do Nascimento et al. 2016; Sinha et al. 2016; Alqerban et al. 2014; Maruo et al. 2016; Duran et al. 2016; Cousley & Turner 2014); em Reabilitação bucal, para intervenções restauradoras (Charette et al. 2016; Barros et al. 2015; Abduo et al. 2015; Patzelt et al. 2014); e em Odontologia Legal, para identificações humanas (Franco et al. 2013; Do Rosário et al. 2012; Silva et al. 2011); processos cíveis

(Silveira et al. 2014); análise de BM (Naether et al. 2012; Thali et al. 2003) e investigações em UHD (Blackwell et al. 2007; Bush et al. 2011a; Sheets et al. 2013; Bush et al. 2011b). Todos esses ramos se beneficiaram da tecnologia 3D, especialmente pela precisão na aquisição digital de imagens da estrutura morfológica dental e maxilofacial. Contudo, faz-se importante notar que a tecnologia 3D também possui potenciais erros metodológicos, que devem ser considerados na investigação da UHD, como a coleta de impressão dental e a confecção de modelos odontológicos em gesso, a digitalização de modelos, e sua manipulação e comparação por meio de softwares.

A presente pesquisa foi justificada para investigar a UHD no contexto de BM comparando pares de modelos odontológicos 3D. Em cada capítulo desta pesquisa o termo "dentição" foi usado com significados diferentes, de acordo com objetivo específico a ser investigado. Em geral, este termo seguiu uma descrição preconizada na literatura científica descrevendo a coroa clínica dos 6 dentes anteriores de cada arcada (Kieser et al. 2007). Diferente dos estudos realizados no passado, (Sognnaes et al. 1982; Rawson et al. 1984), BM não foram utilizadas, mas sim dentições humanas registradas 3D em modelos de gesso. Isto se explica pelo fato de que a UHD é fundamentalmente relacionada à dentição em si, enquanto as BM são relacionadas à dentição e sua relação co a pele humana. Esta abordagem fundamental permite a investigação da morfologia dental sistematicamente adaptada em função de suas partes envolvidas em BM em pele humana. Por este motivo, modelos de gesso digitalizados 3D foram escolhidos como material primário de estudo. O mesmo princípio foi utilizado em pelo menos 6 estudos anteriores. (Sheets et al. 2013; Sheets et al. 2011; Blackwell et al. 2007; Kieser et al. 2007; Bush et al. 2011a; Bush et al. 2011b).

O presente trabalho levou em consideração a limitações dos estudos anteriores, superando-as – em específico, a limitação de adquirir e analisar imagens 3D em 2D (Kieser et al. 2007; Sheets et al. 2011; Sognnaes et al. 1982; Rawson et al. 1984; Nambiar et al. 1995b; Nambiar et al. 1995a) foi superada pesquisando interdisciplinarmente por uma ferramenta nos campos da Engenharia e Design Gráfico. Nestes campos, objetos são digitalizados, sobrepostos e comparados em pares para revelar sua potencial diferença morfológica. As ferramentas disponíveis nestes campos são contidas em softwares, como o Geomagic Studio® (3D Systems®, Rock Hill, SC, USA) e o Cloud Compare® (Telecom Paris Tech® and EDF®, Paris, France). Os mecanismos destes softwares incluem a sobreposição e comparação automatizada de objetos 3D. Esta ferramentas são potencialmente aplicáveis em Odontologia Legal, especialmente para investigar a UHD (Abduo & Bennamoun 2013; Moraes et al. 2014) tendo como objetos comparáveis as dentições humanas. A potencial limitação inerente ao uso desta tecnologia em Odontologia Legal é a ausência da validação de suas ferramentas em dentições. Esta limitação pode ser solucionada com testes científicos quanto às propriedades e acurácia dos softwares para a preparação, orientação e comparação 3D de dentições humanas. Um outro ponto a ser considerado é a dependência de procedimentos que envolvem o examinador, como a sobreposição de imagens pela registro manual de landmarks. Esta limitação é passível de melhoria procurando e testando ferramentas para a sobreposição automática de objetos. Ademais, as limitações na investigação da UHD não são somente relacionadas à comparação de objetos, mas sim à coleta amostral.

Selecionar uma amostra grande o suficiente para representar a população mundial é praticamente inviável. Porém, a literatura sugere que populações estratificadas pela similaridade dental podem ser usadas para reduzir a amostra e, ainda, fornecer inferências mais concretas acerca da UHD. A similaridade dental pode ser baseada tanto na presença de características dentais, como a rotação dental (por exemplo: selecionando indivíduos com a rotação mesiodistal do canino superior esquerdo), quanto pelo alinhamento (por exemplo: selecionando pacientes tratados ortodonticamente) (Sheets et al. 2011; Kieser et al. 2007) e morfologia dental (por exemplo: selecionando indivíduos gêmeos) (Sognnaes et al. 1982). Estes tipos específicos de populações acompanham necessariamente a hipótese de que “se a UHD exite, ela irá se manifestar mesmo entre indivíduos com dentições muito similares). Desta forma, a presente pesquisa considerou a UHD ausente na presença de concordância morfológica entre pares de dentições de indivíduos diferentes. Já a concordância morfológica foi verificada quando os valores de diferença morfológica entre pares de dentições foram menores que o limite estabelecido considerando os erros inerentes à técnica.

Com base no exposto, esta pesquisa objetivou realizar uma análise 3D da morfologia dental, comparando pares de modelos odontológicos digitais 3D em função das partes dentais potencialmente envolvidas em BM em pele humana considerando amostras estratificadas por similaridade dental.

Objetivos e hipóteses

Capítulo 2

OBJETIVO E HIPÓTESE GERAL

Este trabalho objetivou realizar uma análise 3D da morfologia dental em busca da unicidade da dentição humana (UHD) no contexto de marcas de mordida (BM) em pele humana. A hipótese geral relacionada a este objetivo foi a de que "a morfologia da dentição humana anterior é única para cada indivíduo considerando a quantidade de material dental envolvido em BM em pele humana". Para documentar esta pesquisa, uma revisão sistemática foi realizada, indicando o estado atual das pesquisas em UHD. Com base nos resultados desta revisão sistemática, questionamentos foram feitos, gerando objetivos e hipóteses específicos para guiar cada capítulo desta pesquisa.

OBJETIVOS E HIPÓTESES ESPECÍFICOS

1° questionamento: **A UHD pode ser investigada com a tecnologia imaginológica 3D contemporânea?**

1° objetivo: *"Testar a tecnologia imaginológica 3D contemporânea para a comparação morfométrica automática de pares de modelos de gesso 3D digitalizados".*

1ª hipótese: "A tecnologia 3D contemporânea permite a investigação da UHD usando a *comparação morfométrica automática de pares de modelos de gesso 3D digitalizados".*

2° questionamento: **Pode uma amostra ser estratificada significantemente para a população geral a fim de provar a UHD?**

2° objetivo: *"Verificar se uma amostra significante para a população geral pode ser estratificada para provar a UHD".*

2ª hipótese: "Uma amostra significante para a população geral pode ser estratificada para provar a UHD".

3° questionamento: **A dentição humana é única ao se analisar a morfologia da coroa completa dos dentas anteriores?**

3° objetivo: *"Investigar a UHD analisando a morfologia da coroa completa dos dentes anteriores"*.

3^{a} hipótese: *“A dentição humana é única analisando a morfologia da coroa completa dos dentes anteriores”.*

4° questionamento: **A quantidade de material dental analisado influencia na diferença morfológica entre dentições?**
4° objetivo: *“Testar a diminuição da quantidade de material dental analisado em face da diferença morfológica entre dentições”*.
4^{a} hipótese: “A diferença morfológica entre dentições aumenta com o aumento da quantidade de material dental analisado”.

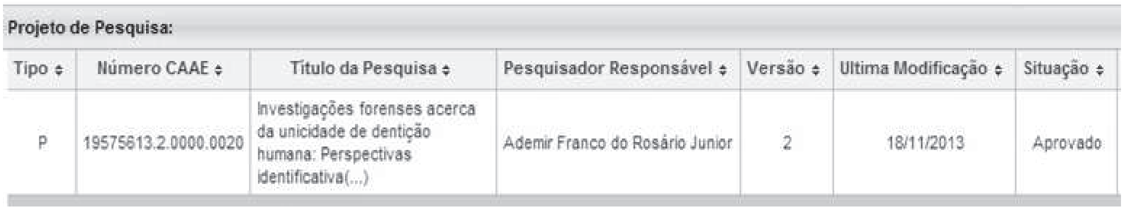
5° questionamento: **A dentição humana permanece única reduzindo sistematicamente a quantidade de material dental analisado dentro do contexto de BM em pele humana?**
5° objetivo: *“Investigar a UHD considerando a quantidade de material dental envolvido em BM em pele humana”*.
5^{a} hipótese: “A dentição humana permanece única quando a quantidade de material dental envolvido em BM em pele humana é analisada”.

ASPECTOS ÉTICOS

A presente pesquisa foi desenvolvida como requerimento parcial para a obtenção do título de Doutor em Odontologia, pela Pontifícia Universidade Católica do Paraná (PUCPR), e do título de doutor em Ciências Biomédicas, pela Katholieke Universiteit Leuven (KU Leuven). A aprovação do Comitê de Ética em Pesquisa da PUCPR foi obtida antes da execução de qualquer procedimento experimental, sendo esta pesquisa registrada na Plataforma Brasil sob o número: 19575613.2.0000.0020 (Figure 1).

Nenhum conflito de interesse foi relacionado à esta pesquisa.

Figura 1 – Aprovação ética para a execução do projeto de pesquisa



Projeto de Pesquisa:

Tipo	Número CAAE	Título da Pesquisa	Pesquisador Responsável	Versão	Ultima Modificação	Situação
P	19575613.2.0000.0020	Investigações forenses acerca da unicidade de dentição humana: Perspectivas identificativa(...)	Ademir Franco do Rosário Junior	2	18/11/2013	Aprovado

Revisão sistemática da literatura envolvendo a unicidade da dentição humana no contexto de marcas de mordida

Capítulo 3

Capítulo baseado no artigo:

Ademir Franco, Guy Willems, Paulo H. C. Souza, Geertruida E. Bekkering, Patrick Thevissen. The uniqueness of the human dentition as forensic evidence – a systematic review on the technological methodology. Int J Legal Med 2015;129(6):1277-1283.

Apresentação oral no 11^{o} Congresso da Sociedade Argentina de Odontologia Forense, Corrientes, Argentina, 2015

Apresentação oral na 10^{a} Semana Acadêmica de Odontologia da Pontifícia Universidade Católica do Paraná, Curitiba, Brasil, 2015

INTRODUCTION

New technological facilities and devices, such as three-dimensional (3D) surface scanners, intraoral cameras and photogrammetry were found to be reliable and accurate pathways for forensic studies (Naether et al. 2012; Sheets et al. 2013). These imaging modalities reveal particular usefulness for the analysis and comparison of human dentitions and allow for the realistic assessment of the uniqueness of the human dentition (UHD) based on dental morphology.

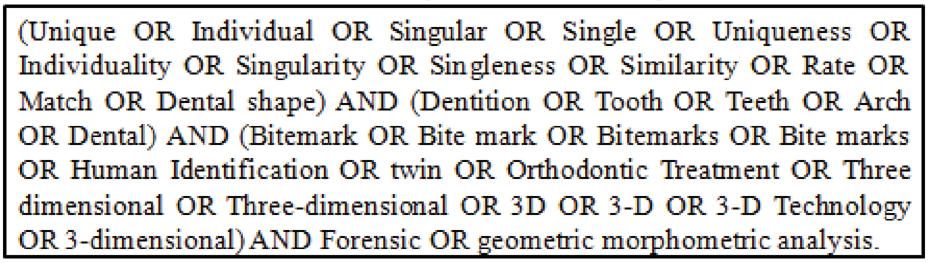
In order to screen and better comprehend the current status of the researches on the UHD as well as their methodology, a systematic literature review was performed, answering the following question: Was the UHD proven?

MATERIAL AND METHODS

Three scientific databases were systematically searched: SCIELO®, MedLine®/PubMed® and LILACS®, from inception up to April 9th 2014.

A search string of keywords was developed from the terms “uniqueness” and “dentition”. In order to detect a larger number of related manuscripts, the term “uniqueness” was modified to “unique”, and additional synonyms were implemented (“individual”, “individuality”, “singular”, “singularity”, “single”, and “singleness”). Using the Medical Subject Headings (MeSH®), the term “dentition” indicated additional terms (“tooth”, “teeth”, arch”, and “dental”). In order to accurately narrow the findings, special terms characterizing fields of interest (“bitemark”, “bite mark”, “bite marks”, “human identification”) and specific populations (“twin” and “orthodontic treatment”) were implemented. Finally, terms were added to retrieve manuscripts already known by the authors and not detected with previous pilot search strings (Figure 2).

Figure 2 – Search string using Boolean operators “AND” and “OR” to combine the terms related to the present research question



(Unique OR Individual OR Singular OR Single OR Uniqueness OR Individuality OR Singularity OR Singleness OR Similarity OR Rate OR Match OR Dental shape) AND (Dentition OR Tooth OR Teeth OR Arch OR Dental) AND (Bitemark OR Bite mark OR Bitemarks OR Bite marks OR Human Identification OR twin OR Orthodontic Treatment OR Three dimensional OR Three-dimensional OR 3D OR 3-D OR 3-D Technology OR 3-dimensional) AND Forensic OR geometric morphometric analysis.

The studies obtained with the established search string were screened and selected by a single examiner based on the following criteria: 1) full-text articles with indexed abstracts reporting on the uniqueness of human dentition were selected. Case reports, ordinary and systematic reviews, short communications, and letters to the editor were not considered; 2) articles written in English, Spanish or Portuguese were included.

Based on the title and abstract information, the included studies were filtered and selected following the recommendations of the Cochrane Handbook for Systematic Reviews of Interventions (Higgins & Green 2006). More specific, articles not fitting to the following criteria were excluded:

- The source and eligibility of the article, comprehending only full-text articles published in journals of dentistry and/or forensic fields.
- The examined sample, including human dental casts and/or human dental impressions.

After full text reading, a second exclusion was performed removing articles which did not aim to prove the uniqueness of human dentition, and which investigated distorted (bitemark) BM on skin.

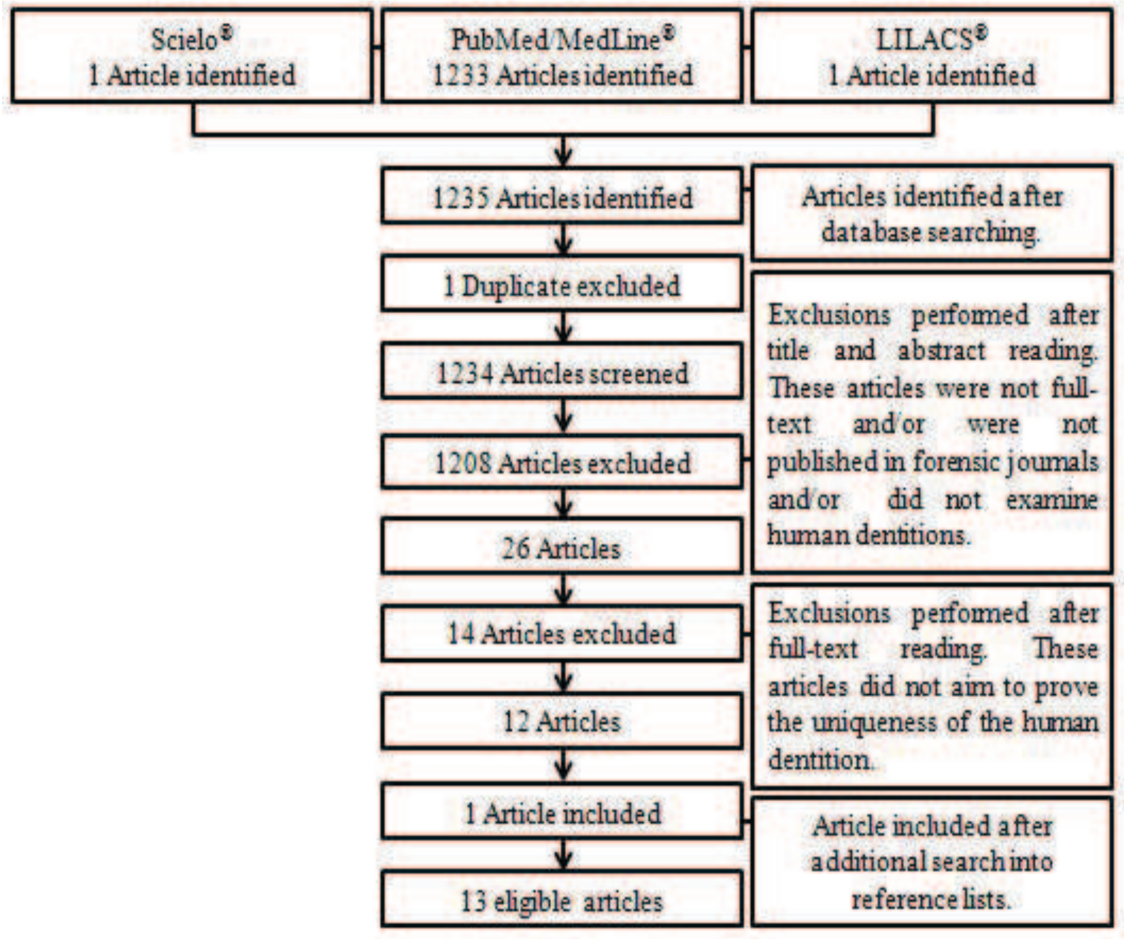
A last search for articles was performed checking the reference lists of the previously included articles. A general overview of included studies was expressed in a diagram flow according to the PRISMA statement standard (The PRISMA Statement 2014).

From the included full-text articles following data were extracted 1) the studied parameters; 2) the sample size; 3) the number of examined teeth; 4) the registration technique used for data collection; 5) the method for data analysis; and 6) the study outcomes.

RESULTS

A general overview of the performed systematic article search and selection was shown in a diagram flow (Figure 3). Twelve articles were selected for inclusion in the current review. One additional article was detected during the search on reference lists becoming eligible after title, abstract, and full-text reading.

Figure 3 – Diagram flow revealing the outcomes for article collection distributing in each filtration procedure



The included articles were published between 1982 and 2014, with the highest number of publications in 2011 (Table 1).

The studied parameters were dental crown shape, size, angulation and dental position. All studies, except one, evaluated a combination of parameters (Table 2). The sample size ranged between 10 and 1099 human dentitions (Table 2). Two articles exclusively examined the anterior teeth of the lower dental arch (Bush et al. 2011b; Sheets et al. 2011). All other articles examined incisors and canines of both dental arches. Additionally, premolars were examined in three articles (Blackwell et al. 2007; Nambiar et al. 1995b; Nambiar et al. 1995a), and two articles added the first and second molars (Nambiar et al. 1995b; Nambiar et al. 1995a) (Table 2). Two and three-dimensional imaging registration techniques were used, alone or combined (Table 2).

Table 1 – List of the eligible articles distributed according to the year of publication

ID	Year	Authors	Title
A	**2014**	Martin-de-Las-Heras S, et al.	A quantitative method for comparing human dentition with tooth marks using three-dimensional technology and geometric morphometric analysis.
B	**2013**	Sheets HD, et al.	Patterns of variation and match rates of the anterior biting dentition: characteristics of a database of 3D-scanned dentitions.
C	**2011**	Bush MA, et al.	Statistical evidence for the similarity of the human dentition.
D	**2011**	Bush MA, et al.	Similarity and match rates of the human dentition in three dimensions: relevance to BM analysis.
E	**2011**	Sheets HD, et al.	Dental shape match rates in selected and orthodontically treated populations in New York state: a two-dimensional study.
F	**2011**	Tuceryan M, et al.	A framework for estimating probability of a match in forensic bite mark identification.
G	**2009**	Martin-de-Las-Heras S, et al.	Comparison of simulated human dermal BM possessing three-dimensional attributes to suspect biters using a proprietary three-dimensional comparison.
H	**2007**	Kieser JA, et al.	The uniqueness of the human anterior dentition: a geometric morphometric analysis.
I	**2007**	Blackwell SA, et al.	3-D imaging and qualitative comparison of human dentitions and simulated bite marks.
J	**1995**	Nambiar P, et al.	Quantitative forensic evaluation of bite marks with the aid of a shape analysis computer program: part 1, the development of "SCIP" and the similarity index.
K	**1995**	Nambiar P, et al.	Quantitative forensic evaluation of bite marks with the aid of a shape analysis computer program: part 2, "SCIP" and bite marks in skin and foodstuffs.
L	**1984**	Rawson RD, et al.	Statistical evidence for the individuality of the human dentition.
M	**1982**	Sognnaes RF, et al.	Computer comparison of BM patterns in identical twins.

ID: Alphabetical identification given for further referencing; Citation of articles starting from 2014 to 1982: (Martin-de-Las-Heras et al., 2014; Sheets et al., 2013; Bush et al., 2011b; Bush et al., 2011a; Sheets et al., 2011; Tuceryan et al., 2011; Martin-de-las-Heras & Tafur, 2009; Kieser et al., 2007; Blackwell et al., 2007; Nambiar et al., 1995b; Nambiar et al., 1995a; Rawson et al., 1984; Sognnaes et al., 1982)

Table 2 – Distribution of eligible articles in relation to the extracted information

Article	Studied dental parameter				Sample Size	Data registration	
	Shape	Size	Angulation	Position		2D	3D
A*	+	+	+	-	13	-	+
B*	+	+	+	-	1099	-	+
C*	-	-	+	+	516	+	+
D*	+	+	+	+	1000	-	+
E*	+	-	-	-	841	+	-
F*	+	+	-	-	15	+	+
G*	+	-	+	-	13	+	+
H*	+	+	-	-	50	+	-
I**	+	+	+	-	42	-	+
J***	+	+	-	-	11	+	-
K***	+	+	-	-	11	+	-
L*	-	-	+	+	397	+	-
M*	+	+	+	-	10	+	-

*2D: Two dimensional; 3D: Three-dimensional; *Incisors and canines were examined; **Incisors, canines and premolars were examined; ***Incisors, canines, premolars, and molars (except third molars) were examined; Sample size expressed as the number of studied human dentitions; Identification from A to M refers to the article specifications in Table 1.*

Two-dimensional images were registered using digital photography (Nambiar et al., 1995b; Nambiar et al., 1995a); flatbed scanning (Sheets et al., 2011; Kieser et al., 2007); and analog radiographs of BM impressions treated with radiopaque powder (Sognnaes et al., 1982; Rawson et al., 1984). In most articles two-dimensional images were produced at 300dpi (or 85µm). Three-dimensional data were registered using diverse types of laser scanners, e.g. Model Maker H40® (3-D Scanners® Ltd., Coventry, UK) (Blackwell et al., 2007), the Minolta VIVID 910® (Konica Minolta Sensing® Inc, Osaka Japan) (Tuceryan et al. 2011), and the Picza Pix-3® (Rolland DG® Corp, Shizouka, Japan) (Martin-de-las-Heras & Tafur 2009). The spatial resolution of laser scanned dentitions was reported in three articles (Sheets et al. 2013; Bush et al. 2011b; Blackwell et al. 2007). In particular 100µm, 10µm and approximately 84µm were referred to, respectively. Digital landmarking processes or superimpositions of digital overlays were used to extract the studied parameters and to compare them in a pool of dental casts, between a dental cast and several BM, or between BM (Table 3).

Table 3 – Distribution of articles according to the data registration and comparison

Articles	Digital Landmarking		Superimposition	Comparing
	Software	Landmarks	Mechanism/Software	
A	-	-	DentalPrint©	DC x BM
B	Landmark©	60	-	DC x DC
C	N/M	N/M	-	DC x DC
D	Landmark©	60	-	DC x DC
E	tpsDIG©	14	-	DC x DC
F	-	-	RapidForm©	DC x BM
G	-	-	DentalPrint©	DC x BM
H	tpsDIG©	14	-	DC x DC
I	3D Rugle3©	42	-	DC x BM
J	N/M	34-36	-	DC x BM
K	N/M	34-36	-	DC x BM
L	N/M	6	-	BM x DC
M	-	-	N/M	BM x BM

DC: Dental casts; BM: BM; N/M: Not mentioned; Identification from A to M refers to the article specifications in Table 1; Landmarks expressed by the number of reference points per dental arch.

The observed statistical methods for data analysis were Procrustes superimposition; Procrustes-sp (size-preserving) superimposition; Comparison of average residual lengths (ARL); Comparison of root mean square scatters (RMSS); and the application of a developed algorithm (Table 4).

Table 4 – Distribution eligible articles in relation to the units for the quantification of differences between human dentitions

Articles	Units for the quantification of differences between dentitions				
	Procrustes	Procrustes-sp	Algorithm	ARL	RMSS
A	-	-	+	-	-
B	+	+	-	-	+
C	-	-	+	-	-
D	+	-	-	-	+
E	+	-	-	-	-
F	-	-	+	-	-
G	-	-	+	-	-
H	+	-	-	-	-
I	-	-	+	-	-
J	-	-	+	+	-
K	-	-	+	+	-
L	-	-	+	-	-
M	-	-	-	-	-

ARL: Average residual length; RMSS: Root mean square scatter; Identification from A to M refers to the article specifications in Table 1.

Four articles proved the uniqueness of human dentition within their respective sample (Sognnaes et al. 1982; Kieser et al. 2007; Rawson et al. 1984; Martin-de-Las-Heras et al. 2014). In the other nine articles positive matches between different dentitions were detected (Sheets et al. 2013; Bush et al. 2011b; Bush et al. 2011a; Sheets et al. 2011; Blackwell et al. 2007; Nambiar et al. 1995a; Nambiar et al. 1995b; Tuceryan et al. 2011; Martin-de-las-Heras & Tafur 2009).

DISCUSSION

The current review showed that no standard consensuses regarding the use of specific variables exist to prove UHD. Bush et al. (2011b) landmarked the mesio and distal borders, as well as the central point, of the anterior teeth in order to collect variables regarding the size, angulation, (incisal edge) shape, and (relative tooth) position parameters. In contrast, Tuceryan et al. (2011) developed a model to extract the contours of the incisal edges from scanned casts for comparisons using superimposition techniques. The single extracted variable provided information regarding the size and the shape parameters. In the present review, the UHD was found by Sognnaes et al. (1982), and Martin-de-las-Heras et al. (2014), both combined three dental parameters; and by Kieser et al. (2007), and Rawson et al. (1984), combining two dental parameters. Neither of them considered all possible dentition parameters and only aimed to detect uniqueness of the dentition for bite mark purposes.

Randomized matching of dental features is one of the most used pathways for the assessment of the similarity between dentitions (Bush et al. 2011a). The influence of the sample size on this study method was investigated by Sheets et al. (2013). The authors systematically increased the sample size along the experimental steps, and observed that the match rates did not increase accordingly. Power analysis tests could arise as a proper pathway to detect a significant sample size for the aimed purposes. However, in all the included articles the sample size was arbitrarily determined. Sheets et al. (2013) justified the absence of power analyses due to the difficulties of performing such calculations on a multivariate basis. Studies designed with small samples, such as Kieser's et al. (2007) (50 dentitions), and Sognnaes' et al. (1982) (10 dentitions), found no matches; while studies with large samples, such as Sheets' et al. (2013) (1099 dentitions), and Bush et al. (2011b) (516 dentitions), detected positive matches. It indicates that the match rate for (a) specific parameter variable(s) or parameter(s) trends to increase with the sample size. Indeed the sample size must enable to cover all the potential variances within the used parameter (variable) and has to extend according to the number of parameters (variables) considered. A way to decrease the size of the investigated sample can be achieved by stratifying the sample on specific populations with similar dental traits. This procedure decreases the variance between the investigated parameters. In this context, Kieser et al. (2007) used 50 orthodontically treated patients and Sognnaes et al. 1982, investigated five pairs of monozygotic twins. In orthodontically treated patients the decreased variance mostly affects parameters related to tooth position, angulation and arch position. In twins, additionally, the tooth shape and size are potentially alike.

Most (n=10) of the included articles examined the six anterior upper and/or lower teeth, because they considered uniqueness of the dentition in the context of BM investigations. However, Blackwell et al. (2007) added premolars into the analysis, explaining that these teeth can appear in bite marks. Nambiar et al. (1995a; 1995b), additionally examined premolars and molars, increasing the number of tooth specific landmarks used to achieve a higher match level.

In the search for uniqueness, many advantages of 3D digital imaging methods were reported. Sheets et al. (2013), and Blackwell et al. (2007), reported the free manipulation of 3D digital dental casts on different axes as an advantage, compared to a 2D image analysis. It allows for a more precise detection and landmarking of reference points. Sheets et al. (2013), Bush et al. (2011a); and Blackwell et al. (2007) highlight the realistic 3D perspective, enabling the placement of a higher number of landmarks. Martin-de-las-Heras et al. (2009), (2013), and Tuceryan et al. (2011), focused on the possibility of accurately extracting digital 3D dental contours using specific software and algorithms. Bush et al. (2011b), referred to the higher spatial resolution, improving image quality of 3D digital scans of dentitions. Later, they pointed out the possibility of performing digital imaging analysis using high-tech software. Based on these findings, it can be concluded that the current technology allows for the digital 3D registration, duplication, manipulation and optimal analysis of human dentitions. Moreover, computed tomography (CT) techniques enable to register digital 3D information of all the teeth available in an investigated individual, adding data of the tooth root(s), pulp, enamel, dentin and cementum. In order to investigate all possible parameters related to uniqueness, digital 3D reconstructions of CT images should be studied (Franco et al. 2013).

All the included articles, using the landmarking process (n=9), investigated the uniqueness of human dentition using the analysis of Procrustes distances, or statistical algorithms. Sheets et al. (2011) explained that Procrustes comprehends a pathway for the interpretation of shapes. The superimposition of non-fitting objects will generate a Procrustes distance, indicating a morphological variation. In the study of Kieser et al. (2007), most similar dentitions generated a Procrustes distance of 0.038, suggesting a very discrete difference in shape, and supporting a potential uniqueness. Recently Sheets et al. (2013), highlighted the role of size information on the search for dental uniqueness using Procrustes-sp (size-preserving), which enables to calculate distances from non-fitting structures. Nambiar et al. (1995a; 1995b), quantified the differences between compared dentitions as average residual length (ARL), while Sheets et al. (2013), and Bush et al. (2011a), expressed root mean square scatters (RMSS). ARL consists on the sum of distances between homologous points of two objects divided by the number of points (Nambiar et al. 1995a). High ARL values indicate high differences between two objects. RMSS is used to quantify data in a multivariate sense during the analysis of the human teeth (Bush et al. 2011a).

During landmarking, reference points are placed on the surface of an object in order to allow for reproducible measurements between the considered points. Kieser et al. (2007) reported as advantages of landmarking the optimal measurement reproducibility, and the possibility of performing geometric morphometric analyses. The geometric morphometrics allow for the quantification of the comparison of subjective data, such as the shape of an object. In geometric morphometrics, landmarks are converted into Cartesian coordinates which enable the morphological capture of a virtual object (Kieser et al. 2007). The whole procedure requires specific software. For semi-automated landmarking Sheets et al. (2013), and Bush et al. (2011a), used Landmark® software (IDAV©, UC Davis, California, USA); Sheets et al. (2011), and Kieser et al. (2007), used tpsDIG® software (SB Morphometrics©, Stony

Brook University, New York, USA); and Blackwell et al. (2007), employed 3D Rugle3® software (Medic Engineering Corporation©, Kyoto, Japan).

Four of the included articles proved the uniqueness of human dentition, but for each of them arguments to doubt the reported results can be given. First, Sognnaes et al. (1982) achieved dental uniqueness examining five identical twin pairs and using a 2D computer-assisted superimposition methodology. It enabled to orientate bite impressions into vertical and horizontal axes and observed the discrepancies between the incisal dental contours. Although a stratified population was examined, the sample size was too small to detect significant results. Further on the imaging facilities and devices available in 1982 hampered the investigation of all possible parameters necessary to prove uniqueness. Second, Rawson et al. (1984) claimed uniqueness of human dentition based on the assumption that tooth position is linearly and uniformly distributed within human dentition. The study of Rawson et al. (1984) was reproduced by Bush et al. (2011b). They revealed a non-uniform distribution of tooth position within human dentition and concluded that inferences about the uniqueness of human dentition with purposes for BM analysis are not supported in an open population.

Third, Kieser et al. (2007), performed a well designed and systematic study, including calibration tests and landmarking. However, a stratified sample of 50 orthodontically treated dentitions of patients aged between 17 and 20 years old was arbitrarily addressed and no inter examiner calibrations were performed. Sheets et al. (2013) commented that Kieser's small sample was potentially influencing the final outcome. Fourth, Martin-de-las-Heras et al. (2013), focused on testing the power of a developed software on discriminating one dentition among a pool of bite impressions. The software revealed statistically significant discriminative power for all the investigated parameters, individually or combined. The study was restricted to the comparison of dental shape, size and angulation used for BM analysis: as such insufficient parameters were compared to enable proof of uniqueness.

In all other articles (n=9) the UHD was not detected. The lack of (1) proper sample stratification; (2) examiner reproducibility; (3) 3D data registration; (4) automated superimpositions; (5) validated 3D morphometric comparison software; and (6) statistical quantification of data comparison appeared as the main limitations to be considered in further researches.

Questionamento: *A UHD pode ser investigada com a tecnologia imaginológica 3D contemporânea?*

Objetivo: *"Testar a tecnologia imaginológica 3D contemporânea para a comparação morfométrica automática de pares de modelos de gesso 3D digitalizados".*

Hipótese: *"A tecnologia 3D contemporânea permite a investigação da UHD usando a comparação morfométrica automática de pares de modelos de gesso 3D digitalizados".*

Testando os softwares imaginológicos contemporâneos para a análise de dentições humanas

Capítulo 4

Capítulo baseado no artigo:

Ademir Franco A, Guy Willems, Paulo H. C. Souza, Sérgio A. Ignácio, Patrick Thevissen. Validation of 3D metric and superimposition tools in three software packages for the morphological analysis of laser scanned anterior dentition models. RBOL. 2016 aceito.

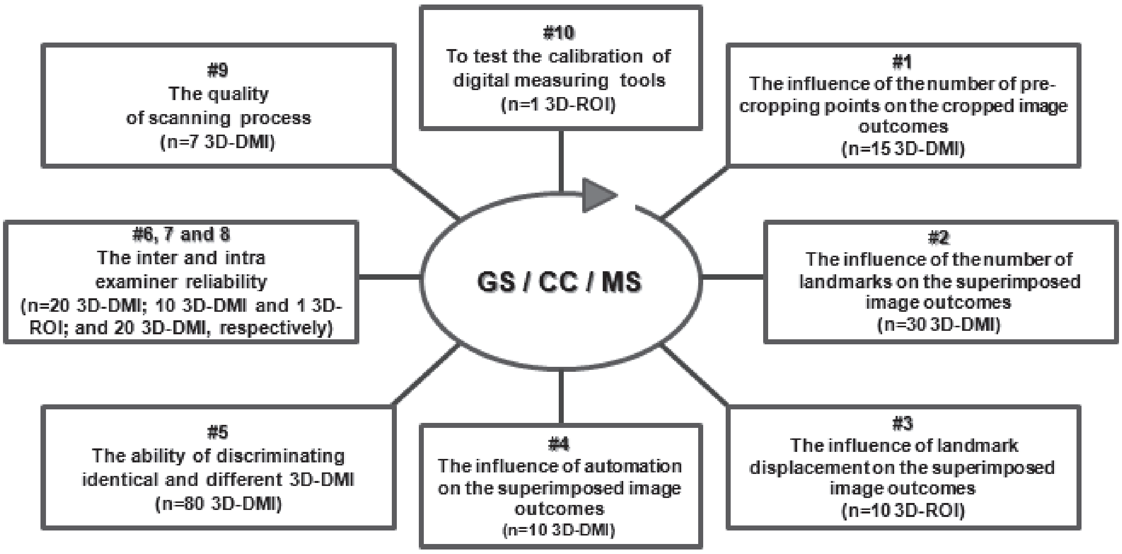
Apresentação oral no 68^{o} Encontro Científico da Academia Americana de Ciências Forenses, Las Vegas, USA, 2016

Efforts were made in the last years to enhance the investigations on the UHD. As previously reported, 2D (Kieser et al. 2007) and 3D (Bush et al. 2011a) analyses of the human dentition were aspired; software were developed (Martin-de-las-Heras & Tafur 2009) and tested (Blackwell et al. 2007); different statistics were implemented (Bush et al. 2011b); and particular populations were sampled (Sognnaes et al. 1982; Sheets et al. 2011). The methodological enhancement also pointed out that pairwise comparisons between dentitions may be the best way to investigate and prove the UHD. In this context, is fundamental to know if the 3D software available currently enable this procedure. The present research aims to test and validate metric and superimposition tools of software packages for the pairwise comparison of dental crown morphology in 3D laser scanned human dentitions.

MATERIAL AND METHODS

A sample of 20 individuals (10 females, 10 males) with an intact permanent anterior upper and lower dentition, without any dental treatment and with the six anterior teeth clinically completely erupted was collected. The maxillary and mandibular dentitions were impressed using manually-mixed alginate (Jeltrate Dustless®, Dentsply®, York, PA, USA) with metallic dental trays (Tecnodent®, São Paulo, SP, Brazil) and casted in dental models with plaster type IV (Durone®, Dentsply®, York, PA, USA. The obtained dental models were casted in rubber mould base formers and each model was manually trimmed in maximum intercuspation (Habib et al. 2007) according to a standard technical procedures (Dofka 1995). Next, the dental models were scanned using the XCAD 3D® (XCADCAM Technology®, São Paulo, SP, Brazil) automated motion device with angular laser scanning, at a precision of <20 microns and a volume capture of 80mm (x-axis) x 50mm (y-axis) x 80mm (z-axis). The 3D dental model images (3D-DMI) were stored as .STL and .OrthoStudio files and imported for analysis in 3 different 3D geometric analysis software namely: Geomagic Studio® (GS) (3D Systems®, Rock Hill, SC, USA); Cloud Compare® (CC) (Telecom Paris Tech® and EDF®, Paris, France); and Maestro 3D Ortho Studio® (MS) (AGE Solutions®, Pontedera, PI, Italy) software. The pool of 40 imported 3D-DMI was used to investigate in 10 tests (Figure 4) the performances of the metric (2 dimensional, 2D) and the superimposition (3 dimensional, 3D) tools of each software.

Figure 4 – Overview of the quantitative research tests performed in the present chapter



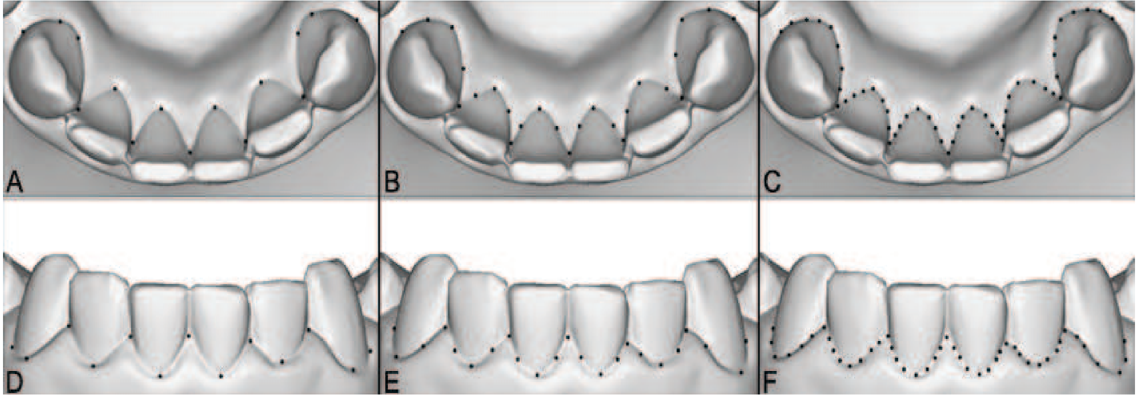
Geomagic Studio® (GS), Cloud Compare® (CC) and Maestro Ortho Studio® (MS) software packaged were used to perform 10 different qualitative research tests, and compared based on the obtained test results. In each test (#) a specific number (n) of three-dimensional dental model images (3D-DMI) or rectangular object images (3D-ROI) were evaluated

The metric tools were available in the 3 software, while superimposition was only integrated in GS and CC. The 2D and 3D test results were compared between the corresponding software. All the analyses were performed using a HP Pavilion® (Hewlett-Packard®, Palo Alto, CA, USA) computer, with 14” screen size and 1366x768 screen resolution.

For reference purposes, a copy of a rectangular object was made impressing, casting and digitalizing it according to the previously mentioned protocols. For uniform analyses a standard positioning of the imported 3D-DMI and the 3D rectangular object image (3D-ROMI) in the studied software was established. The 3D-DMI were positioned in occlusal view with the posterior base border parallel to the horizontal plane. The 3D-ROI was positioned with one of the corners in the center of the screen, enabling the visualization of the vertices in the x-, y- and z- axes. For certain tests, the .STL and .OrthoStudio files of the 3D-DMI and the 3D-ROI were copied using the “copy” and “paste” command tools of the Microsoft Windows® (Microsoft Corp.®, Redmond, WA, USA) operating system.

The 3D analyses required two operator-dependent steps for the pair wise superimposition of 3D-DMI parts of interest: cropping and landmarking. In particular, a manual reference demarcation of the 3D-DMI was necessary in the former placing pre-cropping points and in the latter positioning landmarks. Prior to automated, semi-automated, and manual superimpositions, the manual pre-cropping point positioning along the cemento-enamel junction of the anterior teeth, enabled the software to sort out automatically the anterior tooth crowns. A first test was developed and applied to verify the influence of the number of pre-cropping points placed, on the cropped image outcomes. Three sets of 5 copied mandibular 3D-DMI (n=15) were studied. The first set was cropped after positioning 28 pre-cropping points (15 points on the lingual surface + 13 points on the vestibular surface); the second set after positioning 58 pre-cropping points (25 points on the lingual surface + 23 points on the vestibular surface); and the third set after positioning 94 pre-cropping points (49 points on the lingual surface + 45 points on the vestibular surface) (Figure 5). The cropped 3D-DMI and their respective copies were imported in GS and CC and automatically superimposed. Existing morphological differences in the pair wise 3D-DMI comparisons were quantified in each software.

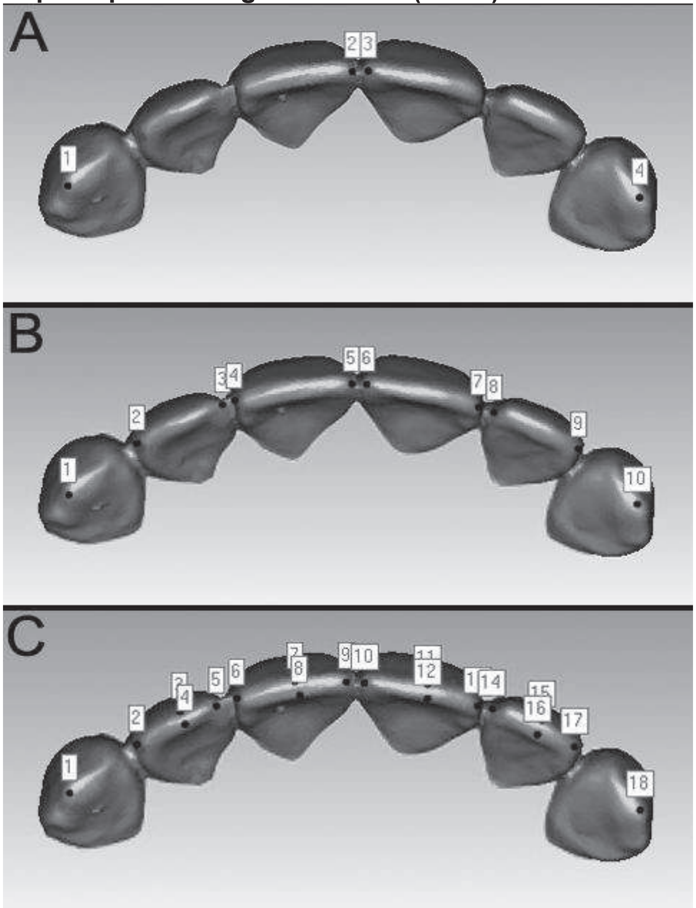
Figure 5 – Pre-cropping point distributions used to test the influence of the number of pre-cropping points on the cropped image outcomes (test 1)



The lingual (panels A, B and C) and vestibular (panels D, E and F) pre-cropping point arrangements, using 28, 58, and 94 pre-cropping points, respectively, are illustrated. In Geomagic Studio® software package the cropping is performed according to curved lines automatically positioned between the pre-cropping points and matched with the gingival contours.

The landmarking procedure was essential prior to the semi-automated and the manual superimpositions. A second test was developed and applied to verify the influence of the number of landmarks used, on the superimposition outcomes. Five different maxillary 3D-DMI were each copied 3 times, and grouped (group A, n=15). Next, 5 new maxillary 3D-DMI were each copied 3 times and grouped (group B, n=15). The 3D-DMI of each image set from group A and B was pair wise superimposed placing 4, 10 and 18 landmarks respectively (Figure 6). In GS and CC the morphological differences between the pair wise compared 3D-DMI were quantified.

Figure 6 – Landmark distributions used to test the influence of the number of landmarks on the superimposed image outcomes (test 2)



The cropped anterior dentitions of the 3D dental model images were landmarked with sets of 4 (A), 10 (B) and 18 (C) landmarks, respectively. The first set included the cusp tips of the canines and the most mesial point of the incisal edges of the central incisors (A). The second set included the landmarks of the first set and added the most mesial and distal point of each incisal incisor edge (B). The third set included the landmarks of the previous two sets and added the most vestibular and palatal points in the center of all incisal edges (C).

A third test was developed and applied to verify the influence of the landmark position on the superimposition outcomes. This test was performed differently in GS and CC. The 3D-ROMI was copied four times (n=5) and imported in GS. Next, the original and the four copied 3D-ROMI were positioned on a background scaled grid. The original 3D-ROMI was landmarked in a fixed region, while the copies were landmarked with increasing landmark displacements (0.5mm, 1mm; 2mm; and 5mm, respectively). Due to the lack of a background grid in CC, the original 3D-ROMI was landmarked in a fixed region and the four copies (n=5) were landmarked using the linear measuring tool with displacements from the fixed region of 0.5mm; 1mm; 2mm; and 5mm, respectively. The morphological differences between the original and copied 3D-ROMI were quantified in each software.

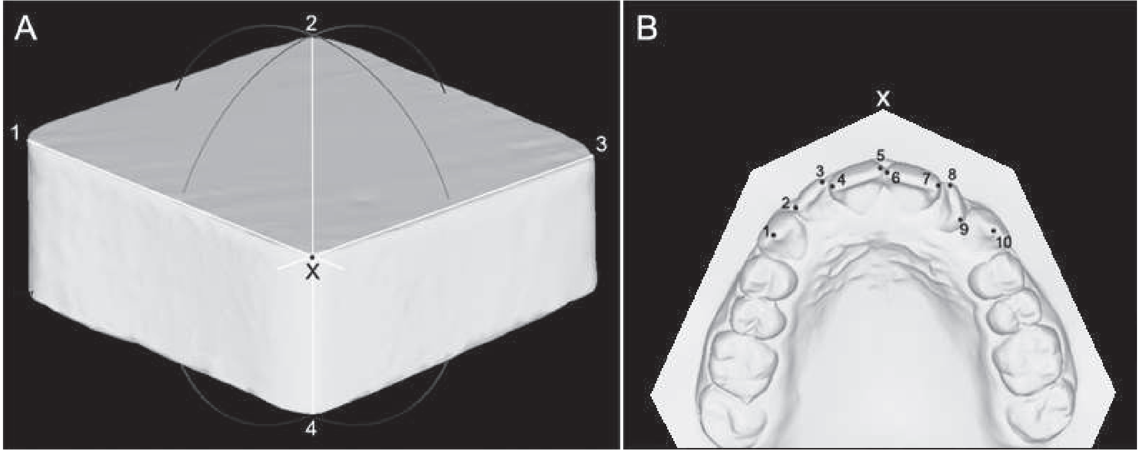
A fourth test was established to verify superimposition differences between automated (no landmark), semi-automated and manual superimpositions. GS and CC allow manual and semi-automated superimpositions. The automated superimposition is only available in GS (the same tool is under research in CC). Between 2 sets of 5 different mandibular 3D-DMI pair wise manual; semi-automated and automated superimpositions were performed. The morphological differences between the pair wise compared 3D-DMI were quantified in each software.

A fifth test was developed and applied to verify the ability of the software to discriminate identical (3D-DMI and their copies) and different 3D-DMI. Forty 3D-DMI (20 maxillary and 20 mandibular) were selected together with 20 (10 maxillary and 10 mandibular) other 3D-DMI and their respective copies. All 3D-DMI (n=80) were cropped, landmarked and pair wise superimposed. The cropping procedure was performed placing 58 pre-cropping points along the cemento-enamel junction of the anterior teeth, always including the highest point at the interdental papillae and the lowest point of the cemento-enamel junction contour. The landmarking procedure was performed placing 10 landmarks on the anterior teeth, in which 8 were distributed in the most distal and most mesial point of the incisal edge of central and lateral incisors and 2 on the cusp tip of canines. The morphological differences between the pair wise compared 3D-DMI were quantified. The mean morphological difference found between identical 3D-DMI was used as threshold to verify if mismatches were occurring among the different 3D-DMI. Specifically, if the pair wise comparison between different 3D-DMI had mean morphological difference below the threshold it was considered a mismatch.

The sixth, seventh and eighth tests were performed to asses inter/intra examiner reliability. In the sixth test 10 3D-DMI (5 maxillary and 5 mandibular) were copied (n=20) and used to test the reproducibility of the cropping procedure. The 3D-DMI and the respective copies were imported in GS and CC and cropped in each software by a first examiner, placing 58 pre-cropping points along the cemento-enamel junction. For the assessment of inter examiner reproducibility, a second examiner performed independently the same procedure. For the assessment of intra examiner reproducibility, the first examiner repeated the same procedure within 14 days. In each software the morphological differences between the pair wise compared 3D-DMI were quantified per examiner.

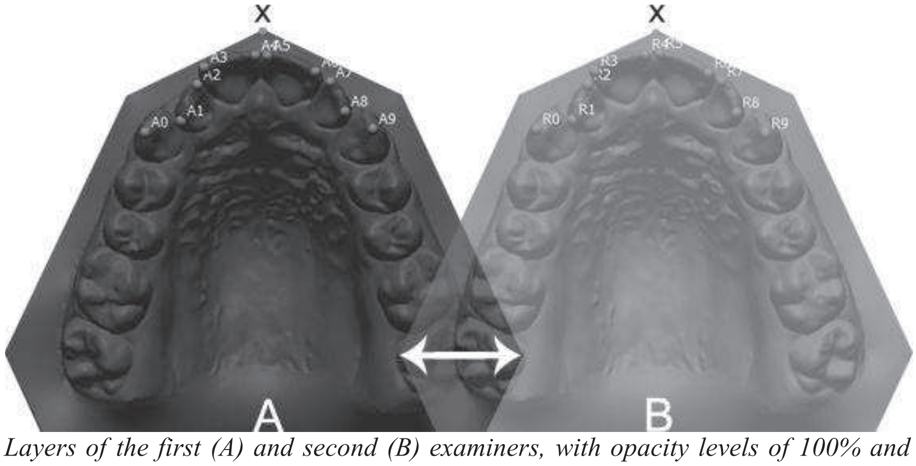
In the seventh test 10 maxillary 3D-DMI were used to 2D test the reproducibility of the landmarking. Ten landmarks were placed by two examiners in each 3D-DMI, using separately GS, CC and MS. On forehand, a third examiner placed a single reference landmark on the most frontal vertex of each 3D-DMI. Screenshots were taken from the landmarked files and implemented in Adobe Photoshop® CS5 (Adobe Systems®, San Jose, California, USA) as image layers. The image layers of the first examiner were kept with 100% opacity levels, while the opacity levels of the image layers of the second examiner were reduced to 50%. The image layers of the first and second examiners were superimposed in 2D, using as references the landmark placed by the third examiner and the posterior base border of the cast in the 3D-DMI parallel to the horizontal plane. A similar procedure was performed using the 3D-ROMI. The first and second examiner placed independently 4 landmarks on each visible vertex, except for the central vertex which was landmarked by a third examiner and used as a reference point (Figures 7 and 8). The screenshots of each examiner were 2D superimposed in Adobe Photoshop® as image layers with different opacity and aligned using the reference landmark placed by the third examiner. The difference between the corresponding landmarks of the first and the second examiner were measured using Photoshop's ruler tool. For the inter examiner reproducibility, the distances measured after the landmarking of the second examiner were compared with the distances of the first examiner, obtained from the 3D-DMI and the 3D-ROMI in each software. For the intra examiner reproducibility, the first examiner repeated the procedures on the 3D-DMI and the 3D-ROMI in each software within 14 days and the distances between both examinations were compared per software.

Figure 7 – Standard positions for landmarking the 3D-ROMI and the 3D-DMI



The 3D rectangular model image (3D-ROMI) (A) and the 3D dental model image (3D-DMI) (B) were landmarked with the reference point (x) for metric analysis and with additional points (from 1 to 10) both for metric and superimposition analysis. For orientation the 3D-DMI were positioned in occlusal view with the posterior base border parallel to the horizontal plane, while the 3D-ROMI was positioned centralizing one of its corners on the screen, allowing the visualization of vertices in x-, y- and z- axes.

Figure 8 – Superimposition of image layers used to test the examiner reliability for the landmarking procedure with Adobe PhotoShop® (test 7)



Layers of the first (A) and second (B) examiners, with opacity levels of 100% and 50%, respectively, will be superimposed with the posterior base of the models and the fixed reference landmark (x) as references. The superimposition procedure is performed dragging one layer over the other using Adobe Photoshop's "move" tool, assuring superimposition within overlap of the reference points

In the eighth test 10 3D-DMI (5 mandibular and 5 maxillary) were copied (n=20) and used to 3D test the reproducibility of the landmarking. The 3D-DMI were imported in GS and CC and landmarked by a first examiner following the set up described in test seven. For the assessment of inter examiner reproducibility, a second examiner performed the same procedure in each software. For the assessment of intra examiner reproducibility, the first examiner repeated the same procedure in each software within 14 days. In both software the original and the corresponding copied 3D-DMI were pair wise superimposed using the landmarks as reference. Appearing morphological differences were quantified per examiner and compared.

Test nine verified the quality of the scanning process. A blind analysis was developed and applied scanning a mandibular 3D-DMI two times with the same scanning device. In the analysis, one of the obtained digital files was copied. The three files (2 scans and 1 copy) were pooled with 4 randomly chosen mandibular 3D-DMI. The 7 3D-DMI were pair wise compared (21 combinations) in each software and the morphological differences were quantified.

The tenth test was developed and applied to assess the calibration of software measurement tools. The rectangular object was measured in situ, with a digital caliper and an ABFO scale #2 and digitally, with the measuring tools of each of the software. Differences between in situ and digital measurements were quantified.

The quantification of morphological differences was automatically performed in GS and CC for all the tests, except tests #7 and #10. The morphological differences were assessed comparing pair wise the spatial position of homologous points in the examined 3D-DMI and reported as a mean morphological difference (bias) with a respective standard deviation (precision), both expressed in millimeters (mm). The quantification of measurement differences was established subtracting the absolute linear measurement values (in mm) from the 3D-DMI or 3D-ROMI pairs respectively compared.

The calculated morphological differences in tests 1, 2, and 4 were assessed for normality using Shapiro-Wilk statistical test. Their mean values were compared between pre-cropping point groups (28 *vs.* 58; 28 *vs.* 94; 58 *vs.* 94); landmark groups (4 *vs.* 10; 4 *vs.* 18; 10 *vs.* 18); and superimposition groups (automated *vs.* semi-automated; automated *vs.* manual; semi-automated *vs.* manual) using Student's t-test for paired samples and Wilcoxon signed rank test assuming normal and not normal distribution. In test 5 the mean values of morphological differences between identical and non identical groups was compared using Student's t-test for independent samples. In tests 6, 7 and 8 the examiner reliability was statistically measured with: Dahlberg's error, which correlates the total variance with error variance and indicates the level of reproducibility; Pearson's correlation coefficient and reliability coefficient, indicating the correlations between the examiner outcomes; and Student's t-test for paired samples to evaluate the systematic error. The outcomes of tests 3, 9 and 10 were assessed with descriptive statistics.

The mean values of the morphological differences in tests 1, 2, 4, 5, 6, 7 and 8 were compared between software using Student's t-test for independent samples and nonparametric Mann-Whitney U test assuming normal and not normal distribution. For all the tests statistical significance was set at $p<0.05$. Student's t-test outcomes indicated larger morphological difference with positive or negative increase in t values. The statistical analyses were performed with SPSS® 23.0 (IBM® Corp., Armonk, New York, USA) software.

After the quantitative approach, the software were qualitatively analyzed based on the integrated tools, their costs and their compatibility with the imported file format and the operational systems.

RESULTS

Normal distribution was observed for all the calculated morphological differences, except for the 18 landmark placement (test 2).

No statistically significant differences were observed comparing the calculated morphological differences within software using a different number of pre-cropping points (test 1), landmarks (test 2), or level of automation (test 4) (Table 5).

Testing the displacement of landmarks (test 3) a wider range in calculated morphological difference between the 3D-ROMI and its copies was observed with an increase in level of landmark displacement (Table 5).

Comparing the mean morphological differences between identical and different 3D-DMI (test 5) revealed no mismatch results below the obtained threshold values, both for the mandible and maxilla within each software (test 5) (Table 5).

Table 5 – Within test results of test #1, #2, #3, #4 and #5 compared with Student's t-test for paired samples and Wilcoxon signed rank test, within each software

#	Variables	Software	MMD	*SD*	*t* (*p*)
1	**Number of pre-cropping points**				
	28 points / 58 points	GS	21.03 / 19.41	2.77 / 6.29	0.97 (0.38)
	28 points / 94 points	GS	21.03 / 17.81	2.77 / 3.52	2.11 (0.10)
	58 points / 94 points	GS	19.41 / 17.81	6.29 / 3.52	0.86 (0.43)
	28 points / 58 points	CC	16.63 / 15.35	3.76 / 6.55	0.41 (0.49)
	28 points / 94 points	CC	16.63 / 16.48	3.76 / 1.67	0.15 (0.88)
	58 points / 94 points	CC	15.35 / 16.48	6.55 / 1.67	0.40 (0.70)
2	**Number of landmarks**				
	4 landmarks / 10 landmarks	GS	0.11 / 0.16	0.08 / 0.19	-0.61 (0.57)
	4 landmarks / 18 landmarks	GS	0.11 / 0.11	0.08 / 0.20	0.02 (0.98)*
	10 landmarks / 18 landmarks	GS	0.16 / 0.11	0.19 / 0.20	0.99 (0.37)*
	4 landmarks / 10 landmarks	CC	1.32 / 0.23	1.22 / 0.19	1.74 (0.15)
	4 landmarks / 18 landmarks	CC	1.32 / 0.72	1.22 / 1.18	-0.67 (0.50)*
	10 landmarks / 18 landmarks	CC	0.23 / 0.72	0.19 / 1.18	-0.40 (0.68)*
3	**Landmark displacement**				
	0.05 mm	GS	0.00	0.04	d/s
	1.00 mm	GS	0.00	0.04	d/s
	2.00 mm	GS	0.00	0.50	d/s
	5.00 mm	GS	0.33	3.03	d/s
	0.05 mm	CC	0.00	0.10	d/s
	1.00 mm	CC	0.02	0.22	d/s
	2.00 mm	CC	0.03	0.37	d/s
	5.00 mm	CC	0.57	1.00	d/s
4	**Level of automation**				
	Automated / semi-automated	GS	0.09 / 0.12	0.05 / 0.09	-1.35 (0.24)
	Automated / manual	GS	0.09 / 0.13	0.05 / 0.09	-2.01 (0.11)
	Semi-automated / manual	GS	0.12 / 0.13	0.09 / 0.09	-1.11 (0.32)
	Semi-automated / manual	CC	0.13 / 0.21	0.10 / 0.10	1.33 (0.25)
5	**Discrimination of 3D-DMI**				
	Different mandibular 3D-DMI	GS	-0.06	1.27	n/a
	Different maxillary 3D-DMI	GS	0.14	1.38	n/a
	Identical mandibular 3D-DMI	GS	0.00	0.01	t/s
	Identical maxillary 3D-DMI	GS	0.00	0.01	t/s
	Different mandibular 3D-DMI	CC	0.17	1.14	n/a
	Different maxillary 3D-DMI	CC	0.27	1.13	n/a
	Identical mandibular 3D-DMI	CC	0.00	0.03	t/s
	Identical maxillary 3D-DMI	CC	0.00	0.04	t/s

*#: test number; MMD: mean morphological difference; t: Student's "t" value; p: significance rate set at 95%; GS: Geomagic Studio®; CC: Cloud Compare®; *: must be interpreted as Wilcoxon's Z value; 3D-DMI: Three-dimensional dental model image; d/s descriptive statistics; t/s threshold setting; n/a: not applicable; SD: standard deviation; MMD and SD expressed in millimeters.*

The inter and intra reliability tests for the cropping (test 6), 2D landmarking (test 7), the 3D landmarking (test 8) indicated optimal reliability between examiners within each software (Table 6). The Outcomes of the examiner reliability test for cropping (test 6) resulted in total variance and error variance equal to zero for all performed statistical tests.

Table 6 – Examiner reliability test results for landmarking with Adobe PhotoShop® (test #7) and software tools (test 8) within each software

#	Test	Software	DE (%)	Reliability coefficient (%)	Pearson's coefficient (%)
7	**Inter**	**GS**	0.46	99.54	99.63
		CC	4.06	95.94	96.56
		MS	3.14	96.86	97.07
	Intra	**GS**	0.97	99.03	99.22
		CC	1.57	98.43	98.73
		MS	1.87	98.13	98.38
8	**Inter**	**GS**	n/a	n/a	n/a
		CC	3.82	96.18	97.97
	Intra	**GS**	n/a	n/a	n/a
		CC	8.27	91.73	95.27

GS: Geomagic Studio®; CC: Cloud Compare®; MS: Maestro Ortho Studio®; DE: Dahlberg's error; Student's t-test for paired samples did not reveal statistically significant differences or systematic error between examiners (p>0.05).

Testing the quality of the scanning process (test 9) indicated no morphological differences in 3D-DMI between the original and rescanned; between the original and copied; and between the copied and rescanned groups.

In the test calibrating the measuring tools (test 10) no differences were observed between the calculated morphological differences in 3D-DMI comparing the in situ and digital measurements.

No difference in performance was observed between software using different number of pre-cropping points (test 1), landmarks (test 2), or level of automation (test 4) (Table 7).

A statistically significant higher mean morphological difference was observed in CC (0.001mm) compared to GS (0.000mm) considering the performance to discriminate identical 3D-DMI (test #5). However, both software performed a perfect discrimination of identical/different 3D-DMI (Table 7).

Inter and intra reliability tests for the cropping (test 6), 2D landmarking (test 7), the 3D landmarking (test 8) were not statistically different between software (Table 7). The qualitative evaluation revealed GS as practically the best performing and most extensive software, because CC was less precise for the discrimination of identical 3D-DMI and did not allow automated superimpositions, while MS was limited by the lack of superimposition and cropping tools (Table 8).

DISCUSSION

Up to the present, several techniques for the analysis of BM were used to prove the relation between the injury and the offender. These techniques varied from overlaying hand drawn transparent foils on photographs, to 3D digital superimpositions (Pretty 2006). The validation of these techniques indicated that the best outcomes for BM analysis probably result from 3D comparison (Naether et al. 2012; Thali et al. 2003). In this context, the present research was developed to test available software packages ability to compare the morphology of 3D laser scanned dentition models. The software containing the most optimal tools and functions will be used to support further studies aiming to prove the uniqueness of the human anterior dentition.

Different studies investigated the UHD (Kieser et al. 2007; Bush et al. 2011a; Bush et al. 2011b; Sognnaes et al. 1982; Sheets et al. 2011). Most of these studies were designed in the context of BM analysis and examined the six anterior teeth (Franco et al. 2015). The methods used in these studies varied, depending on the comparison technique used, the software applied, the modality of image registration, and the morphometric analysis technique utilized (Franco et al. 2015). All the methods commonly consider morphological tooth information, such as the size and shape; and the relation of the tooth in the dental arch, such as its angulation and position (Franco et al. 2015). The outcomes of these studies were not uniformly proving the UHD (Kieser et al. 2007; Sognnaes et al. 1982). Because specific limitations (Franco et al. 2015) of these studies were the lack of 3D image registration and the use of operator-depending procedures the present chapter aimed to select and validate existing object comparison software applicable for unbiased 3D comparison of the human dentition’s morphology.

Similar to previous studies (Kieser et al. 2007; Bush et al. 2011a; Bush et al. 2011b; Sognnaes et al. 1982; Sheets et al. 2011) it was chosen to restrict the area of interest to the six anterior teeth. Therefore cropping the imported 3D-DMI was necessary to select and study the anterior teeth in the collected 3D-DMI. Because the cropping procedure required in the examined software the manual placement of cropping points, this operator-depending procedure was evaluated on the degree of potentially included bias. The first test of the present chapter indicated that no difference were observed placing 28, 58 or 94 pre-cropping points along the cemento enamel junction. It indicated that cropping was a stable procedure, not generating discrepant outcomes. Moreover, no statistically significant difference was observed comparing the performances of the two software. These findings were confirmed in the sixth test, which revealed optimal intra and inter examiner cropping reproducibility.

A second manual procedure necessary in certain 3D geometric analysis software was the landmarking (Franco et al. 2015). This procedure is necessary to align two objects prior to comparison. However, two landmarks can hardly be placed in the exact the same anatomic position over time. In previous research the landmarking was mainly considered in occlusal view, varying the landmark arrangement and number – e.g. 14 (Bush et al. 2011a; Sheets et al. 2011), 24 (Kieser et al. 2007) or 30 (Blackwell et al. 2007) landmarks. The second test of the present chapter demonstrated that the number of landmarks was not statistically significantly influencing the 3D-DMI comparison outcomes. Although a higher number of landmarks contains more positional information, it also contains more operator interventions, increasing the bias. Moreover the third test demonstrated that a systematic error gradually occurs from landmark misplacement of 0.5mm to 5mm, highlighting that even small landmark displacements may interfere with the outcomes. In order to increase the landmarking reliability, landmarking required exhaustive operator training and calibration. Tests seven and eight indicated high examiner reproducibility, proving optimal landmark placement reproducibility in the present chapter. To eliminate the need for manual landmarking in further studies, automated landmarking and or image superimposition procedures are necessary. In the present chapter only GS offered this modality. Until the present, no studies aiming to prove the UHD applied automated 3D-DMI superimpositions.

The fourth test compared the automated superimposition with the semi-automated and the manual superimpositions, revealing no statistically significant difference within (Table 5) and between (Table 7) software. For further studies the automated system is the most adequate, because once it rules out the need for landmarking and reduces the number of operator-depending procedures.

The fifth test simulated investigation procedures enabling to prove the UHD. Indeed, finding no identical 3D-DMI in randomly and pair wise compared sampled subjects suggests proof for the UHD. Based on that, a threshold value was obtained in GS and CC from the comparison of identical 3D-DMI, separately for the mandible and maxilla. The threshold mean and standard deviation were slightly higher in CC than in GS, for the mandible as well as the maxilla (Table 5). Consequently CC had more bias and less precision compared to GS. This inconsistency between software was statistically significant (Table 7). Therefore GS is recommended in the search for identical 3D-DMI.

The ninth test was necessary to verify the quality of the scanning process using the XCAD 3D® (XCADCAM Technology®, São Paulo, SP, Brazil) scanning device. The precision of this device for image acquisition is nearly 20 microns. Other authors reported 3D scanning image precision from 10 (Bush et al. 2011b) to 100 (Sheets et al. 2013) microns, whereas flatbed (2D) scanners reached 85 microns (Bush et al. 2011b). The scanning device used in the present chapter revealed satisfactory outcomes, without increasing the mean error in hundredths of millimeters (<0.00mm). In the same context, the tenth test assessed the calibration of the measuring tools of each software and revealed optimal performances for GS, CC and MS. No difference was detected comparing in situ and digital measurements.

Table 7 – Within results of test 1, 2, 4-8 compared with Student’s t-test for paired samples and Wilcoxon signed rank test, between software

#	Variable	Software	MMD	SD	*t* (*p*)
1	**Number of pre-cropping points**				
	28	GS	21.03	2.77	2.10 (0.06)
		CC	16.63	3.76	
	58	GS	19.41	6.29	1.00 (0.34)
		CC	15.35	6.55	
	94	GS	17.81	3.52	0.76 (0.47)
		CC	16.48	1.67	
2	**Number of landmarks**				
	4	GS	0.11	0.08	-2.09 (0.09)
		CC	1.32	1.22	
	10	GS	0.16	0.19	-0.55 (0.59)
		CC	0.23	0.19	
	18	GS	0.11	0.20	-0.73 (0.46)[a]
		CC	0.72	1.18	
4	**Level of automation**				
	Automated	GS	0.09	0.05	n/a
		CC	n/a	n/a	
	semi-automated	GS	0.12	0.09	-0.23 (0.81)
		CC	0.10	0.10	
	Manual	GS	0.13	0.09	-1.29 (0.23)
		CC	0.21	0.10	
5	**Discrimination of 3D-DMI**				
	Identical 3D-DMI	GS	0.0000	0.00	-3.42 (0.00)
		CC	0.0010	0.00	
	Different 3D-DMI	GS	0.04	0.53	-1.29 (0.20)
		CC	0.22	0.33	
6	**Examiner reliability - Cropping**				
	Inter examiner	GS	0.00	0.00	n/a
		CC	0.00	0.00	n/a
	Intra examiner	GS	0.00	0.00	n/a
		CC	0.00	0.00	n/a
8	**Examiner reliability – Landmarking (ST)**				
	Inter examiner	GS	0.00	0.00	-0.04 (0.96)
		CC	-0.02	0.13	
	Intra examiner	GS	0.00	0.00	1.18 (0.25)
		CC	-0.02	0.06	
7	**Examiner reliability – Landmarking (PS)**				
			MLM		
	Inter examiner	GS	17.99	8.01	0.53 (0.59)
		CC	16.71	7.17	
		GS	17.99	8.01	0.91 (0.36)
		MS	20.45	8.97	
		CC	16.71	7.17	-1.45 (0.15)
		MS	20.45	8.97	
	Intra examiner	GS	18.00	7.95	0.64 (0.52)
		CC	16.46	7.06	
		GS	18.00	7.95	0.87 (0.38)
		MS	20.36	9.02	
		CC	16.46	7.06	-1.52 (0.13)
		MS	20.36	9.02	

#: test number; ST: software tools; PS: Photoshop®; MMD: mean morphological difference; SD: standard deviation; MLM: mean linear measurement; p: significance rate set at 95%; t: Student’s “t” value; GS: Geomagic Studio®; CC: Cloud Compare®; MS: Maestro Ortho Studio®; [a]: Mann-Whitney’s Z value; n/a: not applicable MMD, SD and MLM expressed in millimeters.

Table 8 – Available tools and properties in the three examined software packages

Tool / Property	Advantages and disadvantages	GS	CC	MS
Cropping	3D visualization while cropping	+	-	-
	Cropping with curved lines	+	-	-
Landmarking	3D visualization while landmarking	+	+	-
	Immediate Cartesian coordinate	-	+	-
Superimposition	Automated	+	-	-
Acquisition	Freeware / open source	-	+	-
Compatible operating system	Windows®	+	+	+
	MacOS®	-	+	-
File format	Import .stl files	+	+	-
	Import specific file	+	+	-

+: present; -: absent; GS: Geomagic Studio®; CC: Cloud Compare® (also operating in Linux®); MS: Maestro Ortho Studio®.

Applicability and operating modes of the evaluated software were compared and GS was found to perform superiorly. MS was designed for orthodontic purposes and consequently presented less application tools compared to the GS and CC software, developed for engineering and graphic design. MS missed the essential 3D tools allowing for analysis and comparison of the cropping and the superimposition performances. GS was most advantageous, mainly due to the automated superimposition and to the cropping toolbox, which allowed for simultaneous manipulation and delimitation of curves. CC is certainly useful for the morphological analysis of the human teeth but compared to GS it requires more time and manual work to prepare the images for comparison. CC is freeware and is also compatible with Mac OS® (Apple Inc., Cupertino, California, USA) operating system (Table 8).

Considering its superior toolbox for cropping procedures; its options for automated superimposition; and its most precise discrimination of identical 3D-DMI, GS figured as the most appropriate software for further pairwise comparisons of dentitions in the investigations on the UHD. Its application is recommended in the context of forensic BM and identification research.

Questionamento: *Pode uma amostra ser estratificada significantemente para a população geral a fim de provar a UHD?*

Objetivo: *"Verificar se uma amostra significante para a população geral pode ser estratificada para provar a UHD".*

Hipótese: *"Uma amostra significante para a população geral pode ser estratificada para provar a UHD".*

Características dentais que potencialmente viabilizam a estratificação amostral

Capítulo 5

Capítulo baseado no artigo:

Nikolaos Angelakopoulos, Ademir Franco, Steffen Fieuws, Guy Willems, Patrick Thevissen. Clinically detectable dental identifiers observed in intraoral photographs and extraoral radiographs, validated for human identification purposes. J Forensic Sci. 2016 aceito.

Apresentação oral no 68° Encontro Científico da Academia Americana de Ciências Forenses, Las Vegas, USA, 2016.

INTRODUCTION

Collecting a sample representative for the world population is practically unfeasible studying the UHD. Sample stratification was used to reduce the sample size, narrowing the number of sampled subjects based on specific features available. Besides allowing the reduction of the sample size, the sample stratification also enables stronger assumptions in the search for UHD. It is justified because proving the UHD in subjects with similar dental features is potentially more difficult than proving it randomly in an open population. In the field of fingerprint analysis, sample stratification was based on selecting Caucasian males presenting the same pattern of finger ridges, namely the left loop (Meagher et al. 1999). In forensic odontology, sample stratification was already performed in the search for the UHD. Specifically, monozygotic twins (Sognnaes et al. 1982) and orthodontically treated patients (Sheets et al. 2011; Kieser et al. 2007) were selected. This selection was justified to narrow the selection of subjects that present similar dental morphology and arrangement, respectively. However, several other features are available in Dentistry to possibly base sample stratification on. These features are better known in dental identification as “identifiers”. Specific identifiers can be used to determine populations, e.g. crowding, dental rotation, displacement, diastemas, orthodontics brackets, attrition and prosthetic crowns. Their potential to reduce the sample size depends on the unique information that they possibly contain.

The identifiers are often registered in the routine of Dentistry and Medicine through medical imaging techniques such as radiographs, computed tomography images, and photographs (Silva et al. 2011). Specifically in dentistry, treatment procedures may require particular imaging registrations. Periapical radiographs are often required in endodontics (R. F. Silva et al. 2014), to perform root canal treatment (Forrest & Wu 2010) and in periodontics to investigate the bone status. Panoramic radiographs are more prevalent in orthodontics and maxillofacial surgery in order to have an overview of the maxillofacial bones and teeth prior to diagnosis and treatment planning (Farman 2007).

The detection of dental identifiers that contribute to the significant reduction sample size was aimed in the present chapter. Separately, dental identifiers collected on photographs and radiographs were studied. Consequently, it will reveal the most adequate pathways for sample stratification in the search for UHD.

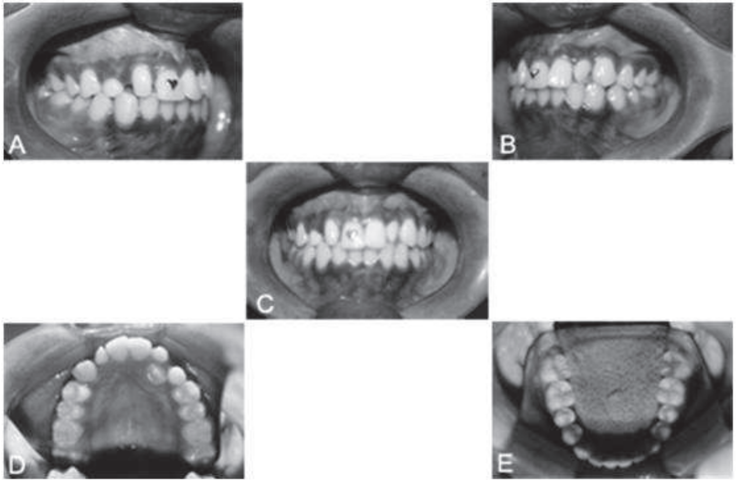
MATERIAL AND METHODS

The studied sample consisted of 1727 subjects (females: 1025; males: 702) with black origin from whom a panoramic radiograph, a lateral cephalometric radiograph and an intraoral photograph series was collected (target set). In each subject, all medical images were registered the same day and collected retrospectively from the database of a private dental clinic in Pretoria, South Africa.

The subjects were aged between 2.3 and 40.1 years, with a mean age of 15.1 years (SD: 4.99 years). The exclusion criteria comprised poor photographic or radiographic image quality.

The intraoral photograph series consisted of 5 photographs taken with a Canon® DS126191 W (Canon, Tokyo, Japan) digital camera mounted with a Canon® EF 18-55mm Macro Lens (Canon, Tokyo, Japan) and a Canon® Macro Ring Lite MR-14EX flash (Canon, Tokyo, Japan) according to the American's Academy of Cosmetic Dentistry Guidelines (Goodlin 2011) (Figure 9). The panoramic- and lateral cephalometric radiographs were taken with a Kodak® 8000C (Kodak, Rochester, New York, EUA) radiographic unit.

Figure 9 – Intraoral photographs taken in five views



A: right lateral view; B: left lateral view; C: frontal view; D: maxillary occlusal view; E: mandibular occlusal view

The variables studied consisted of clinically detectable dental identifiers. They were observed and registered separately for each photograph series, each panoramic radiograph and each lateral cephalometric radiograph. The clinically detectable identifiers were classified in four groups: numbers of teeth present; numbers of prosthetic teeth; tooth specific dental identifiers (Table 9) and general dental identifiers (Table 10).

Table 9 - Description of the tooth specific dental identifiers used in the present chapter

Tooth specific identifier	**Description**
Sound	Hygid tooth, not containing other identifiers
Rotated	Tooth rotated around the vertical axis
Ectopic	Tooth placed out of anatomic site in the dental arch
Displaced	Tooth tilted to mesial, distal, vestibular or lingual sides
Maxillary midline diastema *	Lack of mesial contact between maxillary central incisors
Caries	Decay cavities in the crown
Attrition, abrasion or erosion	Mechanically- or chemically-induced loss of dental tissue on occlusal or incisal surface
Unidentified filling material	Restorative material not identifiable
Calculus deposit	Calcified deposit adhered to the root or crown
Dental piercing *	Aesthetic appliance in the crown
Sealant	Superficial layer of tooth-colored composite in the crown
Single Metallic fixed prosthesis *	Metal crown
Non-metallic fixed prosthesis *	Crown made of tooth-colored material
Bridges and components *	Prosthetic appliance linking two or more prosthetic crowns
Composite filling	Aesthetic tooth-colored material in the crown
Metallic filling	Gold or amalgam filling in the crown
No information	Region/tooth of interest is not visible
Missing tooth	Region of interest is visible but the tooth is absent
Extracted tooth	Tooth is absent leaving empty socket

**: tooth specific identifiers not considered in deciduous teeth.*

The properties established regarding the last two groups were listed and described in table 10. The numbers of present teeth was registered by summing the number of deciduous and permanent teeth observed. The numbers of the restorative prosthetic teeth included teeth restored with a fixed restorative prosthesis. The tooth specific dental identifiers were registered separately for each present deciduous and permanent tooth positions. They contained 19 clinically detectable identifiers, of which 18 were evaluated on each of the 32 permanent tooth positions.

Table 10 - Description of the general dental identifiers used in this chapter, part 1/2

General dental identifier	Description
Oval-shaped crown	Maxillary central incisors with oval-shaped crowns
Square-shaped crown	Maxillary central incisors with square-shaped crowns
Triangle-shaped crown	Maxillary central incisors with triangle-shaped crowns
Atypical shape	Maxillary central incisors with atypical shaped crowns
Not applicable crown shape	Maxillary central incisors are absent or crowns are not visible
Number of diastemas *	Number of observed diastemas
Retained primary dentition	Deciduous teeth observed among fully erupted permanents
Mixed dentition	Deciduous teeth in transition to permanent dentition
Permanent dentition *	Only permanent teeth were observed
Supernumerary dentition	Supernumerary teeth in permanent dentition
Not applicable dentition	Edentulous arches
Open bite	Lack of occlusal contact between maxillary and mandibular teeth
Edge to edge	Occlusal contact between maxillary and mandibular incisor borders
Overjet	Protrusion of the maxillary incisors in relation to the antagonist
Cross bite	In occlusion maxillary teeth are positioned lingual to the mandibular.
Overbite	In occlusion maxillary teeth overlap the mandibular
Not applicable malocclusion	The malocclusion does not fit any of the previous malocclusions
Left canines in Angle's class I *	Tooth #23 occluding between teeth #33 and #34
Left canines in Angle's class II *	Tooth #23 occluding anterior to the position in class I
Left canines in Angle's class III *	Tooth #23 occluding posterior to the position in class I
Not applicable Angle's classification for left canines	Permanent left canines are missing
Left molars in Angle's class I *	Mesiobuccal cusp of #26 occluding on buccal groove of #36
Left molars in Angle's class II *	Cusp of #26 occluding anterior to the position in class I
Left molars in Angle's class III *	Cusp of #26 occluding posterior to the position in class I
Not applicable Angle's classification for left molars	Permanent left molars are missing
Right canines in Angle's class I *	Mesiobuccal cusp of #13 occluding on buccal groove of #43
Right canines in Angle's class II *	Cusp of #13 occluding anterior to the position in class I
Right canines in Angle's class III *	Cusp of #13 occluding posterior to the position in class I
Not applicable Angle's classification for right canines	Permanent right canines are missing
Right molars in Angle's class I *	Mesiobuccal cusp of #16 occluding on buccal groove of #46
Right molars in Angle's class II *	Cusp of #16 occluding anterior to the position in class I
Right molars in Angle's class III *	Cusp of #16 occluding posterior to the position in class I

**: general dental identifiers not considered in deciduous teeth.*

Table 10 - Description of the general dental identifiers used in this chapter, part 2/2

Not applicable Angle's classification for Right molars	Permanent right molars are missing
Oval Maxillary dental arch	Maxillary arch presents oval shape in occlusal view
Tapered maxillary dental arch	Maxillary arch presents tapered shape in occlusal view
Square maxillary dental arch	Maxillary arch presents square shape in occlusal view
Not applicable shape for maxillary dental arch	Maxillary arch is not visible or does not fit the previous shapes
Number of orthodontic brackets	Number of observed orthodontic brackets
Not applicable malocclusion	The malocclusion does not fit the previous description
Oval mandibular dental arch	Mandibular arch presents oval shape in occlusal view
Tapered mandibular dental arch	Mandibular arch presents tapered shape in occlusal view
Square mandibular dental arch	Mandibular arch presents square shape in occlusal view
Not applicable shape for mandibular dental arch	Mandibular arch is not visible or does not fit the previous shapes

The clinically detectable identifier, maxillary midline diastema, referred to the tooth positions #11 and #21 (FDI notation). Fourteen of these clinically detectable identifiers were evaluated in each of the 20 deciduous tooth positions. General dental identifiers where considered as variables referring to the whole dentition.

The general dental identifier considering the shape of central incisors was evaluated according to Williams (1914), considering the relation between incisal and cervical diameter of the tooth. The evaluation was carried out after observing the maxillary right central incisor (#11). In the absence of tooth #11, the contralateral (#21) was observed. If maxillary central incisors were replaced by fixed prosthesis, the shape of the prosthetic crown (#11 or #21) was considered. The shape of the dental arches was classified according to Paranhos et al. (2011), who describe the arch shape in occlusal view.

The general dental variables contained 43 clinically detectable identifiers. Fourteen of them were not considered in deciduous and mixed dentitions.

In the target set (n=1727), the clinically detectable dental identifiers were registered in 895 subjects by a first observer and in the remaining 832 subjects by a second observer. In 308 of these subjects the registration was also performed by the other observer. The set of these subjects is referred to as the source set. The aim is to identify each subject from the source set in the target set. Remark that each subject in the source set also belongs to the target set, but has been scored by the other observer. This reflects forensic identification practice in which the ante-mortem (AM) and post-mortem (PM) evidences are not observed and registered by the same examiner.

For each of the clinically detectable dental identifiers, the distance was quantified between each subject in the source set (n=308) and each of the subjects in the collected target set (n=1727). For clinically detectable dental identifiers referring to counts (the numbers of teeth present, the numbers of prosthetic teeth, the numbers of orthodontic brackets), the distance was the difference in count. For categorical clinically detectable dental identifiers (e.g. crown shape, Angle's class, arch shape), the distance was classified as equal zero (equal identifier) or one (unequal identifier). Because specific clinically detectable dental identifiers were potentially present on multiple teeth, two approaches were distinguished to quantify the distance. First, based on a single feature**,** the number of teeth with the identifier was counted and this count was used as a variable, such that the distance equals the difference in count. This implies that two subjects with the same number of teeth having the identifier were considered to be equal, irrespective which teeth had the feature. Second, based on the pattern of presence of a feature the distance between two subjects equals the number of teeth having a difference in identifier. This implies that two subjects with the same number of teeth having the feature were only considered to be equal if the feature was present at exactly the same tooth/teeth.

The percentage of subjects having in the target set a distance at least as small as the correct subject (in the source set) were referred to as the potential set. The size of the potential set reflects how large a set with candidate matches should be in order not to exclude the correct subject. The mean potential set as well as the percentages of subjects in the inter-observer set for which the potential set was smaller than 20%, 10% and 5% of the reference set were reported, respectively. Analyses have been performed using SAS® 9.2 (SAS Institute Inc., Cary, North Carolina, USA) for Microsoft Windows® (Microsoft Corp., Redmond, Washington, USA) operating system.

RESULTS

The lower the mean potential set, the more useful (most unique) the considered clinically detectable identifier was in identifying the correct subject. Based on a single variable, in photographs, the most unique clinically detectable dental identifier was the number of molars, presenting 14 unique values in the target set and 34.6% of the subjects from the target set registered in the potential set. For the number of molars, the percentage of subjects in the source set for which the potential set is smaller than 20%; 10% and 5%, were 7.6%; 3.9% and 1.6%, respectively (Table 11). In panoramic radiographs, the most unique clinically observable dental identifier was the number of missing teeth, with 28 unique values in the target set and 42% of the subjects from the target set registered in the potential set). For the number of missing teeth the percentage of subjects in the source set for which the potential set is smaller than 20%; 10% and 5%, were 6.7%; 5.9%; and 3.8%, respectively (Table 12). In lateral cephalometric radiographs, the most unique clinically observable dental identifier was the number of displaced teeth, with 5 unique values in the target set and 59.92% of the subjects from the target set registered in the potential set). For the number of displaced teeth, the percentage of subjects in the source set for which the potential set is smaller than 20%; 10% and 5%, were 0.7%; 0.1%; and 0.1%, respectively (Table 13).

Based on the pattern of presence of a feature, in photographs, the most unique clinically observable dental identifier group was the pattern of rotated teeth, with 110 unique values in the target set and 14.9% of the subjects from the target set registered in the potential set. For the number of missing teeth the percentage of subjects in the source set for which the potential set is smaller than 20%; 10% and 5%, were 13.1%; 10.4%; and 9.3%, respectively (Table 11). In panoramic radiographs, the most unique clinically observable dental identifier group was the pattern of displaced teeth, with 406 unique values in the target set and 37.6% of the subjects from the target set registered in the potential set. For the pattern of displaced teeth, the percentage of subjects in the source set for which the potential set is smaller than 20%; 10% and 5%, were 6.1%; 4.6%; and 4.2%, respectively (Table 12). In lateral cephalometric radiographs, the most unique clinically observable dental identifier group was also the pattern of displaced teeth, with 42 unique values in the target set and 54.8% of the subjects from the target set registered in the potential set. For the pattern of displaced teeth, the percentage of subjects in the source set for which the potential set is smaller than 20%; 10% and 5%, were 0.9%; 0.9%; and 0.1%, respectively (Table 13).

Table 11 – Top-5 clinically detectable dental identifiers observed in photographs (based on a single feature and on the pattern of presence of a feature) yielding the smallest mean* potential set, i.e. the percentage of subjects in the target set being as close or closer to the correct subject

CDDI	UV**	Mean potential set		<=20%	<=10%	<=5%
		#Subjects	%***			
Based on a single feature						
Number of molars	14	597.8	34.6%	7.6%	3.9%	1.6%
Number of rotated teeth	13	660.9	38.3%	7.2%	1.2%	0.3%
Number of sound teeth	30	685.8	39.7%	6.1%	3.8%	2.4%
Number of displaced teeth	12	745.3	43.2%	4.7%	0.4%	0.2%
Number of diastemas	18	757.2	43.8%	6.0%	2.5%	1.2%
Based on the pattern of presence of a feature						
Rotated tooth	1010	257.2	14.9%	13.1%	10.4%	9.3%
Sound tooth	366	568.7	32.9%	8.3%	4.6%	4.2%
Displaced tooth	404	629.9	36.5%	5.2%	5.0%	4.7%
Missing tooth	457	803.2	46.5%	4.8%	4.8%	4.6%
Maxillary midline diastema	51	1042.0	60.3%	0.3%	0.3%	0.3%

: mean over all subjects in the target set; CDDI: clinically detectable dental identifier; **: unique values detected in the target set; *: % of subjects in target set; <=20%, 10%, 5%: percentage subjects in source set for which the potential set is smaller than 20, 10 and 5%, respectively*

Table 12 – Top-5 clinically detectable dental identifiers observed in panoramic radiographs (based on a single feature and on the pattern of presence of a feature) yielding the smallest mean* potential set, i.e. the percentage of subjects in the target set being as close or closer to the correct subject"

CDDI	UV**	Mean potential set		<=20%	<=10%	<=5%
		#Subjects	%			
Based on a single Feature						
Number of missing teeth	28	724.7	42.0%	6.7%	5.9%	3.8%
Number of displaced teeth	12	753.3	43.6%	4.5%	1.9%	0.3%
Number of diastemas	16	796.1	46.1%	5.2%	2.5%	1.3%
Number of molars	19	813.9	47.1%	5.9%	5.4%	4.1%
Number of rotated teeth	15	850.7	49.3%	4.3%	2.4%	0.9%
Based on the pattern of presence of a feature						
Displaced tooth	406	649.8	37.6%	6.1%	4.6%	4.2%
Rotated tooth	573	664.6	38.5%	5.0%	4.7%	4.2%
Missing tooth	368	706.8	40.9%	5.9%	5.9%	5.8%
Unidentified filling material	288	945.8	54.8%	3.1%	2.8%	2.7%
Maxillary midline diastema	11	1132.6	65.6%	0.1%	0.1%	0.1%

: mean over all subjects in the target set; CDDI: clinically detectable dental identifier; **: unique values detected in the target set; *: % of subjects in target set; <=20%, 10%, 5%: percentage subjects in source set for which the potential set is smaller than 20, 10 and 5%, respectively*

Table 13 – Top-5 clinically detectable dental identifiers observed in lateral cephalometric radiographs (based on a single feature and on the pattern of presence of a feature) yielding the smallest mean* potential set, i.e. the percentage of subjects in the target set being as close or closer to the correct subject"

CDDI	UV**	Mean potential set		<=20%	<=10%	<=5%
		#Subjects	%			
Based on a single Feature						
Number of displaced teeth	5	1034.7	59.9%	0.7%	0.1%	0.1%
Number of molars	10	1094.8	63.4%	3.5%	3.3%	1.3%
Number of unidentified filling material	9	1212.7	70.2%	2.1%	0.9%	0.5%
Malocclusion	7	1237.1	71.6%	3.9%	2.8%	0.9%
Type of dentition	4	1260.7	73.0%	3.2%	0.1%	0.1%
Based on the pattern of presence of a feature						
Displaced tooth	42	946.1	54.8%	0.9%	0.9%	0.8%
Missing tooth	133	1184.3	68.6%	2.1%	2.0%	1.6%
Unidentified filling material	79	1187.6	68.8%	2.2%	2.2%	1.6%
Sound tooth	15	1624.6	94.1%	0.0%	0.0%	0.0%
Dental piercing	7	1684.4	97.5%	0.1%	0.1%	0.1%

: mean over all subjects in the target set; CDDI: clinically detectable dental identifier; **: unique values detected in the target set; *: % of subjects in target set; <=20%, 10%, 5%: percentage subjects in source set for which the potential set is smaller than 20, 10 and 5%, respectively*

DISCUSSION

The type of dental identifier used in forensic odontology considerably varied in the last decades. For long, unique information from the teeth was mainly obtained from dental treatment identifiers. Currently the challenge consists on retrieving this information from morphological identifiers, because the increased awareness of dental hygiene, which consequently decreased restorative dental treatment worldwide, reduced the need for dental treatment (Athanassouli et al. 1994; Truin et al. 1994). Indeed, the number of unidentified filling material, was the only dental treatment identifier detected among the 5 most unique clinically detectable dental identifiers (less prevalent clinically detectable identifiers in the potential set) for single variables and for the patterns of presence of a feature, observed in photographs, panoramic radiographs and lateral cephalometric radiographs.

The current research highlighted that patterns of identifiers are more unique compared to single identifiers. The scientific literature indicates that the human dentition can be charted in more than 2.5 billion combinations, considering the available number of dental identifiers (Pereira & Santos 2013). Simulating the combination of different dental identifiers revealed that the probability of two persons having the same six missing teeth and five restored teeth is 1 in 59 billion (Senn & Stimson 2010). Quantifying the number of combinations with 16 missing teeth may reach 1 in 600 million (Rajendran & Silvapathasundharam 2012). In this chapter, the uniqueness of an identifier was assessed considering its ability to reduce the reference set. In intraoral photographs, the single identifier “rotation” reduced the sample from 1727 to 597.8 subjects, while the pattern of rotation reduced it to 257.2 subjects. In panoramic radiographs, the single variable “displacement” reduced the sample to 753.3 and the pattern of displaced teeth was reduced to 649.8 subjects. Yet in lateral cephalometric radiographs this phenomenon shrank the sample to 1034.7 and 946.1 subjects, considering the single variable “displacement” and its pattern, respectively. It must be noted that in this chapter the performance of the uniqueness of the considered clinically detectable dental identifiers was overoptimistic because the images in the inter-examiner set were collected from the reference dataset.

Reducing the number of potentially matching subjects using clinically detectable dental identifiers is dependent on the available registration type. In the present chapter the photographic registration revealed more power for reducing the sample if compared to the radiographic registration. The number of molars detected in photographs can reduce the sample to 34.6% when considered as single variable, while in panoramic and lateral cephalometric radiographs the same identifier reduces the sample only to 47.1% and 63.4%, respectively. Radiographs are commonly registered and saved, while photographs are rarely used as registration tool in dental practice. The present outcomes encourage the integration of photographs in the clinical (AM) records, considering that they provide an additional source of clinically detectable identifiers. Moreover dental photographs are easily and quickly taken in daily practice. Because photographs and radiographs have the limitation of registering the dental features at a certain moment in time, an additional longitudinal follow up registration can be recommended. Therefore a photographic registration is preferred because the patient is not charged with ionizing radiation.

Both photographic and radiographic image registration may be performed in different geometric setups. However, usually when photographs are requested for clinical purposes they become part of a single set of five views (angles) (Omanovic & Orchard 2008), providing and combining more dental information if compared to a single radiographic view obtained in panoramic and lateral cephalometric registrations. The devices necessary for registering the dental information in five views has lower cost when compared to the devices necessary for radiographic registrations. Photographs also require less time for image acquisition compared to radiographs. Moreover, exposure to ionizing waves is not necessary in the photographic registration, allowing for the registration of image series. Yet the radiographic registration must be performed only with proper pre-, trans-, or post-therapeutic reasons (American Dental Association Council on Scientific Affairs 2006). On the other hand, the major limitation of intraoral photographs consists on the registration limited to the oral soft tissues and the dental crowns, while in radiographs a wide range of information from dental roots and maxillofacial bones may be detected.

In the context of the present research, the most appropriate technique for image registration in the search for the UHD is the intraoral photographs. This technique is justified as the most appropriate because the crown morphology of the clinically visible anterior teeth is exclusively available for analysis in the context of BM. In photographs the number of molars and the rotated teeth were the most useful identifiers to reduce the sample size considering single and pattern of features, respectively. However, stratifying samples for the investigation of the UHD is not feasible with these identifiers. It is justified because of the inherent characteristics of the identifiers. The number of molars is clearly not relevant in the context of BM analysis, once molars hardly appear in bite patterned injuries. Yet the pattern of rotated teeth requires a highly complex procedure for stratification in the context of BM, in which subjects presenting the exact same pattern of rotated teeth should be sampled – characterizing a practically unfeasible sampling approach. Additionally, despite revealing that the number of molars and the pattern of rotated teeth play a major role in reducing the sample size, is importantant to note that these identifiers may vary with time – e.g. the fisrt may vary with progressive dental loss and the second may vary following orthodontic treatment. Sampling twins and orthodontically treated patients is corroborated as a better practice in the search for the UHD, considering that these subjects are already categorized by dental similarity, and that the similarity of their dental features (dental morphology and arrangement) trends to vary less compared to the identifiers used in the present chapter. Moreover, the dentitions of twins and orthodontically treated patients are similar in general, not considering specific identifiers separately. The opposite was performed in the present research chapter, when specific dental features were considered independently. Finally, twins and orthodontically treated patients present a level of similarity that is important in the context of BM, comprehending especially a similar dental morphology and arrangement, respectively.

Questionamento: *A dentição humana é única ao se analisar a morfologia da coroa completa dos dentas anteriores?*

Objetivo: *"Investigar a UHD analisando a morfologia da coroa completa dos dentes anteriores".*

Hipótese: *"A dentição humana é única analisando a morfologia da coroa completa dos dentes anteriores".*

Investigando a UHD pela análise das coroas dentais anteriores

Capítulo 6

Capítulo baseado no artigo:

Ademir Franco, Guy Willems, Paulo H. C. Souza, Orlando M. Tanaka, Wim Coucke, Patrick Thevissen. Three-dimensional analysis of the uniqueness of the anterior human dentition in orthodontically treated patients and twins. Forensic Sci Int. 2016 Submetido.

INTRODUCTION

As observed in chapter 3, several studies in the field investigated UHD in the context of BM (Blackwell et al. 2007; Bush et al. 2011b; Kieser et al. 2007; Sheets et al. 2011). However, the reported outcomes were possibly biased based on the methodological aspects applied (Franco et al. 2015). Random sampling was one of the issues observed in these studies (Sognnaes et al. 1982; Tuceryan et al. 2011; Blackwell et al. 2007). In chapter 5 was concluded that evaluating a specific population type enables to select subjects presenting similar dental morphology (twins) and arrangement (orthodontically treated patients). Applied in previous studies, sample stratification based on a specific population type did not enable to support a scientific proof of the UHD due to additional methodological research limitations (Kieser et al. 2007; Sheets et al. 2011; Sognnaes et al. 1982). These limitations consisted of 2D image registration techniques used to compare 3D structures (human teeth); operator-depending procedures (landmarking); the lack of operator reproducibility control (intra-/inter-reliability tests); or the lack of proper data analysis (statistics) (Franco et al. 2015).

The current chapter aimed first to establish a threshold sample to measure and consider all the potential faults inherent to the technical procedures involved in the search for the UHD. Second, was aimed to investigate the UHD three-dimensionally (3D) comparing the dental crown morphology of the anterior dentition in stratified samples of orthodontically treated patients, twins, and orthodontically treated twins. Third, was aimed to include a sample of random patients to prove and express the importance of sample stratification in the investigations on the UHD.

MATERIAL AND METHODS

Three groups of dental casts were sampled and 3D digitized. Group 1 was composed by 22 dental casts (11 mandibular and 11 maxillary) of randomly selected patients (7 males and 4 females). Group 2 consisted of 59 maxillary dental casts of orthodontically treated patients (32 males and 27 females), collected after the removal of the orthodontic brackets. Group 3 included 344 dental casts (172 mandibular and 172 maxillary) of 86 pairs of twins, 39 were monozygotic (36 males and 42 females) and 47 were dizygotic (50 males and 44 females). Group 4 comprised 20 dental casts (10 mandibular and 10 maxillary) of 5 pairs of orthodontically treated monozygotic twins (2 males and 8 females) (Table 14).

Table 14 – Subject distribution per samples stratified on dental arch, zygosity and sex

Arch	Group	Zygosity	Male (n)	Female (n)	Subjects (n)	DCF (n)
Maxillary	1	n/a	7	4	11	11
	2	n/a	32	27	59	59
	3	Monozygotic	36	42	78	78
	3	Dizygotic	50	44	94	94
	4	Monozygotic	2	8	10	10
	5	n/a	2	3	5	10
Mandibular	1	n/a	7	4	11	11
	3	Monozygotic	36	42	78	78
	3	Dizygotic	50	44	94	94
	4	Monozygotic	2	8	10	10
	5	n/a	2	3	5	10

DCF: digital cast files; Group 1: random patients; Group 2: orthodontically treated patients; Group 3 twins; Group 4: orthodontically treated twins; Group 5: threshold; n/a: not applicable.

On all included dental casts the permanent anterior teeth were present from canine to canine. Dental casts with clinically visible supernumerary teeth in the anterior region, restorative or prosthetic dental treatment in the anterior teeth, and fixed orthodontic retainers were excluded. Due to the last, the mandibular casts of Group 2 were excluded. In all the groups, the dental impressions were taken by the same operator (author) with alginate (Jeltrate Dustless®, Dentsply®, York, PA, USA) following the instructions of the manufacturer. These impressions were casted with plaster type IV (Durone®, Dentsply®, York, PA, USA) and digitalized using an automated motion device with angular laser scanning (XCAD 3D® (XCADCAM Technology®, São Paulo, SP, Brazil) in resolution of <20 microns. The obtained digital cast files (DCF) were stored in .STL format and imported for morphometric analyses and pair wise comparison in Geomagic Studio® (3D Systems®, Rock Hill, SC, USA) software package (GS). To limit the comparisons to the anterior dentition, a standardized GS cropping procedure was established, placing on each DCF a cropping contour along the cemento-enamel junction of the anterior teeth based on 58 pre-cropping points.

To establish mean threshold values determining when two cropped DCF could be considered morphologically identical one examiner took impressions of

5 different subjects and repeated it after 7 days. The dental impressions were casted, digitized and prepared for analysis according to the procedures previously described and used as reference group (Group 5). The mean thresholds values were a measure of the morphological comparison errors originating from the procedure to obtain the dental impressions, the casts, the DCF, the GS cropping procedure and the GS pair wise morphometric comparison.

Within random (Group 1) and orthodontically treated (Group 2) patients, all possible pair wise DCF comparisons were performed, totalizing 110 (55 per dental arch) and 1711 (only maxillary arch) comparisons, respectively. Specifically in these groups, sub-sampling was necessary to randomly select only the independent pair wise comparisons (in which the same DCF was not repeated). This procedure was repeated 250 times combining independent comparisons. Within twins (Group 3) and orthodontically treated monozygotic twins (Group 4), the DCF were pair wise compared with their respective twin sibling DCF, totalizing 172 (86 for the mandible and 86 for the maxilla) and 10 (5 for the mandible and 5 for the maxilla) comparisons, respectively. Additionally, in Group 3 mono- and dizygotic twin pair DCF were evaluated in function of the zygosity, totalizing 78 (39 for the mandible and 39 for the maxilla) and 94 (47 for the mandible and 47 for the maxilla) comparisons, respectively. In the reference sample (Group 5) the DCF of each subject obtained at moment 1 was pair wise compared with the respective DCF at moment 2, separately for the maxilla and mandible, totalizing 10 comparisons. All the pair wise comparisons were performed with the GS automated superimposition tool.

For each pair wise DCF comparison, GS calculated the observed morphological differences in dental crown morphology of the anterior dentitions. The differences were expressed in millimeters for each of the following quantification values: the maximum positive deviation (max.+); the maximum negative deviation (max.-); the average deviation (ave.); and the standard deviation (SD). The four quantification values were statistically combined and converted in a single value, comprehending the Euclidean distance from origin (zero) obtained with the formula: Distance=$\sqrt{\mathrm{Max}+^2 + \mathrm{Max}-^2 + \mathrm{Average}^2 + \mathrm{Standard\ deviation}^2}$. This procedure enabled to rank the distance values; to verify if distance values were equal; and to detect distance values equal to zero.

UHD was assessed in the four studied Groups (Groups from 1 to 4) comparing their Euclidean distances with the distances obtained in the reference (Group 5). One-way ANOVA (Casella 2008) with log-transformed distances, was applied for the comparisons, separately for maxilla and mandible. Considering the multiple comparisons between groups, a correction for simultaneous hypothesis testing was applied according to Tukey's range test (Tukey 1949). UHD was considered when the mean Euclidean distance of any studied Group was statistically significant higher than the reference Group. In the lack of statistically significant differences sampling quality control was assured performing a post-hoc Power Analysis with a desired effect of 80%.

Quality control for the comparative approach was assured for maxillary and mandibular DCF performing a ROC analysis. In this analysis Group 4 was confronted with all the other groups exploring its threshold potential for classifying non-equal dentitions as non-equal (sensitivity) and equal dentitions as equal (specificity). Confidence intervals for the ROC-curve and for the Area Under the Curve (AUC) were obtained by means of bootstrapping. ROC analyses were also performed systematically including less quantification values.

The statistical tests were performed with significance rate of 5% using S+® 8.0 (Tibco®, Palo Alto, California, USA) software package.

RESULTS

The morphologically most similar maxillary DCF within each Group had Euclidian distances of 5.24 (random patients - Group 1), 1.87 (orthodontically treated patients - Group 2), 2.03 mm (twins - Group 3), 1.88 mm (orthodontically treated twins - Group 4), and 0.66 mm (reference - Group 5). For the mandibular DCF these distances were 5.19 (Group 1), 1.29 mm (Group 3), 1.66 mm (Group 4), and 1.03 mm (Group 5) (Table 15, Figure 10). No equal or zero Euclidian distances were observed.

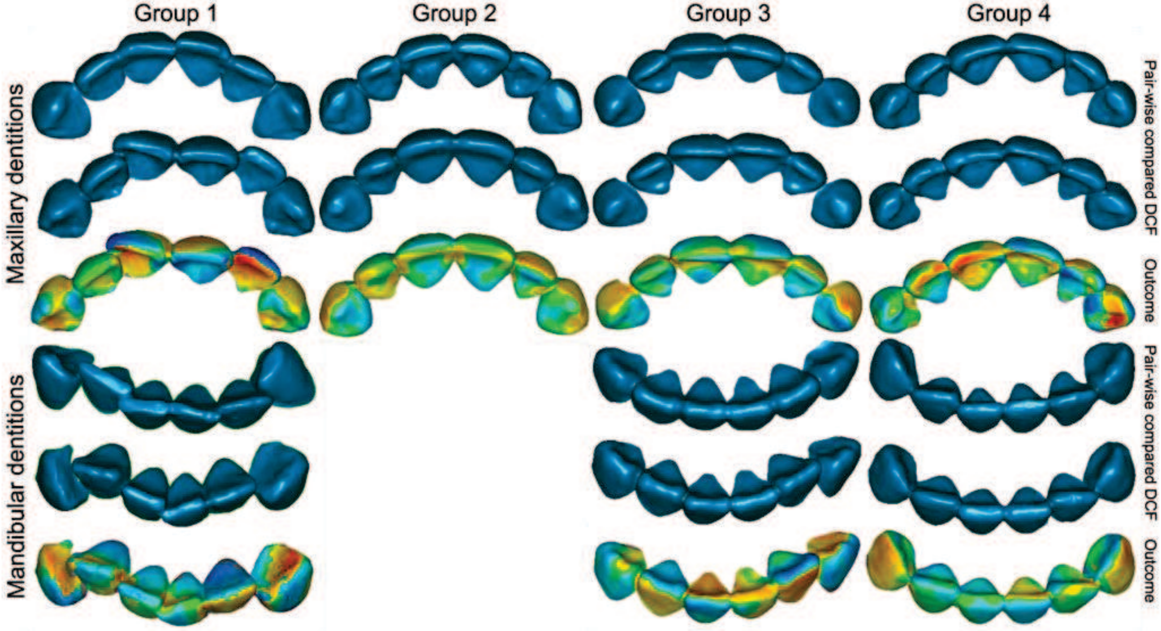
Statistically significant differences ($p<0.05$) were obtained for the maxillary DCF comparisons, compared between Groups 2 and 5, as well as Groups 3 and 5. The mandibular DCF comparisons were statistically significant different comparing Groups 3 and 5 ($p<0.05$). No statistically significant differences were observed comparing Groups 4 and 5 for both dental arches ($p>0.05$) (Table 16; Figure 11 and 12)

Table 15 – Quantifications and distances of the most similar pairs of maxillary and mandibular dentitions detected in each group

Dental arch	Group	Max.+	Max.-	Ave.	SD	Distance
Maxillary	1	3.20	-4.09	-0.01	0.77	5.24
	2	1.11	-1.45	-0.03	0.44	1.87
	3	1.41	-1.43	0.01	0.35	2.03
	4	1.33	-1.29	0.11	0.32	1.88
	5	0.49	-0.45	0.01	0.07	0.66
Mandibular	1	3.96	-3.16	0.35	1.10	5.19
	3	0.88	-0.90	0.03	0.29	1.29
	4	0.83	-1.41	-0.02	0.28	1.66
	5	0.84	-0.59	0.01	0.11	1.03

Group 1: random patients; Group 2: orthodontically treated patients; Group 3: twins; Group 4: orthodontically treated twins; Group 5: threshold; Max.+: maximum positive deviation; Max.-: maximum negative deviation; Ave.: average deviation; SD: standard deviation. Outcomes expressed in millimeters.

Figure 10 – Illustrative representation of the most similar pairs of maxillary and mandibular dentitions and their outcomes for the experimental groups (0, 1, 2 and 3)



The most similar pairs of reference and target maxillary and mandibular dentitions (exclusively blue) of random patients (group 1), orthodontically treated patients (group 2), twins (group 3) and orthodontically treated monozygotic twins (group 4). The outcomes are represented in a color code system ranging from red (max.+) to dark blue (max.-), in which greenish tones corresponds to morphological differences close to zero. Despite not considered statistically significant (p>0.05) compared to the threshold, group 4 revealed clear signs of morphological differences (reddish areas).

Post hoc power analysis demonstrated that in order to obtain statistically significant differences between Groups 4 and 5, sample enlargement with 10 and 51 additional pair wise comparisons would be necessary for the maxillary and mandibular DCF comparisons, respectively (Table 16).

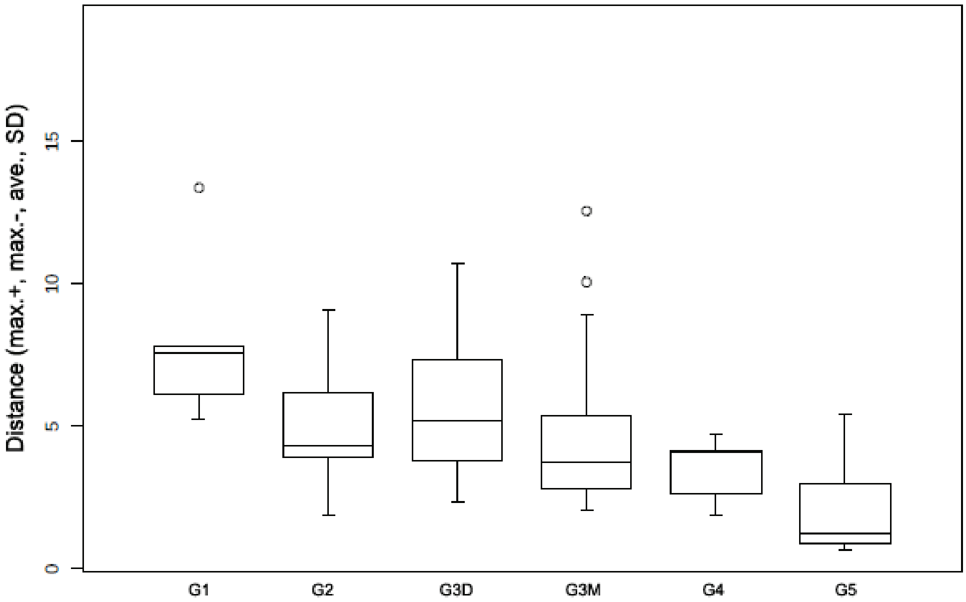
Table 16 – *P*-values obtained from ANOVA comparisons between Groups per dental arch

Dental arch	Comparison	Mean distances	*p*
Maxillary	G1 vs. G2	8.03 vs. 4.74	0.0888
	G1 vs. G3D	8.03 vs. 5.62	0.4196
	G1 vs. G3M	8.03 vs. 4.43	0.0248
	G1 vs. G4	8.03 vs. 3.51	0.0298
	G1 vs. G5	8.03 vs. 2.24	0.0001
	G2 vs. G3D	4.74 vs. 5.62	0.5140
	G2 vs. G3M	4.74 vs. 4.43	0.9631
	G2 vs. G4	4.74 vs. 3.51	0.7531
	G2 vs. G5	4.74 vs. 2.24	0.0001
	G3D vs. G3M	5.62 vs. 4.43	0.0613
	G3D vs. G4	5.62 vs. 3.51	0.2166
	G3D vs. G5	5.62 vs. 2.24	0.0001
	G3M vs. G4	4.43 vs. 3.51	0.9302
	G3M vs. G5	4.43 vs. 2.24	0.0003
	G4 vs. G5	3.51 vs. 2.24	0.0998[a]
Mandibular	G1 vs. G3D	10.79 vs. 4.85	0.0066
	G1 vs. G3M	10.79 vs. 3.79	0.0003
	G1 vs. G4	10.79 vs. 2.70	0.0006
	G1 vs. G5	10.79 vs. 2.09	0.0001
	G3D vs. G3M	4.85 vs. 3.79	0.2148
	G3D vs. G4	4.85 vs. 2.70	0.1941
	G3D vs. G5	4.85 vs. 2.09	0.0040
	G3M vs. G4	3.79 vs. 2.70	0.7562
	G3M vs. G5	3.79 vs. 2.09	0.0723[b]
	G4 vs. G5	2.70 vs. 2.09	0.8150[c]

G1: group 1 (random patients); G2: group 2 (orthodontically treated patients); G3M: monozygotic twins in group 3 (twins); G3D: dizygotic twins in group 3 (twins); G4: group 4 (orthodontically treated twins); G5: group 5 (threshold); [a]: post-hoc Power Analysis indicates that both groups need to be enlarged in 11 comparisons to reach statistically significant difference; [b]: post-hoc Power Analysis indicates that both groups need to be enlarged in 17 comparisons to reach statistically significant difference; [c]: post-hoc Power Analysis indicates that both groups need to be enlarged in 56 comparisons to reach statistically significant difference; p-values from ANOVA test for significance rate of 5%.

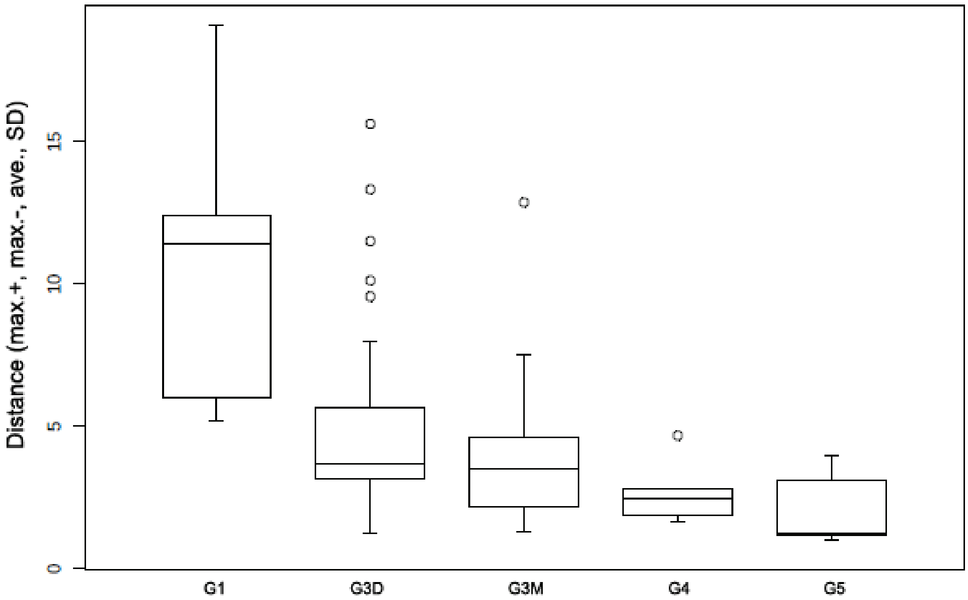
According to the ROC analysis, the of Euclidean distances combining the four quantification values (max.+, max.-, ave., and SD) revealed a sensitivity of 80% and a specificity of 81.6% with an accuracy of 83% for Group 5 when compared to Groups 1, 2, 3 and 4 for maxillary DCF.

Figure 11 – Euclidean distances calculated on the combination of the four quantification values (Max.+, Max.-, Ave., SD) and the *p*-values testing comparisons between the studied and reference groups for maxillary DCF



DCF: digital cast files; G1: group 1 (random patients); G2: group 2 (orthodontically treated patients); G3M: group 3 (monozygotic twins); G3D: group 3 (dizygotic twins); G4: group 4 (orthodontically treated monozygotic twins); G5: group 5 (threshold); Max.+: maximum positive deviation; Max.-: maximum negative deviation; Ave.: average deviation; SD: standard deviation; p-values from ANOVA test comparing groups may be consulted in Table 16.

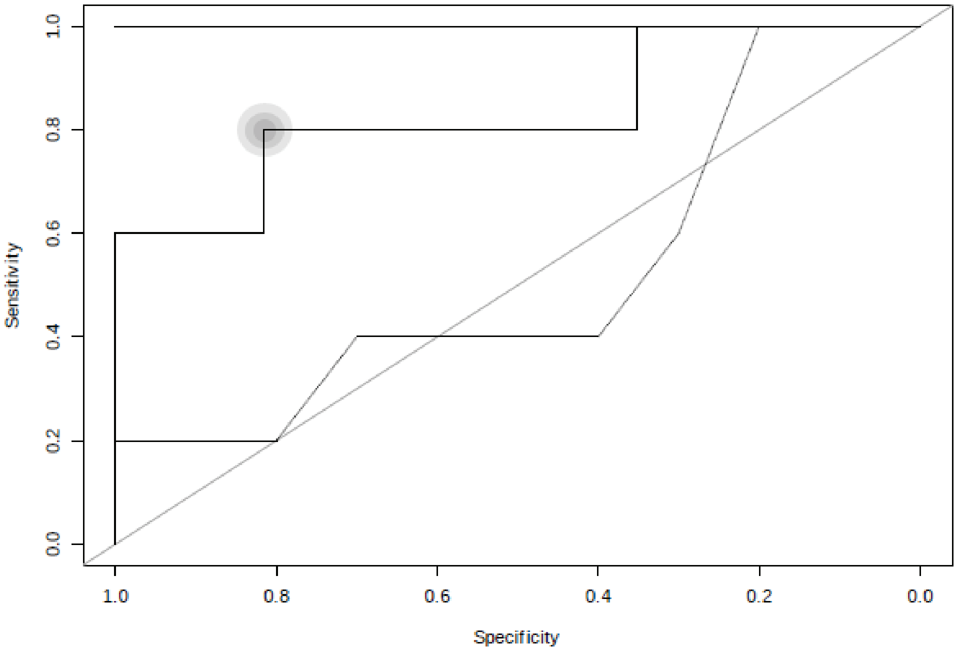
Figure 12 – Euclidean distances calculated on the combination of the four quantification values (Max.+, Max.-, Ave., SD) and the *p*-values testing comparisons between the studied and reference groups for mandibular DCF



DCF: digital cast files; G1: group 1 (random patients); G3M: group 3 (monozygotic twins); G3D: group 3 (dizygotic twins); G4: group 4 (orthodontically treated monozygotic twins); G5: group 5 (threshold); Max.+: maximum positive deviation; Max.-: maximum negative deviation; Ave.: average deviation; SD: standard deviation; p-values from ANOVA test comparing groups may be consulted in Table 16.

For the mandibular DCF sensitivity reached 80% and specificity 66.7%, with an accuracy of 81% (Figures 13 and 14). These outcomes were not modified combining less quantification values.

Figure 13 – ROC curve expressing the threshold potential of Group 5 to correctly classify maxillary DCF as non-equal (sensitivity) or equal (specificity) based on the combination of the four quantification values (Max.+, Max.-, Ave., SD)

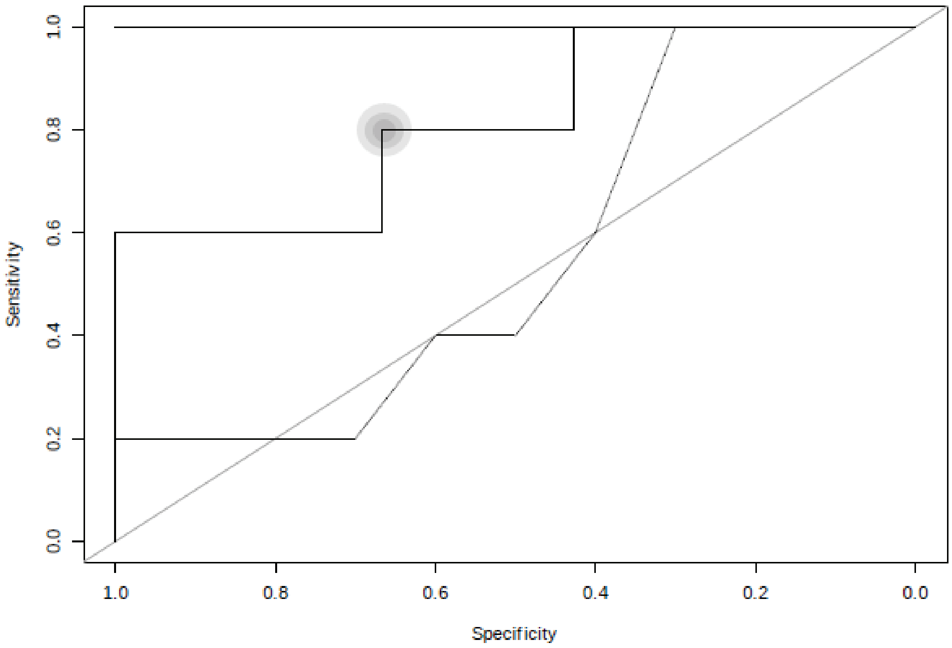


The maximized potential prediction (grey circle) indicates that Group 5 reached sensitivity of 80% - for classifying non-equal maxillary dentitions as non-equal and specificity of 81.6% - for classifying equal maxillary dentitions as equal (specificity). The Area Under the Curve (AUC, vertical arrow) suggests classification accuracy of 83%. Analyses combining less quantification values negligibly changed the current values.

DISCUSSION

In the present chapter the UHD was investigated mainly in the context of BM. Thus, the studied samples were stratified on the number of teeth (six per dental arch), the dental part (tooth crowns), and the type of population (orthodontically treated patients and twins). In these groups, beside general morphological information, dental shape, size, angulation and mutual tooth position were considered. This information was registered in life-size 3D DCF, enabling to capture all dental characteristics used in BM practice (Johansen & Bowers 2000). Opposite to all studies included in a related systematic review (Franco et al. 2015), currently the entire dental crown morphology was considered instead of only their incisal edges, because in BM the exact amount of tooth area that contacts and impresses the human skin may vary from case to case. Accordingly, the current study set up included that all the morphological evidences at the dental crown level possibly involved in BM.

Figure 14 – ROC curve expressing the threshold potential of Group 5 to correctly classify mandibular DCF as non-equal (sensitivity) or equal (specificity) based on the combination of the four quantification values (Max.+, Max.-, Ave., SD)



The maximized potential prediction (grey circle) indicates that Group 5 reached sensitivity of 80% - for classifying non-equal mandibular dentitions as non-equal and specificity of 66.7% - for classifying equal mandibular dentitions as equal. The Area Under the Curve (AUC, vertical arrow) suggests classification accuracy of 81%. Analyses combining less quantification values negligibly changed the current values

The initial analysis within each group enabled to rank the Euclidian distance values in order to screen the most similar pairs of DCF in the sample. The smallest distances resulted below 5.24 mm for the maxilla and below 5.19 mm for the mandible (Table 15). This analysis also indicated no equal Euclidean distance values between the pair wise comparisons. However, equal Euclidian distances only indicated that the combined quantification of all morphological differences calculated by GS between two pair wise DCF comparisons were equal. Theoretically, zero is the Euclidean distance value indicating that two compared DCF are equal and proves lack of uniqueness, but in the performed research measuring and operator errors need to be taken into account. The factors potentially biasing the performed pair wise DCF comparisons were 1) the confection of dental impressions and dental casts, 2) the 3D scanning process; 3) the 3D digital cropping; and 4) the GS comparison measure error. In this context, Group 5 was created combining and quantifying these factors to establish a threshold enabling to classify non-equal and equal DCF (separately for the mandible and the maxilla). This threshold was used as reference in the search for equal DCF in the studied Groups 1, 2, 3 and 4.

Randomly selected patients (Group 1) were included in the present chapter not to prove uniqueness within this population but yet to highlight the importance of sample stratification on studies in the field. Based on that, it became more relevant to compare Group 1 with the other experimental groups (Groups 2, 3 and 4) than with the threshold group (Group 5). As observed the mean Euclidean distances obtained in Group 1 were the highest in this study. However, the mean Euclidean distance for maxillary DCF (8.03) was only statistically different ($p<0.05$) when compared to monozygotic twins (Group 3) and orthodontically treated monozygotic twins (Group 4). Yet the mean Euclidean distance for mandibular DCF (10.79) was statistically different ($p<0.05$) compared to all the groups. This finding suggest that sample stratification for maxillary DCF better succeeds using samples (Group 3) and sub-samples (Group 4) of monozygotic twins, while for mandibular DCF orthodontically treated patients may be used as well. A secondary finding obtained from these outcomes suggests that UHD is more difficult to be proved within the maxillary anterior teeth, which required stronger sample stratification to reach significantly statistical difference from the random population. As expected, when compared to Group 5, Group 1 revealed significantly statistical differences ($p<0.05$) for maxillary and mandibular DCF. However, as previously observed, proving UHD in a random population (Group 1) is less difficult than in populations stratified by the similarity in dental arrangement and morphology, such as orthodontically treated patients (Group 2) and twins (Groups 3 and 4), respectively.

All the Euclidean distance values obtained from orthodontically treated patients (Group 2) revealed significantly statistical difference compared to the reference Euclidean distances (Group 5) ($p<0.05$). Previous studies used 2D registration techniques and landmarking procedures to report outcomes obtained from orthodontically treated patients (Kieser et al. 2007; Sheets et al.

2011). Their results were contradictory. Kieser et al. (2007) (Kieser et al. 2007), found no match between pair wise comparison and Sheets et al. (2011) (Sheets et al. 2011) detected matches. The present chapter differs in set up from both previous studies, mainly due to the use of 3D imaging techniques combined with automated image superimposition. While 3D imaging enables the investigation of morphometric information without restricting morphological dental evidences, automated superimposition eliminates the bias related to operator-depending procedures. Moreover, Sheets et al. (2011) (Sheets et al. 2011), reported outcomes for mandibular dentitions, while in the current study maxillary dentitions were examined. Sheets et al. (2011) (Sheets et al. 2011) justified the use of mandibular dentitions explaining that "*fewer matches would result in the mandibles due to the higher incidence of crowding and malalignment*". This statement supports the choice of maxillary dentitions in the current study, confirming the current sample stratification based on selecting subjects with most similar dental traits. Opposite to Sheets et al. (2011) (Sheets et al. 2011), in the current study no match between dentitions was found, proving UHD in a stratified sample.

The study of twins (Group 3) and orthodontically treated monozygotic twins (Group 4) included maxillary and mandibular dentitions. In twins (Group 3) higher mean Euclidean distances both for mono- and dizygotic twins were observed in relation to the reference group (Group 5) (Table 15). Significantly statistical difference was observed from these comparisons, except for mandibular DCF of monozygotic twins ($p>0.05$). Despite that, mandibular DCF of monozygotic twins slightly varied out of statistical significance ($p=0.07$), presenting clear clinically visible differences (Figure 10). Sognnaes et al. (1982) (Sognnaes et al. 1982) performed the only study on the UHD sampling twins. The authors used bite impressions registered in wax compared through photography (2D), probably including high inherent research bias. Similar to the current reports for twins (Group 3), Sognnaes et al. (1982) (Sognnaes et al. 1982) did not find matches between dentitions. Oppositely in the present chapter, when a higher level of stratification was applied using orthodontically treated monozygotic twins (Groups 4) no significantly statistical difference was observed in relation to the reference group (Group 5) ($p>0.05$) both mandibular and maxillary DCF (Table 15). According to the Power Analysis outcomes, the lack of significant differences found in Groups 3 and 4 was possibly related to the unequal (Group 3) and small (Group 4) sample size compared to Group 5. Specifically, the addition of pair wise comparisons revealed to generate significantly statistical differences, indirectly suggesting UHD in Groups 3 and 4. Moreover these samples reveal clinically significant morphological differences in the pair wise compared DCF with the lowest Euclidean distance values in the experimental sample groups (Figure 10). Both considerations enable to conclude that UHD was observed in the present chapter, but a larger sample size with more equally sized groups is necessary to provide statistical significance where it was not found.

Ranking the studied groups based on their mean Euclidian distance, indicated that orthodontically treated monozygotic twins (Group 4) presented the most similar DCF, followed by the monozygotic twins of Group 3, the dizygotic twins of Group 3, the orthodontically treated patients (Group 2), and the randomly selected patients (Group 1) (Figure 12 and 13). As expected monozygotic twins had lower mean Euclidean distance values compared to dizygotics. The literature suggests a genetic control over dental morphology (Osborne et al. 1958; Lundstrom 1963). Despite that, no significantly statistical difference was observed in relation to zygosity both for maxillary and mandibular DCF. It indicates that probably the choice of dental arch does not guarantee to detect less or more equal DCF, and consequently does not positively contribute in the proposed sample stratification. In fact the current study revealed that the best sample stratification may be achieved combining the standard alignment of tooth position found in orthodontically treated patients (Group 2) and the similar dental morphology observed in twins (Group 3), resulting in subjects (Group 4) that have Euclidean distances closest to the reference threshold (Group 5).

The ROC analysis was used to test Group 5 as potential reference threshold. This analysis was based on the dichotomization of the unique versus the not unique outcomes (Hajian-Tilaki 2013). In the maxilla, sensitivity and specificity outcomes reached 80% and 81.6%, respectively, revealing a balanced power for reference threshold (Group 5) as a classifier of uniqueness. In the mandible, these outcomes reached 80% and 66.7%, respectively, suggesting an unbalanced power and indicating that Group 5 had better performance classifying unique dentitions and more deficiency for classifying non unique dentitions. However, for both maxillary and mandibular dentition the level of accuracy expressed by the AUC reached 83% and 81%, respectively, classifying the overall threshold power of Group 5 as good (Khouli et al. 2009). This outcome did not change modifying the number of included quantification values provided by GS (max.+, ave., and SD; max.-, ave. and SD; and ave. and SD). It indicates that in future researches less quantification values may be tested and used. In practice it is translated in a faster data collection and analysis. Future researches should focus on overcoming the limitations found in the present chapter, such as sampling higher number of orthodontically treated monozygotic twins, avoiding the need for statistical power inferences. Further on the possible bias related to the manual confection of dental impressions and casts and the semi-automated digital cropping could be excluded using intraoral scanning and automated algorithms, respectively.

The present chapter provided evidence for UHD based on the evaluation of the complete six anterior tooth crowns. These results need further investigation in the context of BM analysis because it still has to be proven that the UHD remains if only tooth parts (incisal parts of anterior teeth) are analyzed.

Questionamento: *A quantidade de material dental analisado influencia na diferença morfológica entre dentições?*

Objetivo: *"Testar a diminuição da quantidade de material dental analisado em face da diferença morfológica entre dentições".*

Hipótese: *"A diferença morfológica entre dentições aumenta com o aumento da quantidade de material dental analisado".*

Testando a influência da quantidade de material dental analisado na diferenciação morfológica entre dentições

Capítulo 7

Capítulo baseado no artigo:

Ademir Franco, Guy Willems, Paulo H. C. Souza, Sérgio A. Ignácio, Patrick Thevissen. Three-dimensional validation of the impact of the quantity of teeth or tooth parts on the morphological difference between twin dentitions. J Forensic Odontostomatol. 2016;34(1):27-37.

INTRODUCTION

BM analysis and dental identification rely on the quality and quantity of the dental evidences available. Quality of dental evidences is not only related to the standards for image registration but mainly to their classification, such as the morphological, treatment-related and pathological evidences. Specifically, evidences based on tooth morphology will be more prevalent than those based on dental treatment and pathology in the near future (Franco et al. 2013). On the other hand, the quantity of evidences is directly related to the amount of tooth material investigated, which is translated in the amount of teeth and tooth parts available for analysis. Morphology revealed evident distinctive power – according to chapter 6, considering the tooth crowns of the six anterior teeth.

In the context of the present research, UHD was studied for application in BM impressed on human skin. In BM analysis, the quality of evidences is usually reduced due to the 2D image acquisition (Dorion 1982; Senn & Weems 2013) and to the restriction of depending exclusively on tooth morphology (lack of more combinations with other evidences, such as the treatment and pathologically related features). The analysis of BM evidences varied according to the technology available at that moment, including the 2D investigation of the shape of the contours of incisal edges using manually produced transparent foils (Luntz & Luntz 1973) and digitally produced overlays (hollow contours) (Johansen & Bowers 2000); the 2D investigation of dental and arch size (e.g. mesio-distal diameter and intercanine distance, respectively) and angulation (e.g. level of dental rotation in the arch) using digitally produced overlays (Johansen & Bowers 2000); and the 3D investigation of morphological information using digital superimpositions (Thali et al. 2003; Martin-de-las-Heras & Tafur 2009). In relation to the quantity of evidences, BM analysis is limited twice. First, on the investigation of the (6) anterior teeth of each dental arch, because they are mainly involved in the bite (reduced amount of teeth) (Kieser et al. 2007; Rawson et al. 1984; Sognnaes et al. 1982; Sheets et al. 2011; Bush et al. 2011b; Bush et al. 2011a). Second, on the investigation of incisal edges , because they are the parts impressed in the bitten skin (reduced amount of tooth parts) (Dorion 1982).

The impact of the quantity of teeth and tooth parts on the morphological difference of human dentitions was never scientifically tested. In this context, studying twins reveals to be a proper approach considering that they have more similar dental and maxillofacial traits compared to random subjects. Additionally, this population enables the pair wise comparison of dental morphology between siblings following controlled and systematic modifications in the quantity of teeth and tooth parts. Based on that, the present chapter aims to quantify the differences between dentitions of twin siblings considering all the morphological information available (not only specific shape and metric related information – overcoming the limitation of the previous studies in the field) and investigating different quantities of teeth and tooth parts.

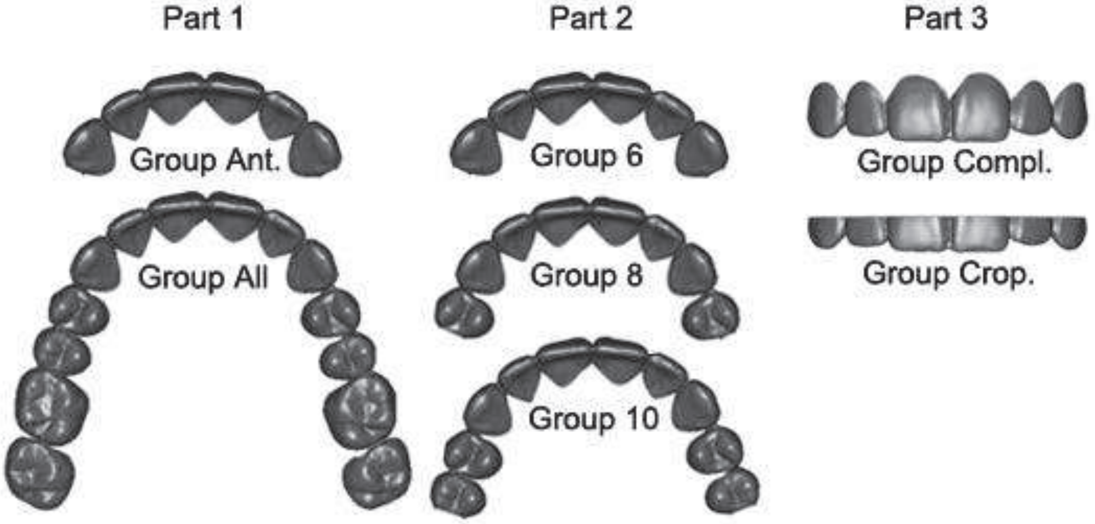
MATERIAL AND METHODS

The studied sample consisted of 86 pairs of twins (n=172), of which 39 pairs (n=78) were monozygotic (M) and 47 pairs (n=94) were dizygotic (D). From each of the included subject (n=344) dental impressions of the maxillary (n=177) and the mandibular arch (n=177) were taken and scanned in digital cast files (DCF) according to the set up described in the previous chapter. These DCF were duplicated (n=688) using the computer copying and pasting command tools of the operating system (Windows 10®, Microsoft Windows, Redmond, USA). The analysis was performed using Geomagic Studio® (3D Systems®, Rock Hill, SC, USA) software package (GS).

The study was divided in 3 parts (Figure 15). In Part 1, the DCF from the original 86 pairs of twins (n=172) were copied. Using GS the original images were cropped with a remaining area of interest equal to the clinically visible dental crowns of the anterior teeth (Group Ant.). The copied images were cropped with a remaining area of interest equal to the clinically visible dental crowns of all present teeth (Group All). In Part 2, monozygotic twin pairs with the permanent teeth in complete gingival eruption were selected (14 mandibular 19 maxillary pairs of dentitions). The DCF of these subjects were cropped including 10 tooth crowns, namely the anterior teeth and the first and second premolars (Group 10). This group was duplicated twice. The DCF of the first duplicate were cropped including 8 tooth crowns, namely the anterior teeth and the first premolars (Group 8), while in the second duplicate the DCF were cropped including 6 tooth crowns, namely the anterior teeth (Group 6). Part 3 used the same sample of Part 2. The DCF were cropped remaining the complete dental crowns of the anterior teeth (Group Compl.) and were duplicated. The duplicate DCF were cropped with a section parallel to the horizontal plane at the level of the highest interdental papilla (Group Crop.). All the crown cropping procedures were performed in GS, placing pre-cropping points along the cemento enamel junction of all the teeth included in the areas of interest.

As described in chapter 6, within each group all the possible pair wise morphologic comparisons between DCF were accomplished using GS automated superimposition. The same software quantified the pair wise differences between DCF, also the same formula previously reported to combine the four quantification values expressed by GS was used. The log-transformed differences between DCF were compared between groups using a linear mixed model with Sidak (1967) correction for multiple hypotheses. The statistical tests were performed with significance rate of 5% using S+® 8.0 (Tibco®, Palo Alto, California, USA) software package.

Figure 15 – DCF areas of interest used for morphological comparison in each study part



DCF: digital cast files; Part 1 – Group Ant.: anterior dentition; Group All: entire dentition; Part 2 – Group 6: anterior dentition; Group 8: anterior dentition and first premolars; Group 10: anterior dentition and first and second premolars; Part 3 – Group Compl.: anterior dentition with complete crowns; Group Crop.: anterior dentition with partial crowns. DCF in Part 1 and 2 represented in 2D occlusal view and in Part 3 in 2D buccal view. Occlusal and buccal views are merely illustrative. Entire dental crowns were used and compared in a 3D environment in all study parts.

RESULTS

In study Part 1, the mean Euclidian distance observed comparing DCF in Group All was statistically significantly higher than the mean Euclidian distance observed comparing DCF in Group Ant., both for the maxilla and the mandible (p=0.0001) (Table 17; Figure 16).

In study Part 2, the mean Euclidean distance observed comparing DCF in Groups 6, 8 and 10 gradually increased in the maxilla as well as the mandible. No statistically significant differences were observed between Groups (p>0.05) (Table 17; Figure 17).

In Part 3, the mean Euclidean distance observed comparing DCF in Group Compl. was statistically significant higher than Group Crop., both for the maxilla (p=0.002) and the mandible (p=0.012) (Table 17; Figure 18).

Table 17 – Comparison of mean Euclidean distances, arch specific for each studied group

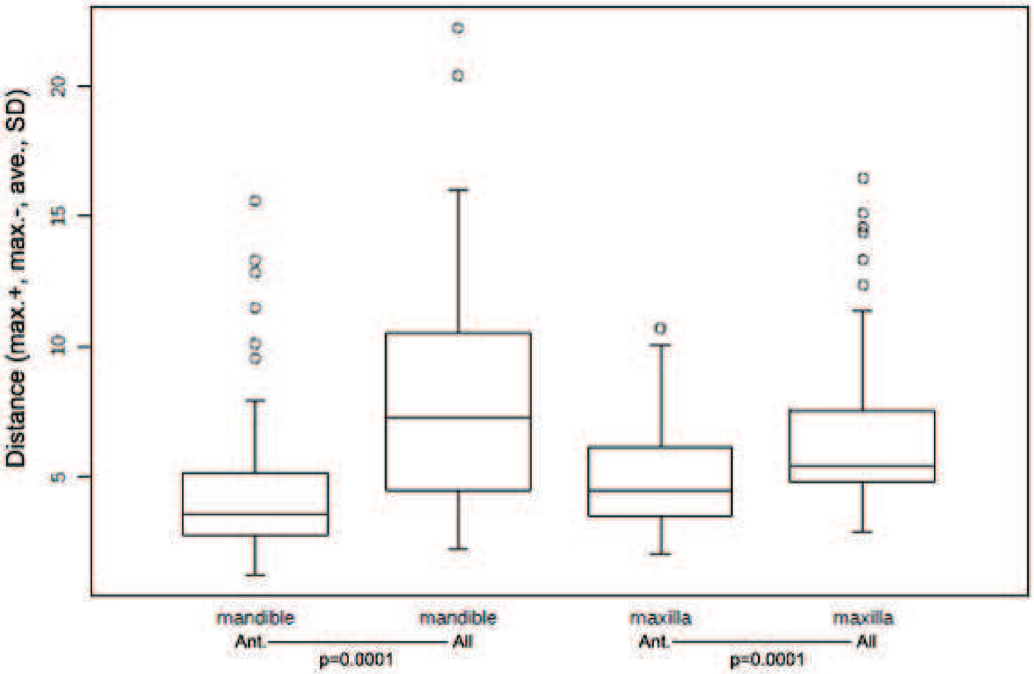
Dental arch	Part	Groups	Mean	*P*
Maxillary	1	Ant. vs. All	4.98 vs. 6.43	0.0001
	2	6 vs. 8	3.38 vs. 3.54	0.9088
		6 vs. 10	3.38 vs. 3.64	0.7843
		8 vs. 10	3.54 vs. 3.64	0.9931
	3	Compl. vs. Crop.	3.38 vs. 2.57	0.0027
Mandibular	1	Ant. vs. All	4.29 vs. 7.89	0.0001
	2	6 vs. 8	2.95 vs. 3.17	0.8858
		6 vs. 10	2.95 vs. 3.51	0.5145
		8 vs. 10	3.17 vs. 3.51	0.9135
	3	Compl. vs. Crop.	2.95 vs. 2.21	0.0122

Part 1 – Group Ant.: anterior dentition; Group All: entire dentition; Part 2 – Group 6: anterior dentition; Group 8: anterior dentition and first premolars; Group 10: anterior dentition and first and second premolars; Part 3 – Group Compl.: anterior dentition with complete crowns; Group Crop.: anterior dentition with partial crowns. p-values obtained with a linear mixed model using Sidak (1967) correction for multiple hypotheses. Significance rate set at 5%.

DISCUSSION

Forensic dentistry is currently using the hypothesis that the increase in quantities of teeth and tooth parts provides more dental evidence, enabling to detect more morphological differences between compared subjects. Twin subjects were sampled to allow for the detection of quantitative morphological differences between pair wise compared siblings with the smallest influence of possible qualitative differences present between the examined sibling pairs. It justifies not using randomly selected subjects or copied DCF files. In the first the highest pair wise qualitative morphological differences are obtained. In the second zero morphological difference will be observed between corresponding DCF.

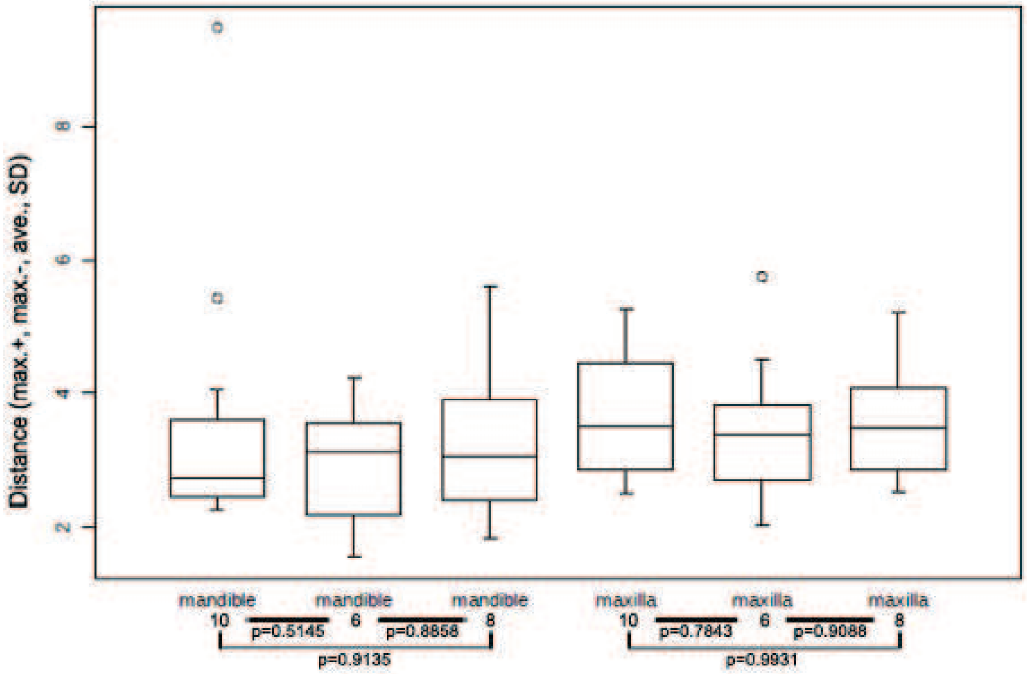
Figure 16 – Boxplots expressing the Euclidean distance of all pair wise DCF comparisons separate for the mandible and maxilla in Groups Ant. and All



DCF: Digital cast files; Group Ant.: anterior dentition; Group All: entire dentition; Mean Euclidean distance for maxillary DCF: 4.98 (Group Ant.) and 4.43 (Group All); Mean Euclidean distance for mandibular DCF: 4.29 (Group Ant.) and 7.89 (Group All); Max.+: maximum positive deviation; Max.-: maximum negative deviation; Ave.: average deviation; SD: standard deviation; p-values obtained with a linear mixed model using Sidak (1967) correction of multiple hypotheses considering a significance rate set at 5%; Difference between the mean Euclidean distance of Groups Ant. and All for maxillary and mandibular DCF: 0.0001 (p).

In the context of the present chapter, lack of distinctive distinction between dentitions is translated as the absence of morphological difference (Euclidean distance = zero) between pair wise compared DCF. In particular, the mean Euclidean distances with highest distinctive power (6.43 for maxillary and 7.89 mandibular DCF) were observed comparing entire dentitions (Group All, Part 1). Oppositely, anterior dentitions with partial crowns (Group Crop., Part 3) revealed the lowest distinctive power (2.57 for maxillary and 2.21 for mandibular DCF). In general, it suggests that more tooth quantity contributes to an increase in Euclidean distances, making dentitions potentially more distinctive.

Figure 17 – Boxplots expressing the Euclidean distance of all pair wise DCF comparisons separate for the mandible and maxilla in Groups 6, 8 and 10

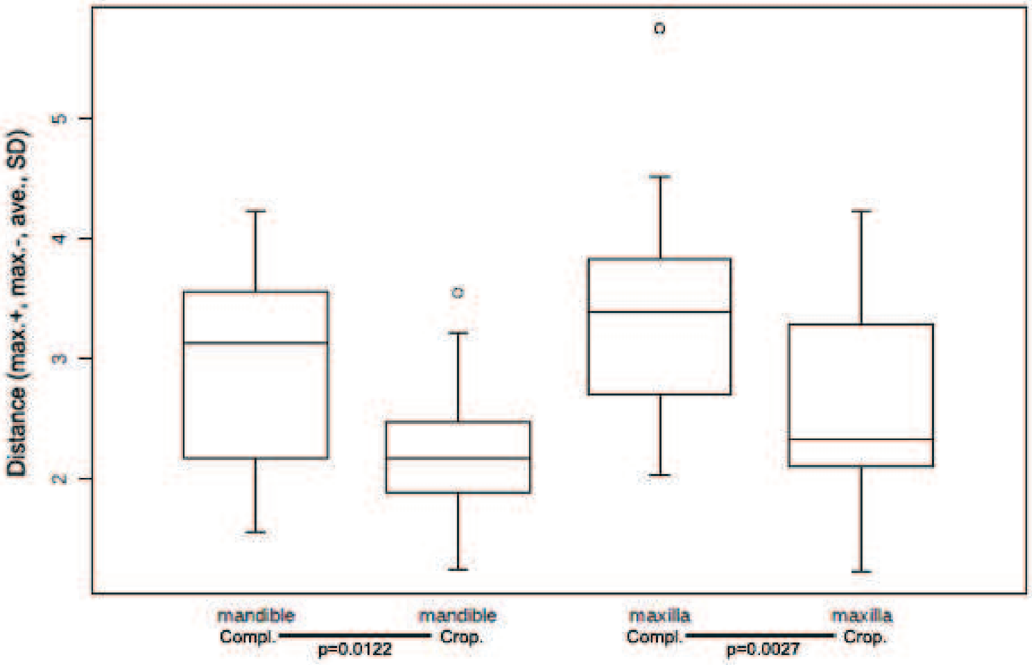


DCF: Digital cast files; Group 6: anterior dentition; Group 8: anterior dentition and first premolars; Group 10: anterior dentition and first and second premolars; Mean Euclidean distance for maxillary DCF: 3.38 (Group 6), 3.54 (Group 8), and 3.64 (Group 10); Mean Euclidean distance for mandibular DCF: 2.95 (6), 3.17 (8), and 3.51 (10); Max.+: maximum positive deviation; Max.-: maximum negative deviation; Ave.: average deviation; SD: standard deviation; p-values obtained with a linear mixed model using Sidak (1967) correction of multiple hypotheses considering a significance rate set at 5%; Difference between the mean Euclidean distance of Groups 6 and 8 for maxillary DCF: 0.9088 (p); Difference between the mean Euclidean distance of Groups 6 and 10 for maxillary DCF: 0.7843 (p); Difference between the mean Euclidean distance of Groups 8 and 10 for maxillary DCF: 0.9931 (p); Difference between the mean Euclidean distance of Groups 6 and 8 for mandibular DCF: 0.8858 (p); Difference between the mean Euclidean distance of Groups 6 and 10 for maxillary DCF: 0.5145 (p); Difference between the mean Euclidean distance of Groups 8 and 10 for maxillary DCF: 0.9135 (p).

In Part 1, the clear statistical significant difference ($p<0.05$) between entire (Group All) and anterior (Group Ant.) DCF (Figure 16) points out, that substantial increase in tooth quantity provides more morphologically different dental evidence. Specifically, the proportion in the number of teeth between groups (Group. All/Group Ant.) increased with 133.33%, making the proportion of mean Euclidean distances increase with 29% for maxillary and 83% for mandibular DCF (Table 17). In Part 2 morphological differences were also observed gradually including first (Group 8) and second (Group 10) premolars but no statistically significant results were achieved between these group comparisons ($p>0.05$). The proportions in the number of teeth increased with 33.33% between Groups 8 and 6; 66%

between Groups 10 and 6; and 25% between Groups 10 and 8. Consequently, the proportions of mean maxillary Euclidean distances increased with 4% (Group 8/Group 6); 7% (Group 10/Group 6); and 2% (Group 10/Group 8), while the proportions of mean mandibular Euclidean distances increased with 7% (Group 8/Group 6); 18% (Group 10/Group 6); and 10% (Group 10/Group 8).

Figure 18 – Boxplots expressing the Euclidean distance of all pair wise DCF comparisons separate for the mandible and maxilla in Groups Compl. And Crop.



DCF: Digital cast files; Group Compl..: anterior dentition with complete crowns; Group Crop.: anterior dentition with partial crowns; Mean Euclidean distance for maxillary DCF: 3.38 (Group Compl.) and 2.27 (Group Crop.); Mean Euclidean distance for mandibular DCF: 2.95 (Group Compl.) and 2.21 (Group Crop.); Max.+: maximum positive deviation; Max.-: maximum negative deviation; Ave.: average deviation; SD: standard deviation; p-values obtained with a linear mixed model using Sidak (1967) correction of multiple hypotheses considering a significance rate set at 5%; Difference between the mean Euclidean distance of Groups Compl. And Crop. for maxillary DCF: 0.0027 (p). Difference between the mean Euclidean distance of Groups Compl. And Crop. for mandibular DCF: 0.0122 (p).

In Part 3, statistically significant differences between groups ($p<0.05$) were observed (Table 17; Figure 18). Specifically in this part, the analysis of proportion in quantity of tooth material is less accurate compared to the previous study parts, because the anterior dentition with partial crowns (Group Crop.) were horizontally cropped at the level of the highest interdental papilla, which discretely varies between twin subjects. Assuming that the anterior dentitions were cropped in half – generating a difference in tooth material of

50% between both groups (Group Crop./Group Compl.), the proportion of mean Euclidean distances increased in 31% and 33% for maxillary and mandibular DCF, respectively. The analysis of all study parts revealed that higher Euclidean distance values were observed comparing a larger quantity of tooth material. However, in Parts 1 and 3 statistically significant findings ($p<0.05$) were obtained differently from Part 2 ($p>0.05$). This phenomenon is justified by the proportion of tooth material included for analysis. In Parts 1 and 3 the proportion of tooth material increased at least in 50% between groups, increasing the mean Euclidean distances up to 83% (mandibular DCF of Part 1) and generating statistically significant results. In Part 2 the proportion of increase in tooth material varied between 25-66.66%, increasing the mean Euclidean distances only up to 18% (mandibular DCF between Groups G6 and G10). These evidences suggest that the inclusion of premolars in the anterior dentition provides additional morphological information that are considered too small to impact the mean Euclidean distances with statistical significance. The opposite is observed for the inclusion of all the available teeth in the dental arch (Part 1) and for the analysis of complete (instead of partial) anterior crowns (Part 3).

From a forensic point of view, the use of anterior teeth combined with premolars and molars results in more power to distinguish dentitions. It confirms the hypothesis that more tooth material allows for more combination of evidences (Pereira & Santos 2013) contributing to a better distinction between subjects. Even in the absence of statistically significant findings (Part 2), the gradual increase in morphological difference observed adding premolars, represents a clinically significant finding. It suggests that these minor morphological differences can be useful in the forensic practice. They allow for possible matches between a BM and suspect dentitions based on the comparison of the clinically detected premolar morphology. In parallel, the amount of tooth quantity is not exclusively restricted to the number of teeth, but involves also the amount of tooth material available. In Part 3 the analysis of complete anterior crowns (Group Compl.) increased the morphological difference with 31-33% compared to partial crowns (Group Crop.). More specifically, the gingival half (50%) of the dental crown seems to provide more distinctive morphological information compared to the incisal (50%). While the incisal half generated up to 33% of morphological difference between DCF, the gingival part is responsible for generating the remaining difference (up to 67%). It is justified due to the inherent genetic influence on the quality of evidences that discretely varies between twin siblings. The quality of evidences may also be modified by non-genetic influence depending on the tooth part studied, such as nail biting habit (affecting the incisal part) and periodontal disease (affecting the gingival part). In BM practice the outcomes of study Part 3 are even more tangible because the dental analysis is commonly restricted to the incisal part of the crown (Dorion 1982) (part that impresses the bitten surface). The current findings suggest that the use of partial anterior crowns hampers the distinction between dentitions compared to the analysis of complete crowns. However, uniqueness of partial anterior crowns (Group Crop.) remains unproved, indicating the need for further investigations.

Comparing the increase of tooth material between maxillary and mandibular DCF, the mean Euclidean distances increased most regarding the latter. Specifically, in Part 1 the proportion of mean Euclidean distances between mandibular and maxillary DCF increased with 41%. In Part 2 it increased with 2% (Group 8/Group 6); 10% (Group 10/Group 6); and 7% (Group 10/Group 8). In Part 3 the increase was 1%. These findings suggest that mandibular DCF trend to be more morphologically different than maxillary DCF in function of the quantity of tooth material considered. It points out that mandibular DCF have more unique power. Consequently, they may enable a better distinction between persons (both for human identification and BM analysis) compared to the maxilla. Sheets et al. (2011) justify this finding explaining that dental crowding is more common in the mandibular arch, possibly increasing the morphological difference when compared to the maxillary arch. On the other hand, the lower distinctive power of maxillary DCF must be considered an important finding for potential sample stratification in studies proving the UHD, because apparently uniqueness is more hardly proved within maxillary DCF. Despite these evidences, the morphological difference between dental arches was only prominent in study Part 1 (41% increase). In study Part 2 (2-10% increase) and Part 3 (1% increase) the reduced Euclidean difference between arches suggests that predilection for analysis of specific dental arch in dental identifications and BM must be avoided.

In the next chapter further research will consider transporting the investigation of the UHD UHD to a level closer to the dentition parts involved in forensic BM practice (on human skin). It is justified because the present chapter revealed that the quantity of dentition material influence over the quantified morphological difference between DCF. Based on that, it is necessary to focus on systematic modifications in the quantity of material material located at the incisal edges of the anterior dentition.

Questionamento: *A dentição humana permanece única reduzindo sistematicamente a quantidade de material dental analisado dentro do contexto de BM em pele humana?*

Objetivo: *"Investigar a UHD considerando a quantidade de material dental envolvido em BM em pele humana".*

Hipótese: *"A dentição humana permanece única quando a quantidade de material dental envolvido em BM em pele humana é analisada".*

Testando a influência da quantidade de material dental possivelmente envolvido em marcas de mordida na unicidade da dentição humana

Capítulo 8

Capítulo baseado no artigo:
Ademir Franco, Guy Willems, Paulo Henrique Couto Souza, Wim Coucke, Patrick Thevissen.
UHD three-dimensionally assessed for forensic BM analysis. J Forensic Leg Med. 2016
Submetido.

INTRODUCTION

The biomechanics behind the biting phenomenon is complex (Nambiar 1994), involving not only the action of teeth, but also the position of the tongue (Dorion 1982), the occlusion, the relation between dental arches during biting (Nambiar 1994), the bite intention (attacking or defending) (Whittaker & McDonald 1989), the reaction of the injured person (Nambiar 1994), and the type of the bitten material and its underlying structure (Webster 1982). BM on skin are typically presenting two arches facing each other (Dorion 1982; Senn & Weems 2013). These arches correspond to the anterior maxillary and mandibular dentitions of the inflicted biter (Senn & Weems 2013), and contain separate indentations related to the involved anterior teeth (Dorion 1982). Due to these factors and their mutual interactions the depth of the skin indentations caused by the incisal dentition edges highly varies.

Recent studies with 3D dental registration, analyzed the anterior dental crown morphology 1 up to 3mm from their incisal edges (Martin-de-las-Heras & Tafur 2009; Martin-de-Las-Heras et al. 2014). Studies using 2D technology were limited to investigate geometric contours of the incisal dentition edges in occlusal view (Kieser et al. 2007; Sheets et al. 2011). Based on that, the 3D investigation on the UHD within small quantities of the anterior teeth, namely 1 to 3 mm from their incisal edge, brings the research to the BM level. Moreover, the analysis of slices at the considered distances from the incisal dentition edges of the anterior teeth mimic the current analyses in forensic BM practice,. Indeed, currently mainly the 2D digital superimposition of dental overlays from the anterior dentition in occlusal view on 2D photographs of the BM, is used (Bowers & Johansen 2000). Knowing if the UHD remains with a reduced amount of dentition material is essential for the validity of forensic BM investigations because only then proof is given that just a single suspect is able to inflict a particular bite.

The present chapter aims to investigate the UHD in the anterior dentition based on systematically reducing the incisal 3D quantity of dentition material analyzed; and using a slice of the analyzed anterior dentition.

MATERIAL AND METHODS

Four-hundred forty-five dental casts were obtained and digitalized as dental cast files (DCF) as described in chapter 6. The DCF were divided in 4 study Groups (I, II, III and IV) (Table 18).

Table 18 – Sample distribution of the studied groups per dental arch, sex and zygosity

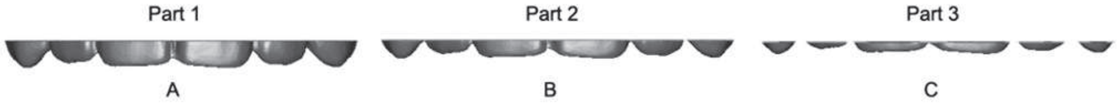
Group	Sample	Arch		Sex		Zygosity		n
		Maxill.	Mand.	Male	Female	Mono-	Di-	
I	Random subjects	11	11	7	4	n/a	n/a	22
II	Orthodontically treated subjects	59	0	32	27	n/a	n/a	59
III	Twins	172	172	38	48	39	47	344
IV	Orthodontically treated twins	10	10	2	8	5	0	20
V	Threshold subjects*	10	10	2	3	n/a	n/a	20

*DCF: digital cast files; Maxill.: maxillary; Mand. Mandibular; n/a: not applicable; n: sample size; A total of 2.013 pair wise comparisons were performed: 110 in Group I; 1.711 in Group II, 172 in Group III, 10 in Group IV, and 10 in Group V. In Groups I and II the pair wise comparisons were performed matching all the DCF in the sample, while in Groups III and IV the comparisons were performed exclusively between twin siblings. Yet in Group V, the pair wise comparisons were performed with the DCF obtained from the same patients in moment 1 and 2. *In Group V, the threshold was developed performing the dental impression and digitalization of dental casts of five subjects in two different times. The sample distribution for twins (Groups III and IV) based on sex is expressed as pairs of monozygotic (mono-) and dizygotic (di-)siblings.*

The inclusion and exclusion criteria, the software used for comparisons of DCF and the quantification of differences between DCF were considered and performed as described in chapter 6. In total, 2.013 comparisons were performed, 110 in Group I; 1.711 in Group II, 172 in Group III, 10 in Group IV. The 5 most similar pairs of DCF of each group were selected and used in this study.

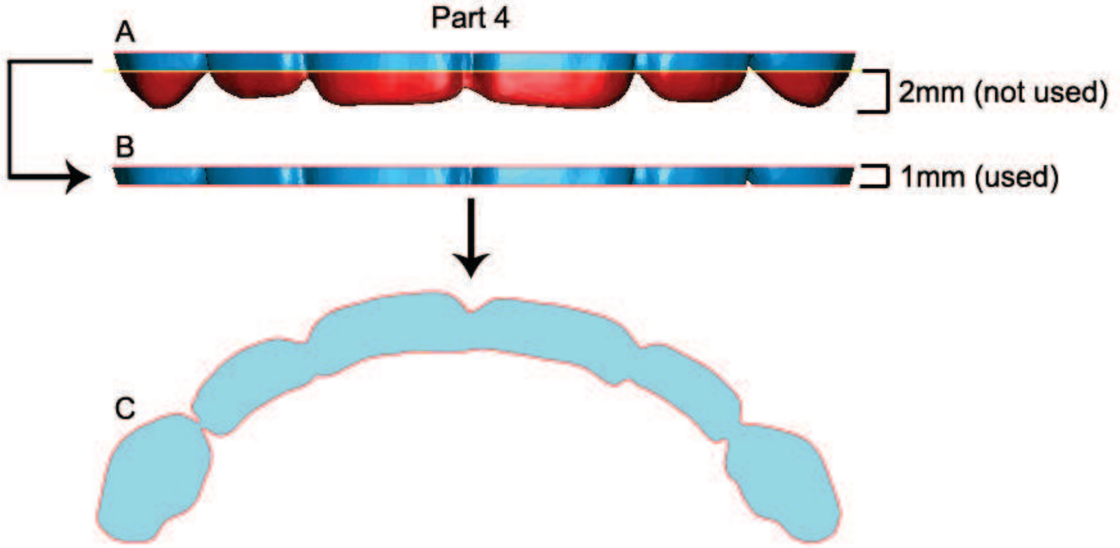
Twenty additional DCF were obtained from 5 patients from whom dental impressions and related casts were made twice within a period of 7 days. This Reference Group (Group V) served to establish thresholds, enabling to quantify the errors obtained and included during the cast and DCF acquisition, study part specific. In part 1, all the DCF were cropped leaving maximally 3mm from the incisal edges of the anterior dentition (calculated from the incisal edge of the highest tooth). In part 2, only the groups with Euclidean distances above the threshold (considered unique) in part 1were used. The DCF of these groups were re-imported in the software and cropped, remaining a tooth portion of maximally 2mm from the incisal edges of the anterior teeth. In part 3, the same DCF were re-cropped keeping maximally 1mm tooth material from the incisal edges (Figure 19). In part 4, all the groups were used and the DCF were cropped keeping a slice of 1mm at the level maximally 2mm from the incisal edge of the anterior dentitions (Figure 20). In each study part, a threshold value was established using Group V.

Figure 19 – Tooth parts of interest of the DCF used in study parts 1, 2 and 3 (3D analysis)



Images A, B and C correspond to the tooth parts of interest of the digital cast files (DCF) remaining 3 (part 1), 2 (part 2) and 1mm (part 3) from the incisal edges, respectively.

Figure 20 – Tooth part of interest of the DCF in study part 4 (simulated 2D analysis)



In study part 4, the digital cast files (DCF) cropped 3mm from the incisal edges (part 1) were cropped at their gingival portion (A), remaining a slice of 1mm (B). In axial (occlusal) view (C), these slices mimic the bidimensional registration of forensic BM indentations.

Statistically, the comparison between the studied groups was performed applying One-way ANOVA (Casella 2008) with log-transformed distances, separately for maxilla and mandible. A correction for simultaneous hypothesis testing was applied according to Tukey's range test (Tukey 1949). The human dentition was considered unique when the mean Euclidean distance of any studied Group was statistically significantly higher than the respective reference Group. A Receiver Operator Characteristic (ROC) analysis was performed to assess the threshold potential of Group V – differently cropped in each study part, for detecting equal (sensitivity) and non-equal dentitions (specificity). In this context, the Area Under the Curve (AUC) was obtained by means of bootstrapping. The statistical tests were performed with significance rate of 5% using S+® 8.0 (Tibco®, Palo Alto, California, USA) software package.

RESULTS

In part 1 (3mm at incisal edges), only the mean Euclidean distances obtained for the maxillary (4.35) and the mandibular (4.83) DCF of Group I (randomly-selected subjects) was higher than, and statistically different from the Euclidean distances from the respective threshold (Group V) ($p<0.05$) (Table 19).

The ROC analysis revealed sensitivity and specificity of 100% and 70% for maxillary DCF (87%), and 80% and 73.3% for mandibular DCF (AUC: 78%), respectively (Figure 21).

In parts 2 and 3 (2mm or 1mm at incisal edges, respectively), the mean Euclidean distances of the maxillary (part 2: 3.36, part 3: 2.86) and mandibular (part 2: 3.99; part 3: 3.94) DCF of Group I remained above the respective thresholds with statistical significant difference ($p<0.05$) (Table 22). In both parts the sensitivity and specificity reached 100% for maxillary and mandibular DCF (AUC: 100%) (Figures 23 and 24).

In part 4 (slice of 1mm) only Group I was above the respective threshold and statistically significant different from it ($p<0.05$) (Table 19). The ROC analysis revealed sensitivity and specificity of 80% and 55% for maxillary DCF (AUC: 71%), and 80% and 67% for mandibular DCF (AUC: 76%), respectively (Figure 25).

DISCUSSION

No consensus exists on the depth of the indentations in BM on human skin, but studies with bite impressions on foodstuff (Webster 1982) and wax (Martin-de-las-Heras & Tafur 2009) suggest that the incisal edges may penetrate from 1 to 3mm. The depth on indentations in the living depends not only of the biting force applied, but also on the visco-elastic properties of the skin.

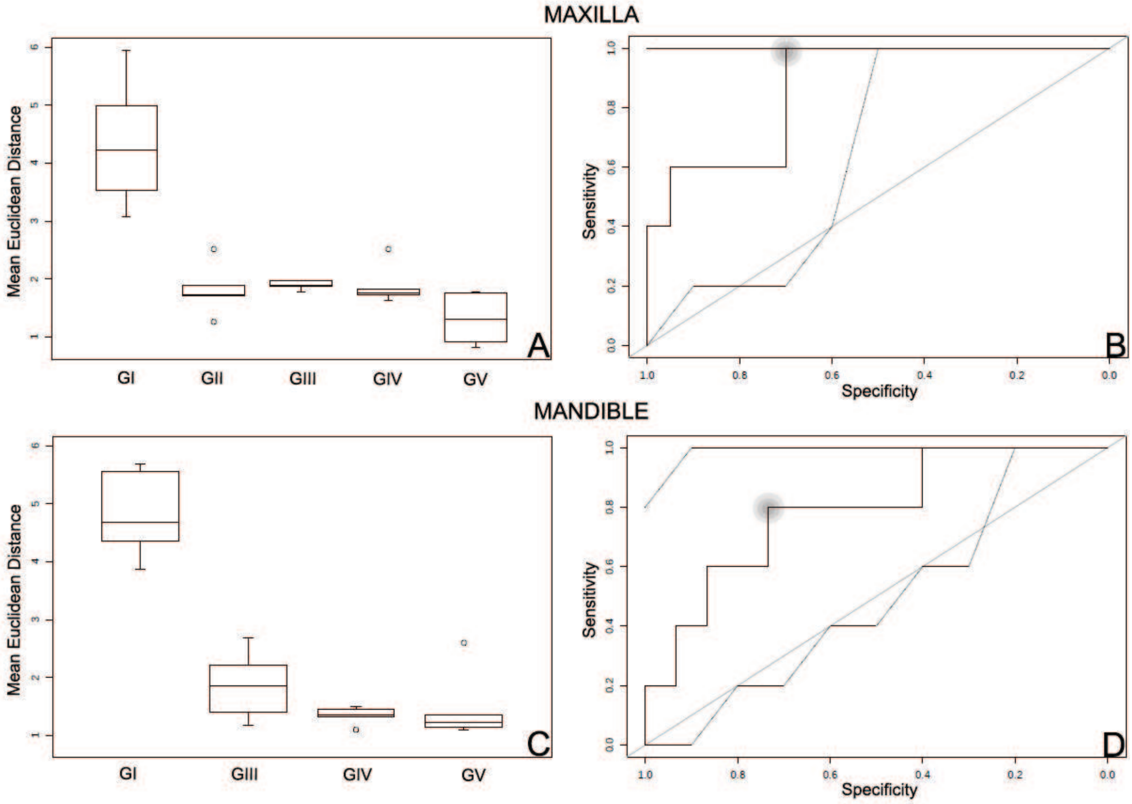
Table 19 - Significance level of comparison between the Study Groups I, II, III and IV and the respective Threshold Group (Group V) per study part separate for each dental arch

Part	Dental arch	Comparison	Mean (SD)	*P*
1	**Maxilla**	GI vs. GV	4.35 (1.55) vs. 1.31 (0.45)	0.0001*
		GII vs. GV	1.82 (0.45) vs. 1.31 (0.45)	0.1836
		GIII vs. GV	1.90 (0.07) vs. 1.31 (0.45)	0.0835
		GIV vs. GV	1.88 (0.35) vs. 1.31 (0.45)	0.1041
	Mandible	GI vs. GV	4.83 (0.77) vs. 1.48 (0.63)	0.0001*
		GIII vs. GV	1.86 (0.60) vs. 1.48 (0.63)	0.4959
		GIV vs. GV	1.34 (0.15) vs. 1.48 (0.63)	0.9910
2	**Maxilla**	GI vs. GV	3.36 (1.01) vs. 1.12 (0.61)	0.0035*
	Mandible	GI vs. GV	3.99 (0.30) vs. 1.41 (0.24)	0.0001*
3	**Maxilla**	GI vs. GV	2.86 (0.86) vs. 1.07 (0.60)	0.0049*
	Mandible	GI vs. GV	3.94 (0.56) vs. 1.08 (0.45)	0.0004*
4	**Maxilla**	GI vs. GV	4.46 (1.12) vs. 1.78 (0.82)	0.0018*
		GII vs. GV	2.00 (0.48) vs. 1.78 (0.82)	0.8981
		GIII vs. GV	2.21 (0.57) vs. 1.78 (0.82)	0.6663
		GIV vs. GV	2.20 (0.69) vs. 1.78 (0.82)	0.7095
	Mandible	GI vs. GV	5.89 (1.50) vs. 1.45 (0.40)	0.0001*
		GIII vs. GV	1.66 (0.52) vs. 1.45 (0.40)	0.8505
		GIV vs. GV	1.45 (0.19) vs. 1.45 (0.40)	0.9990

*Part 1: digital cast files (DCF) cropped with remaining tooth part of interest of 3mm from the incisal edges; Part 2: DCF cropped with remaining tooth part of interest of 2mm from the incisal edges; Part 3: DCF cropped with remaining tooth part of interest of 1mm from the incisal edges; Part 4: DCF cropped with remaining tooth part of interest of 1mm slice excluding the incisal edges. GI: randomly-selected subjects; GII: orthodontically treated subjects; GIII: twins: GIV: orthodontically treated twins; GV: respective threshold (obtained specifically for each study part).Study parts 2 and 3 only compared GI because this was the only study group in which the UHD was observed in study part 1. Mean and standard deviation (SD) of the Euclidean distances obtained in each group. P value calculated from ANOVA test with significance rate of 5%. *statistically significant difference (p<0.05).*

A human bite power may reach 37.72kg (Paphangkorakit & Osborn 1997), depending on the size of the muscle, the bone morphology and the articulation between dental arches. On the other hand, the elasticity of the skin decreases considerably (Bush et al. 2010) from a subjected power of 13.5kg and responds to skin rupture with higher biting forces. However, the human skin responds heterogeneously, depending on the underlying tissue (Bush et al. 2010), the age and the weight of the victim (Bush et al. 2009). This information demonstrates that there is not concrete proof of the depth of intrusion of the dentition during biting and the depth of the remaining indentation on human skin. Thus, in this chapter a systematic selection of the 3D quantity of remaining tooth parts of interest was established (parts 1, 2 and 3). Moreover, study part 4 was designed to mimic the currently overall established BM practice, which relies on the 2D registration of the BM, registering and comparing the contours of the BM and transparent overlays of the incisal edge of the suspect's dental casts (Bowers & Johansen 2000). In this part, BM contours were mimicked by selecting a slice (1mm) of the DCF cropped axially at maximally 3mm from the incisal dentition edges. The slice was selected at this region because theoretically it includes the BM contours that could be generated after intruding the incisal dentition edges between 2 and 3 mm. Similarly, the literature describes the production of 2D comparison overlays from 3D dental casts (Las Heras et al. 2005).

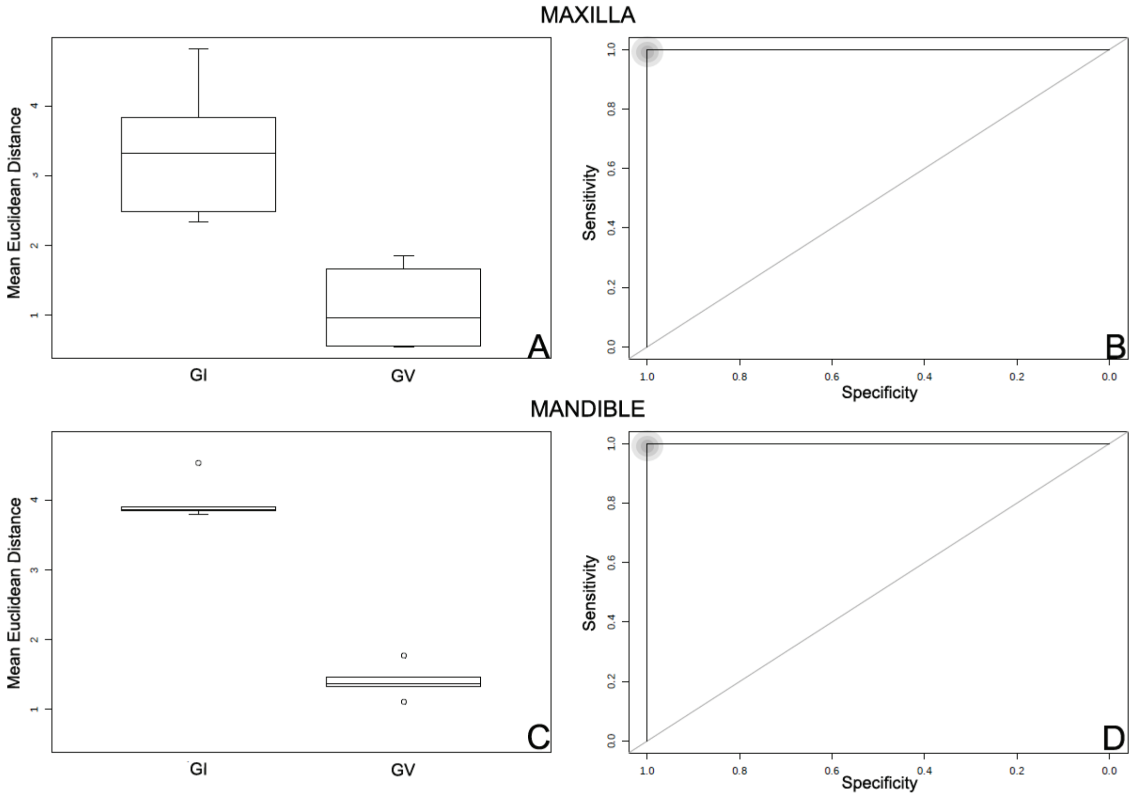
Figure 21 – Euclidean distances of the Groups I, II, III, IV and V obtained pair wise comparing DCF with remaining tooth part of interest of 3mm from the incisal edges (study part 1), expressed in Boxplots (A, C) and ROC curves (B, C)



A, C: Boxplots showing the difference in Euclidean distances between the study Groups I (randomly-selected subjects), II (orthodontically treated subjects), III (twins), IV (orthodontically treated twins), and V (threshold) combining the four quantification values (maximum positive deviation, maximum negative deviation, average, standard deviation). B: The maximized potential prediction (grey circle) indicates that Group V reached sensitivity of 100% - for classifying equal maxillary (B) digital cast files (DCF) as equal and specificity of 70% - for classifying non-equal maxillary (B) DCF as non-equal (specificity). D: The maximized potential prediction (grey circle) indicates that Group V reached sensitivity of 80% - for classifying equal mandibular (D) DCF as equal and specificity of 73.3% - for classifying non-equal mandibular (D) DCF as non-equal (specificity).The Area Under the Curve (AUC) reached accuracy of 87% and 78%, for maxillary (B) and mandibular (D) DCF, respectively.

This procedure was enabled by a software with tools specifically developed for slicing digital models and improving the 2D analysis in the BM practice (Martin-de-las-Heras & Tafur 2009). The authors matched the obtained 3D dental overlays digitally with photographs of BM on pig skin (Martin-de-las-Heras & Tafur 2009). However, the 3D dental overlays were generated from the impression of dental casts on wax (Martin-de-las-Heras & Tafur 2009), creating an indirect procedure. The study performed in the present chapter has the advantage of generating the possibly involved dentition parts and a slice, mimicking concerned dentition contours directly from the 3D DCF, tackling any potential distortion involving indirect procedures.

Figure 22 – Euclidean distances of the Groups I and V obtained pair wise comparing DCF with remaining tooth part of interest of 2mm from the incisal edges (study part 2), expressed in Boxplots (A, C) and ROC curves (B, C)

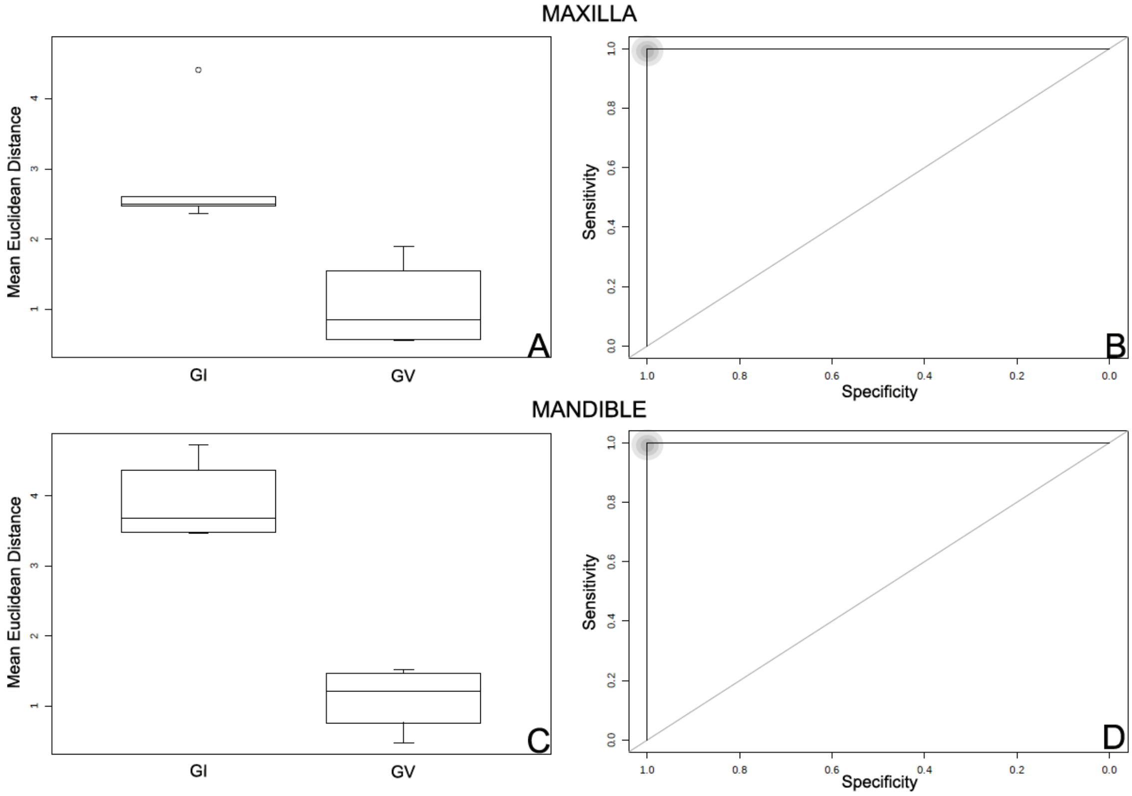


A, C: Boxplots showing the difference in Euclidean distances between the study Groups I (randomly-selected subjects) and V (threshold) combining the four quantification values (maximum positive deviation, maximum negative deviation, average, standard deviation). B: The maximized potential prediction (grey circle) indicates that Group V reached sensitivity of 100% - for classifying equal maxillary (B) digital cast files (DCF) as equal and specificity of 100% - for classifying non-equal maxillary (B) DCF as non-equal (specificity). D: The maximized potential prediction (grey circle) indicates that Group V reached sensitivity of 100% - for classifying equal mandibular (D) DCF as equal and specificity of 100% - for classifying non-equal mandibular (D) DCF as non-equal (specificity).The Area Under the Curve (AUC) reached accuracy of 100% for maxillary (B) and mandibular (D) DCF.

Moreover the present chapter differed from earlier published studies (Rawson et al. 1984; Nambiar et al. 1995a; Nambiar et al. 1995b; Sheets et al.

2013; Bush et al. 2011b; Blackwell et al. 2007; Bush et al. 2011a), using not exclusively DCF from randomly-selected subjects, but also from three populations stratified on dental similarity.

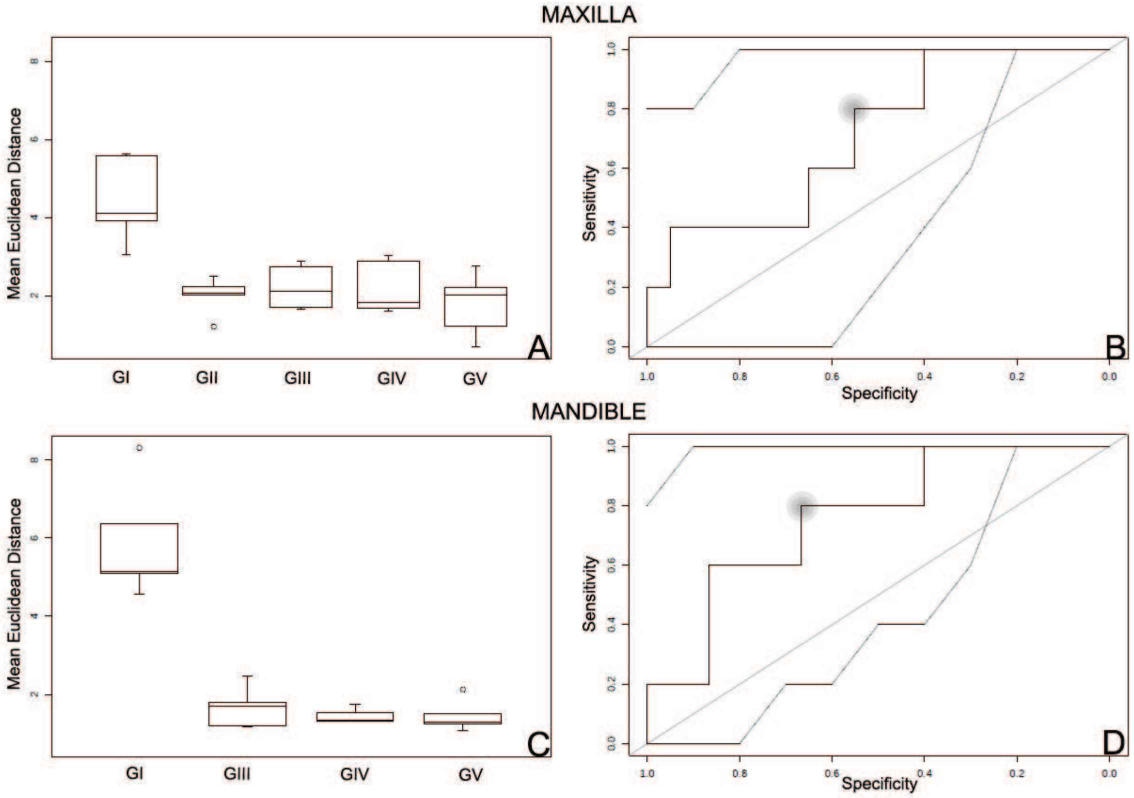
Figure 23 – Euclidean distances of the Groups I and V obtained pair wise comparing DCF with remaining tooth part of interest of 1mm from the incisal edges (study part 3), expressed in Boxplots (A, C) and ROC curves (B, C)



A, C: Boxplots showing the difference in Euclidean distances between the study Groups I (randomly-selected subjects) and V (threshold) combining the four quantification values (maximum positive deviation, maximum negative deviation, average, standard deviation). B: The maximized potential prediction (grey circle) indicates that Group V reached sensitivity of 100% - for classifying equal maxillary (B) digital cast files (DCF) as equal and specificity of 100% - for classifying non-equal maxillary (B) DCF as non-equal (specificity). D: The maximized potential prediction (grey circle) indicates that Group V reached sensitivity of 100% - for classifying equal mandibular (D) DCF as equal and specificity of 100% - for classifying non-equal mandibular (D) DCF as non-equal (specificity).The Area Under the Curve (AUC) reached accuracy of 100% for maxillary (B) and mandibular (D) DCF.

Orthodontically treated subjects were addressed due to presenting similar dental alignment (or a "*lower level of individuality*") - (Kieser et al. 2007) and twins were addressed due to having a genetic control over dental morphology (Osborne et al. 1958). Related to the latter, is currently known that morphological dental variations are found even among monozygotics (Lundstrom 1963; Osborne et al. 1958; Townsend et al. 1988), theoretically resulting in similar but not identical dentitions.

Figure 24 – Euclidean distances of the Groups I, II, III, IV and V obtained pair wise comparing DCF with remaining tooth part of interest a slice of 1mm not including the incisal edges (study part 4), expressed in Boxplots (A, C) and ROC curves (B, C)



A, C: Boxplots showing the difference in Euclidean distances between the study Groups I (randomly-selected subjects), II (orthodontically treated subjects), III (twins), IV (orthodontically treated twins), and V (threshold) combining the four quantification values (maximum positive deviation, maximum negative deviation, average, standard deviation). B: The maximized potential prediction (grey circle) indicates that Group V reached sensitivity of 80% - for classifying equal maxillary (B) digital cast files (DCF) as equal and specificity of 55% - for classifying non-equal maxillary (B) DCF as non-equal (specificity). D: The maximized potential prediction (grey circle) indicates that Group V reached sensitivity of 80% - for classifying equal mandibular (D) DCF as equal and specificity of 66.7% - for classifying non-equal mandibular (D) DCF as non-equal (specificity).The Area Under the Curve (AUC) reached accuracy of 71% and 76%, for maxillary (B) and mandibular (D) DCF, respectively.

In this context, a third population was addressed with orthodontically corrected changes in the phenotype expression of the dental arrangement (orthodontically treated monozygotic twins). A second stratification was performed selecting the five most similar pairs of DCF in each studied group. Consequently, it narrowed and equalized the number of subjects in each stratified group. This high level of sample stratification on dental similarity is reflected in the sensitivity and specificity obtained comparing each group with its corresponding threshold (Group V). A perfect classification of equal (sensitivity: 100%) and non-equal (specificity: 100%) DCF was obtained in Group I (randomly-selected subjects). It reveals that even retaining the 5 most similar dentitions (minimal Euclidean distances), Group I presented a distinguishable discrepant dental morphology related to its respective threshold. In relation to the other groups, the sensitivity and specificity of group V decreased up to 80% and 70%, respectively. However, it maintained an acceptable discriminative accuracy for the threshold expressed in AUC values above 71%. The only exception was observed in the 2D BM mimicking registration (part 4), in which Group V presented a specificity of 55% and 67% for maxillary and mandibular DCF, respectively. This may be explained by the influence of the limited quantity of tooth material considered in study part 4. Although in Group I no equal DCF were found, even on BM level, no UHD may be claimed, because a randomly chosen individual can potentially be part of one of the stratified subgroups in which was proven that UHD does not exist. .

In the present research chapter the UHD behaved the same analyzing the maxillary and mandibular dentitions. It suggests that no predilection for dental arches should be made in BM cases, indicating that all the available dental information should be analyzed (Dorion 1982). On the other hand, the present research was limited by the lack of mandibular DCF from orthodontically treated subjects, encouraging the use of this material in further studies in the field. Another limitation consisted on the manually collection of dental impressions and the confection of dental casts in plaster (operator dependency). This limitation was tackled by incorporating the potential error during the manual confection of dental casts in the threshold. However, future researches could be performed replacing the manual confection of dental casts by intra-oral scanning. Moreover, the present research was designed in the context of BM without analyzing the BM itself, but yet investigating fundamentally the UHD from dentitions. Proving the match between dentitions of different subjects indicates that a single BM may belong to more than one biter. In the present chapter, the comparison between dentitions was performed using dental casts scanned as DCF and analyzed on BM level by selecting as area of interest the incisal edges of the anterior teeth. The quantity of dental material analyzed was diminished in each study part based on the assumed tooth parts that are potentially impressed in BM on skin. This process is fundamental for undisputable BM investigations because BM are characterized by variations in depth of indentations. Moreover, this chapter demonstrated how highly similar the dentitions from different subjects can be when their teeth are well aligned (Group II) or when their teeth present similar morphology (Group III).

These results indicate that forensic BM analysis must not be disregarded, but performed after case selection. This was also advised in the literature in the sense that "*not every case is suitable for analysis*" (Barsley et al. 2012). In fact, the case selection should be based on distinctive individual tooth or arch characteristics. Further studies should be conducted exploring the limits of case selection in BM analysis. The present chapter suggests that BM cases involving suspects with similar dental arrangement and morphology should be classified as non suitable for analysis.

Considerações finais

Capítulo 9

A presente pesquisa foi motivada com base na incerteza que alicerçava a unicidade da dentição humana (UHD) no contexto de marca de mordidas em pele humana. Os resultados obtidos em cada um dos capítulos apresentam importância e valor específico, separadamente. Porém, quando combinados eles permitem responder questionamentos que permaneceram obscuras no decorrer das pesquisas realizadas no campo da Odontologia Legal.

Questionamentos e respostas específicos:

1º questionamento: **A UHD pode ser investigada com a tecnologia imaginológica 3D contemporânea?**

Sim. A tecnologia imaginológica 3D contemporânea permite o aprimoramento da metodologia previamente utilizada nas pesquisas acerca da UHD. Os testes técnicos realizados com o objetivo de apontar o melhor software para investigar a UHD mostraram que a imaginologia 3D combinada com a sobreposição e comparação morfométrica automatizadas viabilizou a otimização os estudos em UHD.

2º questionamento: **Pode uma amostra ser estratificada significantemente para a população geral a fim de provar a UHD?**

Não. A abordagem mais efetiva para estratificar a amostra consiste na padrão de similaridade dental. Neste contexto, pacientes tratados ortodonticamente e gêmeos exercem papel fundamental, apresentando alinhamento e morfologia dental similar, respectivamente. Diferentemente da estratificação por características dentais, realizada no capítulo 5, a estratificação por similaridade dental permite considerar a morfologia da dentição anterior de uma maneira mais abrangente e mais facilmente executável.

3º questionamento: **A dentição humana é única ao se analisar a morfologia da coroa completa dos dentas anteriores?**

Sim. Os experimentos iniciais realizados nesta pesquisa revelaram que a UHD permaneceu quando analisadas a coroas clinicas da dentição anterior de pacientes tratados ortodonticamente e gêmeos. Contudo, quando extrapolados para BM, estes resultados podem não ser palpáveis, pois em BM o registro da morfologia dental em pele humana inclui, geralmente, apenas borda incisal dos dentes anteriores.

4º questionamento: **A quantidade de material dental analisado influencia na diferença morfológica entre dentições?**

Sim. A quantidade de material dental analisado influencia na diferenciação morfológica entre dentições. Especificamente, maior diferenciação morfológica foi observada aumentando a quantidade de material dental analisado. Quando extrapolado para BM estes resultados podem indicar que a dentição humana pode não única analisando-se apenas a borda incisal dos dentes anteriores.

5º questionamento: **A dentição humana permanece única reduzindo sistematicamente a quantidade de material dental analisado dentro do contexto de BM em pele humana?**

Não. As bordas incisais dos dentes anteriores de pacientes tratados ortodonticamente e de gêmeos revelaram não ser únicas, confirmando a influência da quantidade de material dental analisado na UHD. Este achado sugere que a análise de BM deve ser realizada ainda com mais cautela do que usual, considerando-se a seleção precisa de casos. Pesquisas em BM não devem ser descartadas, mas sim encorajadas e continuamente realizadas no meio científico.

Questionamento e resposta geral:

A morfologia dental humana é única para cada indivíduo no contexto de BM?

Não. A UHD não foi comprovada para o contexto de BM utilizando sobreposições e comparações 3D digitais automatizadas por pares e amostras altamente estratificadas de indivíduo com similaridade no alinhamento e morfologia dental.

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