UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

LIZA LIMA RAMENZONI

ANÁLISE DAS BANDAS DE HUNTER-SCHREGER COMO NOVO MÉTODO BIOMÉTRICO DE IDENTIFICAÇÃO HUMANA

Dissertação apresentada à Faculdade de Odontologia, da Universidade Estadual de Campinas, para obtenção do título de Mestre em Biologia Buco-Dental, Área de Histologia e Embriologia.

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Orientador: Prof. Dr. Sérgio Roberto Peres Line

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SUMÁRIO

RESUMO	**1**
ABSTRACT	**2**
1. INTRODUÇÃO	**3**
2. ARTIGO	**6**
3. CONCLUSÃO	**28**
4. REFERÊNCIAS BIBLIOGRÁFICAS	**29**
5. ANEXOS	**33**

RESUMO

O esmalte dental é caracterizado por camadas de prismas em direções alternadas regularmente. Estas camadas sucessivas formam as Bandas de Hunter-Schreger (HSB) que aparecem como faixas claras e escuras quando vistas sob forte iluminação lateral. Neste presente trabalho avaliamos a singularidade das HSB em dentes humanos como um método biométrico para identificação pessoal, já que as diferenças no padrão das HSB em dentes ainda não foi estudada. A amostra foi composta de 274 incisivos inferiores. Os procedimentos seguintes foram executados: os dentes foram fotografados em uma lupa esteroscópica e fibra óptica acopladas. O contraste das imagens após digitalização, foi aumentado utilizando Corel Photo Paint 9® e então as mesmas imagens foram analisadas em software de identificação automatizado de base biométrica (Verifinger 4.2 SDK / Fingersec®). O software gerou uma lista de comparações de dados biométricos com uma medida de semelhança (comparações entre "minutias"). As medidas de similaridade do banco de dados foram comparadas em uma matriz de semelhança. Analisamos também a variação das espessuras médias das Bandas desde que este parâmetro é muito variável e pode ser usado para confirmar a identificação. Os resultados demonstraram que o padrão de HSB é altamente variável e único para cada dente analisado. HSB não puderam ser observadas em 4,5% dos dentes examinados. Dentes sem HSB não foram incluídos no banco de dados. Dentes com 0 ou 1 minutias totalizaram 3,3% da amostra. Nestes casos, a distinção pode ser feita através de comparação visual simples. Assim, as medidas biométricas das HSB provaram ser um método com alta potencialidade para identificação pessoal, desde que o tecido do esmalte resiste condições ambientais extremas e as imagens são obtidas facilmente. Estas características fazem das HSB um modelo potencialmente útil para análise forense utilizando medidas físicas ou biológicas pessoais, dando uma descrição correta do indivíduo.

ABSTRACT

Dental enamel is characterized by layers of prisms with regularly alternating directions. These successive layers form Hunter-Schreger Bands (HSB) that appear as dark and light bands when viewed under strong illumination. In the present study, we evaluate the HSB singularity in human teeth as a biometric-based method for personal identification since differences in HSB patterns have never been studied. The sample was composed of 274 lower incisors. The following procedures were performed: the teeth were photographed at low magnification, the contrast of the captured images was increased using Corel Photo Paint 9® and then analyzed in automated biometrics-based identification software (Verifinger Demo 4.2 SDK / Fingersec®). The software generated a list of biometric data comparisons with a similarity measure (minutiae matching). The storage of database comparisons could be represented with a similarity matrix. We also analyzed the thickness of the bands since this parameter is very variable and could be used for the identification. The results demonstrated that the pattern of HSB is highly variable and unique for each tooth. HSB bands could not be observed in 4.5 % of the teeth examined. Teeth without HSB could not be included in the database. Teeth having 0 or 1 minutiae comprised 3.3% of our sample. In these cases, the inspection was simply done by visual comparison. Thus, the biometric measurements of HSB proved to be a valuable method for personal identification, since enamel can resist extreme environmental conditions and the images could be easily obtained. These characteristics make HSB a potentially useful model for personal physical or biological measurements to give a correct description of an individual.

1. INTRODUÇÃO

Dentro da medicina legal, várias técnicas puderam fornecer subsídios de real valor para resolução de problemas médicos legais na identificação humana. O processo de identificação é usualmente feito com o uso de fotografias, comparação de imagens radiográficas, impressões digitais e mais recentemente com a análise de DNA (Weicheng & Tieniu 1999). Entretanto, métodos de identificação pós-morte, possuem certas limitações e podem deixar de ser eficientes quando os corpos investigados sofreram decomposição ou putrefação impossibilitando seu reconhecimento em investigações periciais, o que haveria de acontecer, por exemplo, em cadáveres extremamente carbonizados ou tendo estes poucos vestígios de fragmentos de tecidos neste caso já calcificados (Perry *et al.* 1988).

A análise biométrica de um indivíduo é um dos métodos mais usados durante o processo de identificação. A biometria recorre a técnicas de identificação baseadas em características físicas, biológicas ou comportamentais de um indivíduo. Atualmente, metodologias de identificação e verificação baseadas em biometria (impressão digital, íris, reconhecimento facial) são analisadas em sistemas automatizados e softwares que têm a capacidade de distinguir características pessoais de indivíduos com alto grau de acerto.

Para identificar um único indivíduo baseando-se em métodos biométricos, as seguintes características são desejáveis: alta unicidade para cada indivíduo, fácil transmissão da característica, fácil obtenção, invariável ao tempo (sem modificações significantes durante um período de tempo), ser passível de ser adquirido com método menos invasivo possível e ser distinguido por pessoas sem muito treinamento especial (Weicheng e Tieniu 1999).

Um dos mais conhecidos procedimentos biométricos usados para identificação humana é a impressão digital. Esta é conhecida por ser única para cada indivíduo podendo, portanto, ser precisa para métodos de comparação e posterior identificação pessoal (Weicheng e Tieniu, 1999).

As linhas observadas na impressão digital podem se dividir em duas vias formando uma bifurcação. A bifurcação o os pontos finais das linhas são conhecidos como **"**Minutia**",** e na maioria dos casos estes pontos de singularidade são usados como referências e parâmetros de entrada de algoritmos. Assim, as imagens das impressões digitais são traduzidas em representações numéricas (Hiratsuka & Oshino 2002, Nikodemuszszekely & Szekel 1993). Através deste algoritmo, os dados numéricos podem ser armazenados em um arquivo, permitindo posteriormente uma simples e rápida comparação entre a medida biométrica em consideração com as demais pertencentes ao banco de dados (Weicheng e Tieniu 1999).

Recentemente a análise de DNA tem se tornado um método poderoso para identificação humana. Este método, entretanto, é relativamente dispendioso e requer um tempo que impede uma obtenção e disponibilizarão dos resultados prontamente. Além disso, o sucesso da análise DNA é também altamente dependente da preservação do espécime.

O órgão dentário é apontado como uma das peças fundamentais na identificação humana devido ao esmalte dental, considerado o tecido com maior quantidade de mineral no corpo (Ten Cate 1994). Esta natureza altamente mineralizada, proporciona ao esmalte alta resistência e dureza, permitindo que o órgão dentário suporte condições extremas de degradação como a exposição a altas temperaturas, umidade e pressão excessivas (Sweet *et al* 1995, Valenzuela *et al* 2000). As características dentais provaram ser particularmente disponíveis para a identificação de vítimas de carbonização em alta temperatura em desastres em massa, quando o reconhecimento visual e a identificação através da impressão digital não são mais possíveis (Valenzuela *et al* 2000). O uso de fichas odontológicas no processo de identificação em cadáveres nestes casos é imprescindível para obtenção de um correto resultado (Clark 1994).

Na maior parte das espécies de mamíferos, incluindo humanos, o esmalte é caracterizado por apresentar camadas de prismas de hidroxiapatita dispostas regularmente em direções alternantes formando um ângulo de até aproximadamente 90º. (Von Koenigswald *et al* 1987). Estas camadas sucessivas de prismas formam as Bandas de Hunter-Schreger (HSB) que aparecem como camadas claras e escuras quando observadas sobre uma iluminação de baixa potência em uma lupa esteroscópica (Koenigswald 1994). As bandas claras e escuras podem ser invertidas mudando a direção da fonte de iluminação. Este fenômeno óptico ocorre devido ao fato dos prismas funcionarem como fibras ópticas quando expostos sob uma fonte de luz direta.

Considera-se que as HSB existentes na maior parte das espécies de mamíferos, servem como uma estrutura funcional adaptativa que poderia aumentar a resistência, durabilidade e proteção do esmalte contra a formação e propagação de quebras, trincas e rachaduras (Koenigswald 1980, Stefen 1999, Stefen 2001, Koenigswald *et al* 1987).

Em estudo no laboratório de Morfologia, verificamos que quando observadas sob forte iluminação lateral, as faixas claras e escuras das HSB se assemelham a uma impressão digital. Esta similaridade nos motivou a proporcionar este presente trabalho que teve como objetivo principal avaliar a singularidade das HSB como um método de identificação humana, utilizando para isto um programa automatizado de identificação de base biométrica, o mesmo usado para impressões digitais. Desta forma podemos avaliar as variações de padrão das HSB como um método eficaz e complementar na identificação em investigações forenses.

2. ARTIGO

Esta tese está baseada na resolução CCPG/001/98/UNICAMP que regulamenta o formato alternativo para teses de Mestrado e Doutorado e permite a inserção de artigos científicos de autoria e co-autoria do candidato. Por se tratar de pesquisa envolvendo dentes e seres humanos, este estudo foi submetido à apreciação da Comissão de Ética em Pesquisa da Faculdade de Odontologia de Piracicaba (UNICAMP), em 21/06/2001 (protocolo nº 046/2001) (ANEXO1). Desta forma, esta tese será composta do seguinte artigo:

Capítulo 1: "Automated biometrics-based personal identification of the Hunter-Schreger bands of dental enamel". Proceedings of the Royal Society B, aceito para publicação (ANEXO 2).

Automated biometrics-based personal identification of the Hunter-Schreger bands of dental enamel

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Abstract

The use of automated biometrics-based personal identification systems is a ubiquitous procedure in present times. Biometrics has certain limitations, such as in cases when bodies are decomposed, burned, or only small fragments of calcified tissues remain. Dental enamel is the most mineralized tissue of organisms and resists postmortem degradation. It is characterized by layers of prisms of regularly alternating directions, known as Hunter-Schreger bands (HSB). In this article we show that the pattern variation of the HSB, referred here as toothprint, can be used as a biometric-based parameter for personal identification in automated systems.

Keywords: Forensic science, Hunter-Schreger bands, Dental enamel

Introduction

Human identification is becoming increasingly important in modern life. It may be required in simple procedures such as logging into a computer network or in more complex situations like post-mortem identification and criminal analysis. It is usually achieved by the use of passwords, physical tokens, photographs, iris and dental patterns, fingerprint and more recently DNA analysis (Weicheng & Tieniu 1999). Commonly these identification methods fail or have certain limitations and may not be efficient when bodies are decomposed, burned, or in cases when only small fragments of calcified tissues are left (Perry *et al.* 1988).

The term 'biometrics' is used to refer to identification techniques based on physical characteristics. They are sometimes referred to as 'positive identification', because they are claimed to provide greater confidence that the identification is accurate. Biometric-based identification/verification methodologies such as fingerprint verification, iris scanning and facial recognition have been steadily improved and refined in automated systems and software, which have the capacity to reliably distinguish individuals.

To uniquely identify an individual based on biometric information, the following characteristicS of biometric data are desirable: highly unique to each individual, easily transmittable, able to be acquired as no intrusively as possible and distinguishable by humans without much special training (helpful to manual intervention) (Weicheng & Tieniu 1999). DNA testing is an example that revolutionized human identification in forensic science. This method, however, is relatively expensive, time consuming and the results are not promptly available. The success of DNA analysis is also highly dependent on the preservation of the specimen.

Another well-known and widely used biometric is fingerprint. Fingerprint is unique for each individual and can be a precise method for personal identification (Perry *et al.* 1988, Sweet *et al.* 1995). In the identification process, every fingerprint can be broken down into two basic features, called ridges and valleys. In a typical fingerprint these features will appear as clear and dark lines. Minutiae are points at the ending lines and at the bifurcations when one line splits up in two. Minutiae are the major parameter used for algorithm extraction in softwares of automated fingerprint identification systems (Hiratsuka & Oshino 2002, Nikodemuszszekely & Szekel 1993).

In most mammalian species, including humans, dental enamel is characterized by layers of prisms of regularly alternating directions known as Hunter-Schreger bands (HSB). HSB appear as dark and light bands under low-powered light microscopy (Koenigswald 1994, Koenigswald & Pfretzchner 1987). This phenomenon occurs because enamel prisms function like optic fibers when exposed to a directed source of light (Whittaker & Rothwell1984). When observed from the outside the dark and light lines of HSB in dental enamel closely resemble a fingerprint as they form minutiae. Due to the similarity with fingerprint the pattern of HSB will be referred here as toothprint. This similarity prompted us to use a fingerprint identification/verification software to evaluate the HSB singularity in human teeth as a biometric-based method for personal identification.

Materials and Methods

a) Documentation and analysis of HSB. The sample was composed of 274 lower incisors supplied by the museum of the Department of Morphology - Piracicaba Dental School, State University of Campinas, SP, Brazil. We have also performed *in situ* analysis of the HSB pattern in central inferior incisors directly from the mouth of 30 individuals.

This research project is in agreement with the resolution 196/96 from National Committee of Health Department (BR) and was approved by the ethical Committee in Research of the Piracicaba Dental School.

HSB were observed with a binocular microscope (magnification of 10-20 X) and light shinning obliquely on the tooth (optic fiber - Fiber Light®). HSB were clearly visible from outside in the cervical to the mid coronal regions of the teeth (average area of 6 mm^2).

HSB were documented with a Pentax MZ-M photographic camera and ILFORD 50 ASA (Kodac®). Digital images of the negatives were contrasted with Corel Photo Paint 9 program (Corel Corporation ®). After contrast enhancement, the images were extracted on automated biometrics-based personal identification software (Verifinger Demo 4.2 SDK / Fingersec®). Verifinger recognition algorithm follows the commonly accepted fingerprint identification scheme, which uses a set of specific fingerprint points (minutiae). The software uses enhanced algorithmic solutions, which improve the system performance and reliability such as adaptive image filtration algorithm, which eliminates noises, ridge ruptures and stuck ridges. The Verifinger software is fully tolerant to fingerprint translation and rotation, and can recognize a fingerprint from any part of it since the software does not require presence of the fingerprint core or delta points in the image. The software provides a numeral score of similarity (bits). Higher scores are given to more similar characters, and lower or negative scores for dissimilar characters. The similarity scores were plotted on similarity matrices, which express the similarity between two data points. The similarity scores given by the software were plotted on similarity matrixes. The similarity matrix was numbered from 1 to 262 (according to the tooth number), in the ordinate and in the abscissa (N x N). The similarity scores were divided into 4 groups: Group I: 2000 to 1001 bits (very high similarity), Group II: 1000 to 101 bits (high similarity), Group III: 100 to 10 bits (low similarity), Group IV: 9 to 0 bits (very low similarity).

b) Analysis of HSB thickness. The average thickness (μm) of HSB in the central portion of 70 human lower incisors was measured using the ImageMaster TotalLab Software version 2.00 (Amersham Pharmacia Biotech).

c) Effect of temperature. To test the effect of tooth burning on the visualization of HSB teeth were incubated for 1 h on a heat oven at temperatures ranging from 100ºC to 500ºC.

Results

HSB could be examined and photographed without special preparation directly from the mouth of 30 individuals (Fig.1) and from 262 isolated incisors. Incisors teeth were chosen because they are frontal, thereby easy to observe.

The similarity matrix shown on Fig. 2 represents the matching of each tooth with the teeth stored on database. The best match (i.e. the higher score), was always achieved when a tooth was compared with itself on the database. It indicates that toothprint analysis as fingerprint exhibits a high discrimination power.

The light and dark bands of a toothprint can be reversed, creating a negative image, by changing the direction of the light source. In order to assess the reliability and robustness of this biometrics in identification/verification procedures the toothprints of 100 teeth were captured with the light source directed to the mesial side of the teeth and stored in a new database. The toothprints of these 100 teeth were also captured in a distinct occasion with the light directed to the distal side of the teeth. The toothprints of distally illuminated teeth were then compared with the database composed by the mesially illuminated teeth. In this case the best match was also always achieved when a tooth was compared with its template (i.e. negative image) on the database (Fig. 3).

We have also performed an *in vivo* analysis directly from the mouth of 30 individuals. The comparison of right-left upper and lower central incisors showed that a toothprint is distinct from its homologous tooth on the opposite side (not shown). This indicates that a toothprint pattern is highly specific, been unique for each teeth of an individual.

HSB bands could not be observed in 4.5 % of the teeth examined. Teeth without HSB could not be included in the database. Scanning electron microscope analysis confirmed absence of HSB in these teeth. Although most teeth presented HSB, 3.3 % of the teeth analyzed had only 0 or 1 minutia. These teeth gave low similarity scores, thereby increasing the false acceptance and rejection rates in the identification process.

Different from fingerprint the average thickness of HSB are variable, and this feature can be used as an additional parameter for automated identification (Fig.4). After the use of two automated systems the identification of the few remaining teeth with low similarity scores can be made by simple visual comparison. This work can be performed by individuals without much special training (Fig.5, electronic supporting material).

HSB were clearly observed in the enamel of isolated teeth heated at temperatures of up to 300ºC for 1h (Fig. 6, electronic supporting material). Above this temperature enamel becomes opaque, probably due to the incorporation of smoke residues resulting from the burn of organic matrix. This hinders the diffusion of the directed light throughout HSB.

Discussion

Teeth have been extensively used as a source of information in human identification, especially when the soft tissues cannot provide reliable information (Holland *et al* .2003, Stefen 2001). We show here that HSB can be observed and recorded directly from the mouth (Fig. 1) and from isolated teeth or even in tooth fragments collected in the field. Modern life is characterized by the concentration of large populations in urban areas with the gathering of people in huge office buildings, schools, theaters, and mass transportation systems. Unfortunately, with that comes an increase in the necessity of new and diverse methods of forensic investigation to identify the victims of mass incidents and perpetrators of terrorist acts. The attacks on the World Trade Center (WTC) towers on September 11, 2001 set a dramatic example. This event represented the single largest terrorist-related mass fatality incident in the history of the United States. More than 2,700 individuals of varied racial and ethnic background lost their lives that day. Through the efforts of thousands of citizens, including recovery workers, medical examiners, and forensic scientists, the identification of only approximately 1,500 victims had been accomplished through June 2003 (Holland *et al.* 2003). Toothprint is a highly robust biometric-based procedure for personal identification that could be useful in mass disasters. The analysis of HSB pattern or toothprint may complement fingerprint and DNA as well as others methods of identification. Accordingly, toothprint recording would be specially recommended for individuals working in dangerous occupations such as firefighters, soldiers, jet pilots, divers, and people that live or travel to politically instable areas.

HSB withstand extreme environmental conditions and are preserved after skin decomposition. HSB could be clearly observed and recorded in teeth burned at temperatures of up to 300°C for 1 h. Experimental evidences indicate that teeth can withstand much higher temperatures in burned corpses. The preservation is achieved due to the protective effect of the tongue, mastication muscles and other soft tissues in the face and mouth (Valenzuela *et at.* 2000). Furthermore, well-preserved HSB have been observed from the outside in fossil teeth dating from several hundred thousand to over sixty million years (Koenigswald 1994, Stefen 2001, Ferretti 1999, Line & Bergqvist *in press*). Toothprint analysis is non-invasive, accurate and can be readily performed in automated systems. Toothprint is, therefore, a suitable physiological biometric trait for human personal identification and verification.

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FIGURE LEGENDS

Fig. 1: HSB could be examined and photographed without special preparation directly from the mouth (A, B). After contrast enhancement by Corel Photo Paint 9 (Corel Corporation ®) (C) the images were extracted on automated biometrics-based personal identification software (Verifinger Demo 4.2 SDK / Fingersec®) (D).

Fig. 2: The similarity matrix: N x N (N: numbered teeth from 1 to 262). Toothprints were divided into four groups according to the similarity scores: Group I: 2000 to 1001 bits (very high similarity) ( ), Group II: 1000 to 101 bits (high similarity) ( ), Group III: 100 to 10 bits (low similarity) ( ), Group IV: 9 to 0 bits (very low similarity) ( ). The darker line on the main diagonal shows that the highest scores (i.e. the best match), were always achieved when a tooth was compared with itself on the database.

Fig. 3: Similarity matrix comparing 100 toothprints. The images of each tooth were captured with light source directed to the mesial (abscissa) and distal (ordinate) sides. Toothprints were divided into four groups according to the similarity scores. Group I: 2000 to 1001 bits (very high similarity) ( ), Group II: 1000 to 101 bits (high similarity) ( ), Group III: 100 to 10 bits (low similarity) ( ), Group IV: 9 to 0 bits (very low similarity) ( ). The darker line on the main diagonal shows that the higher scores (i.e. the best match), were always achieved when a tooth was compared with its negative image on the database.

Fig. 4: Average thickness (µm) of HSB in 70 human lower incisors.

FIGURES

Figure 1

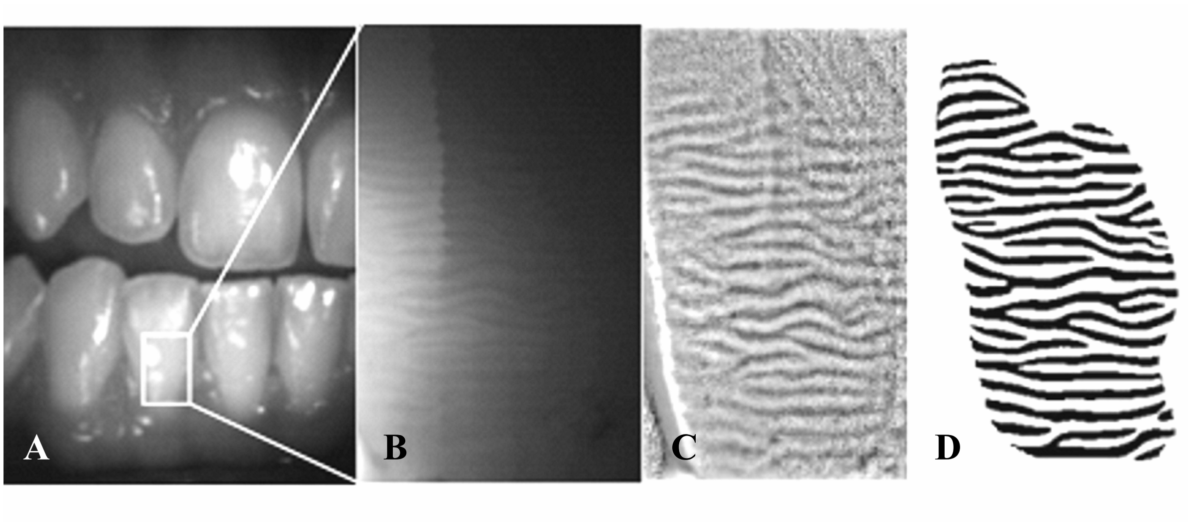


Figure 2

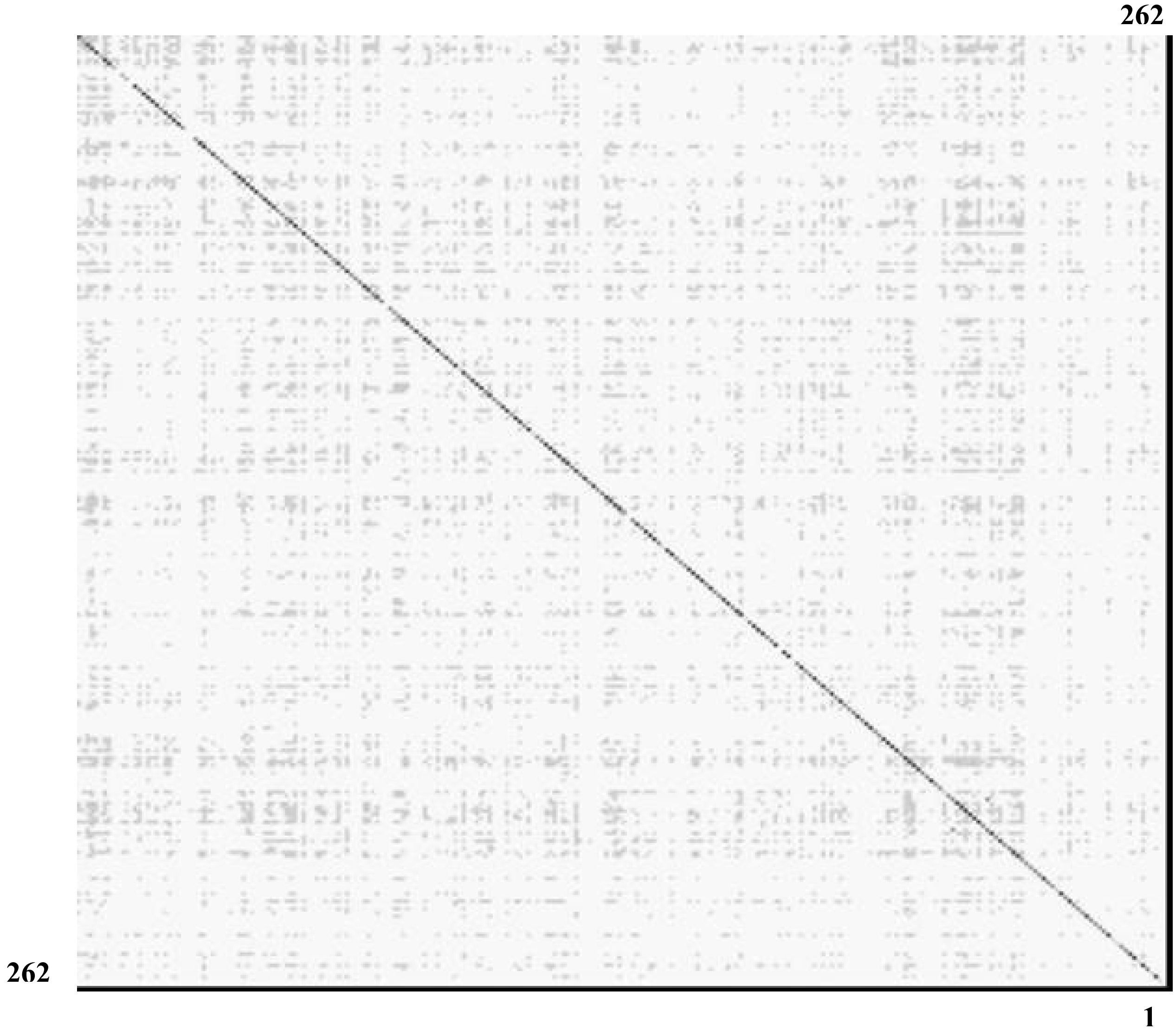


Figure 3

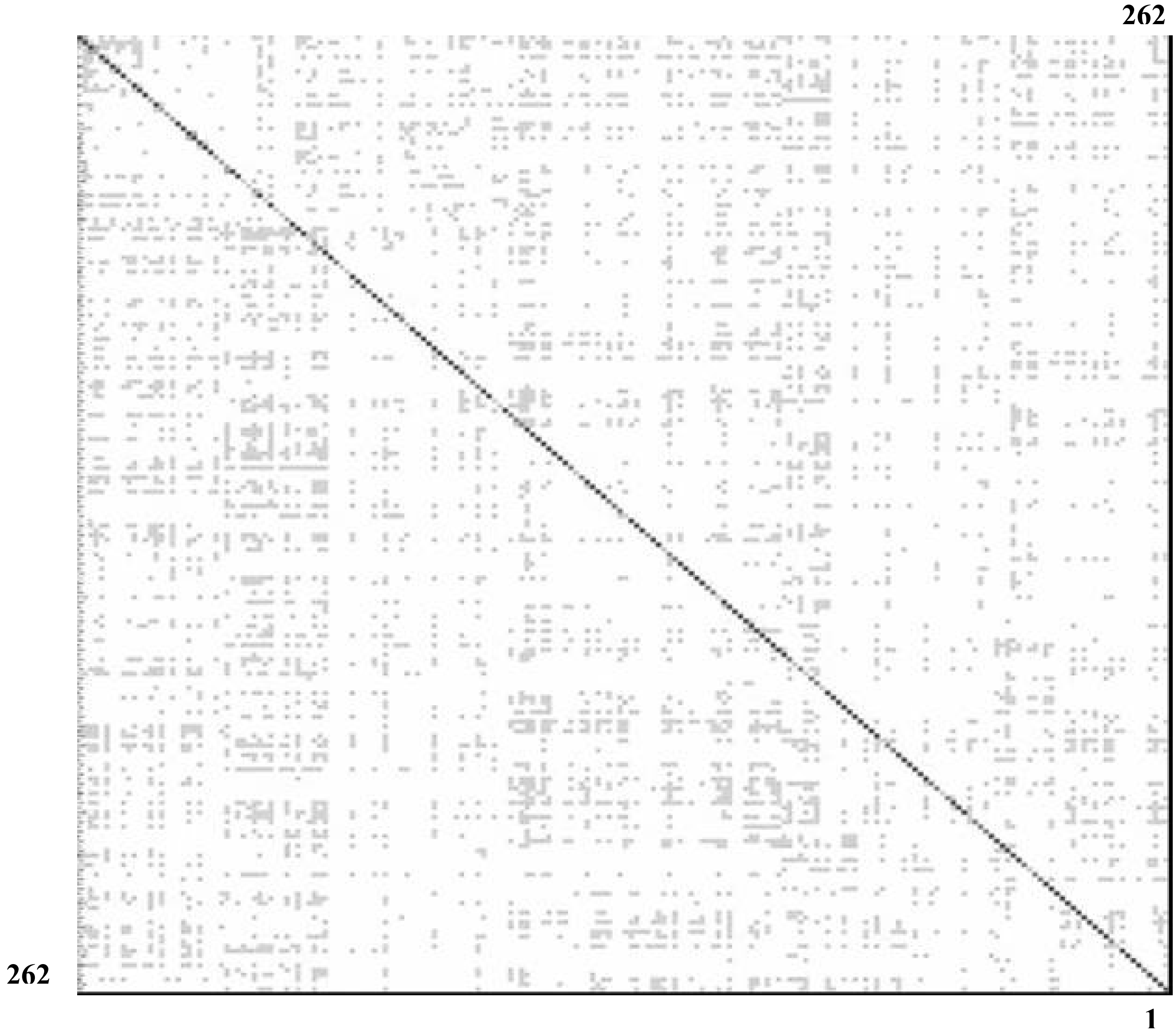
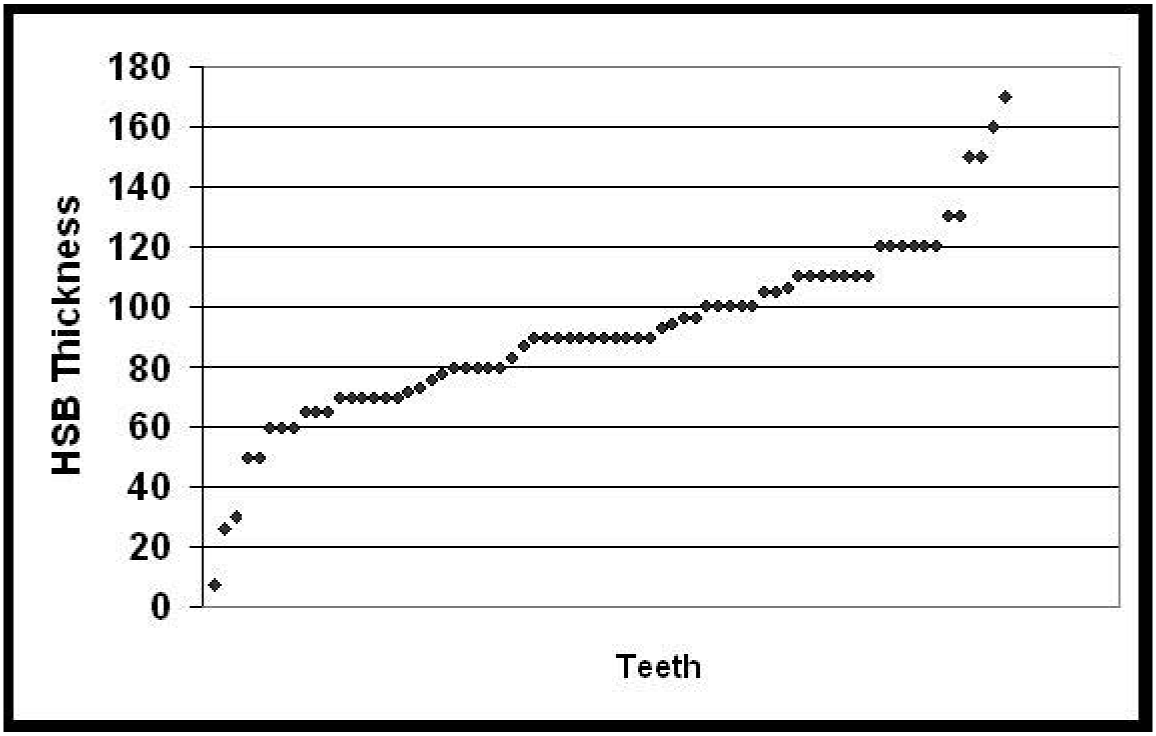


Figure 4



ELETRONIC SUPPORTING MATERIAL

FIGURES LEGENDS

Figure 5. Find the match. The image on the center is a fragment of one of the 10 toothprints around it. The quiz was designed to show that identification might be performed by people without much special training. The answer is on the end of on-line supporting material.

Figure 6. Effect of incineration at 300ºC for 1h on toothprint observation. A) Contrast enhancement by the Corel Photo Paint 9 software (Corel Corporation ®). B) Toothprint extracted by the Verifinger Demo 4.2 SDK software (Fingersec®).

Answer to the quiz of figure 5 = # 10

Figure 5

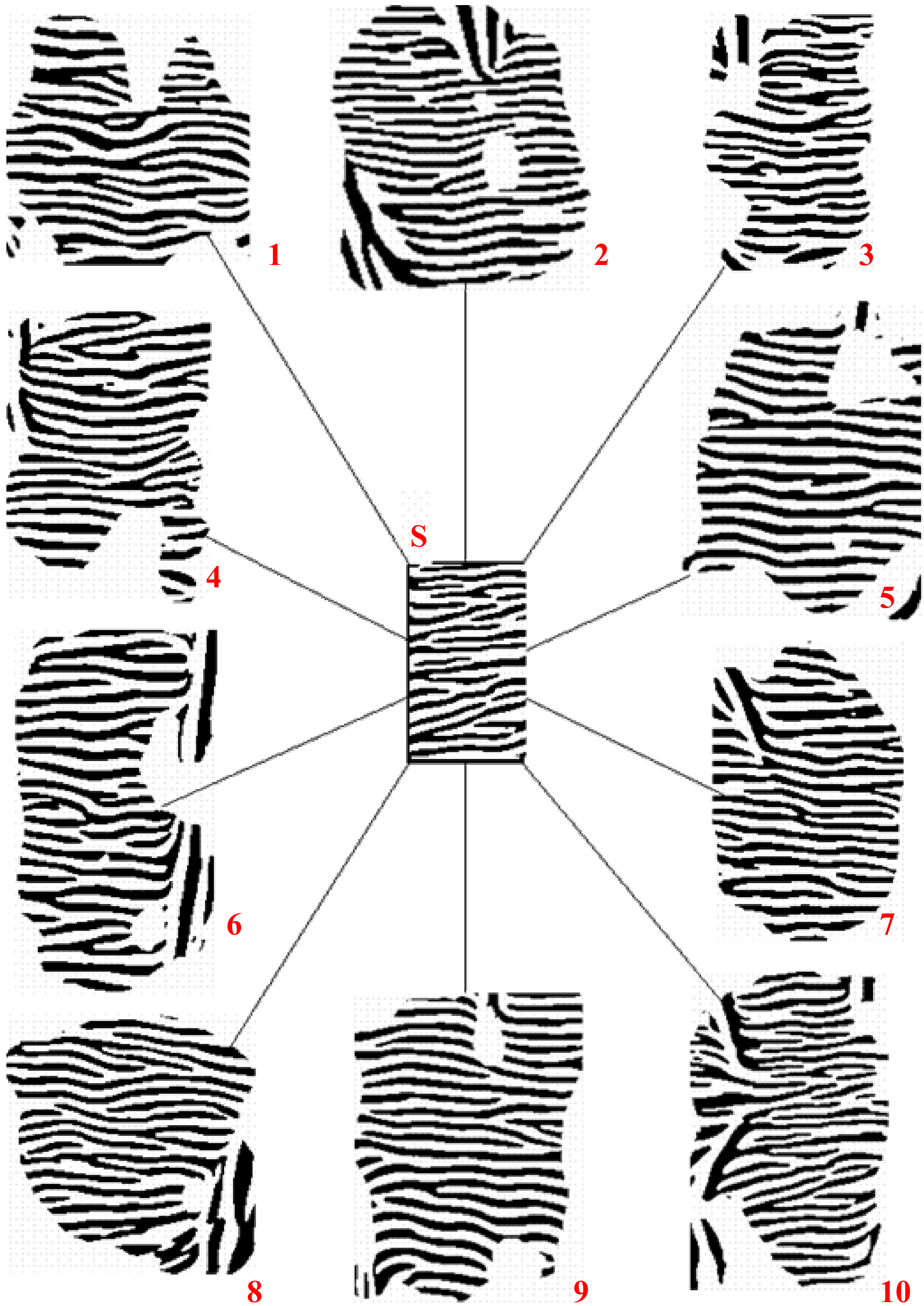
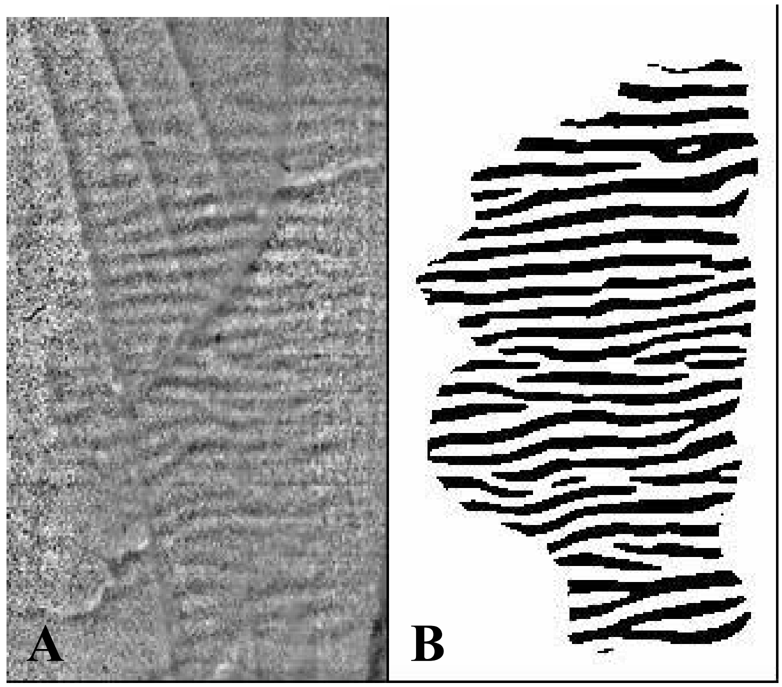


Figure 6



3. CONCLUSÃO

A análise das HSB mostrou ser um método biométrico preciso na identificação pessoal. O padrão de HSB pode complementar métodos como impressão digital ou DNA como também outros métodos de identificação forense de vítimas de incidentes em massa e desastres naturais. A análise é precisa, não invasiva e pode ser executada prontamente em sistemas automatizados. Portanto, as HSB constituem uma característica biométrica satisfatória para identificação pessoal humana.

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Anexo 1

UNICAMP

COMITÊ DE ÉTICA EM PESQUISA

UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ODONTOLOGIA DE PIRACICABA

MENTE · MANV · CORDE

CERTIFICADO – 2ª VIA

Certificamos que o Projeto de pesquisa intitulado "Estudo das bandas de Hunter-Schreger como um novo método biométrico de identificação humana", sob o protocolo nº **046/2001**, da Pesquisadora ***Liza Lima Ramenzoni***, sob a responsabilidade do Prof. Dr. **Sérgio Roberto Peres Line**, está de acordo com a Resolução 196/96 do Conselho Nacional de Saúde/MS, de 10/10/96, tendo sido aprovado pelo Comitê de Ética em Pesquisa – FOP, em 21/06/2001.

Piracicaba, 11 de março de 2004

We certify that the research project with title "Study of the Hunter-Schreger Bands for new method of human identification, protocol nº **041/2001,** by Researcher ***Liza Lima Ramenzoni***, responsibility by Prof. Dr. **Sérgio Roberto Peres Line**, is in agreement with the Resolution 196/96 from National Committee of Health/Health Department (BR) and was approved by the Ethical Committee in Resarch at the Piracicaba Dentistry School/UNICAMP (State University of Campinas) in June/21/2001.

Piracicaba, SP, Brazil, March 11 2004

Cinthia Machado Tabchoury

Profa. Dra. Cinthia Pereira Machado Tabchoury
Secretário
CEP/FOP/UNICAMP

Prof. Dr. Jacks Jorge Júnior
Coordenador
CEP/FOP/UNICAMP

Anexo 2

ARTICLE IN PRESS

PROCEEDINGS OF THE ROYAL SOCIETY B

Proc. R. Soc. B
doi:10.1098/rspb.2005.3409

Automated biometrics-based personal identification of the Hunter–Schreger bands of dental enamel

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The use of automated biometrics-based personal identification systems is a ubiquitous procedure in present times. Biometrics has certain limitations, such as in cases when bodies are decomposed, burned, or only small fragments of calcified tissues remain. Dental enamel is the most mineralized tissue of organisms and resists post-mortem degradation. It is characterized by layers of prisms of regularly alternating directions, known as Hunter–Schreger bands (HSB). In this article, we show that the pattern variation of the HSB, referred here as toothprint, can be used as a biometric-based parameter for personal identification in automated systems.

Keywords: forensic science; Hunter–Schreger bands; dental enamel

1. INTRODUCTION

Human identification is becoming increasingly important in modern life. It may be required in simple procedures such as logging into a computer network or in more complex situations like post-mortem identification and criminal analysis. It is usually achieved by the use of passwords, physical tokens, photographs, iris and dental patterns, fingerprint and more recently DNA analysis (Weicheng & Tieniu 1999). Commonly these identification methods fail or have certain limitations and may not be efficient when bodies are decomposed, burned, or in cases when only small fragments of calcified tissues are left (Perry *et al.* 1988).

The term 'biometrics' is used to refer to identification techniques based on physical characteristics. They are sometimes referred to as 'positive identification', because they are claimed to provide greater confidence that the identification is accurate. Biometric-based identification/-verification methodologies such as fingerprint verification, iris scanning and facial recognition have been steadily improved and refined in automated systems and softwares, which have the capacity to reliably distinguish individuals.

To uniquely identify an individual based on biometric information, the following characteristic of biometric data are desirable: highly unique to each individual, easily transmittable, able to be acquired as no intrusively as possible and distinguishable by humans without much special training (helpful to manual intervention) (Weicheng & Tieniu 1999). DNA testing is an example that revolutionized human identification in forensic science. This method, however, is relatively expensive, time consuming and the results are not promptly available. The success of DNA analysis is also highly dependent on the preservation of the specimen.

Another well-known and widely used biometric is fingerprint. Fingerprint is unique for each individual and can be a precise method for personal identification (Perry *et al.* 1988; Sweet &Sweet. 1995). In the identification process, every fingerprint can be broken down into two basic features, called ridges and valleys. In a typical fingerprint, these features will appear as clear and dark lines. Minutiae are points at the ending lines and at the bifurcations when one line splits up in two. Minutiae are the major parameters used for algorithm extraction in softwares of automated fingerprint identification systems (Nikodemuszszekely & Szekel 1993; Hiratsuka & Oshino 2002).

In most mammalian species, including humans, dental enamel is characterized by layers of prisms of regularly alternating directions known as Hunter–Schreger bands (HSB). HSB appear as dark and light bands under low-powered light microscopy (Koenigswald & Pfretzchner 1987; Koenigswald 1994). This phenomenon occurs because enamel prisms function like optic fibres when exposed to a directed source of light (Whittaker & Rothwell 1984). When observed from outside, the dark and light lines of HSB in dental enamel closely resemble a fingerprint as they form minutiae. Due to the similarity with fingerprint the pattern of HSB will be referred here as toothprint. This similarity prompted us to use a fingerprint identification/verification software to evaluate the HSB singularity in human teeth as a biometric-based method for personal identification.

2. MATERIAL AND METHODS

(a) *Documentation and analysis of HSB*

The sample was composed of 274 lower central incisors supplied by the museum of the Department of Morphology, Piracicaba Dental School, State University of Campinas, SP, Brazil. We have also performed *in situ* analysis of the HSB

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(*a*) (*b*) (*c*) (*d*)

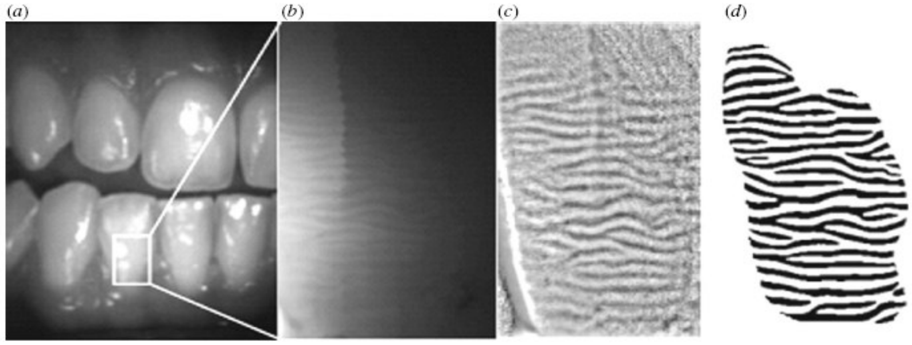

Figure 1. HSB pattern documented *in vivo* with fibre light source. (*a*, *b*) Bands were visible from the cervical to the mid coronal region of the tooth. (*c*) Contrast enhancement by the COREL PHOTO PAINT 9 (Corel Corporation). (*d*) Extracted images on automated biometrics-based personal identification software (VERIFINGER DEMO 1.4 SDK/FINGERSEC).

pattern in central inferior incisors directly from the mouth of 30 individuals.

This research project is in agreement with the resolution 196/96 from National Committee of Health Department (BR) and was approved by the ethical Committee in Research of the Piracicaba Dental School.

HSB were observed with a binocular microscope (magnification of 10–20×) and light shinning obliquely on the tooth (optic fibre–Fibre Light). HSB were clearly visible from outside in the cervical to the mid coronal regions of the teeth (average area of 6 mm^2).

HSB were documented with a Pentax MZ-M photographic camera and ILFORD 50 ASA (Kodac). Digital images of the negatives were contrasted with COREL PHOTO PAINT 9 program (Corel Corporation). After contrast enhancement, the images were extracted on automated biometrics-based personal identification software (VERIFINGER DEMO 4.2 SDK/FINGERSEC). VERIFINGER recognition algorithm follows the commonly accepted fingerprint identification scheme, which uses a set of specific fingerprint points (minutiae). The software uses enhanced algorithmic solutions, which improve the system performance and reliability such as adaptive image filtration algorithm, which eliminates noises, ridge ruptures and stuck ridges. The VERIFINGER software is fully tolerant to fingerprint translation and rotation, and can recognize a fingerprint from any part of it since the software does not require presence of the fingerprint core or delta points in the image. The software provides a numeral score of similarity (bits). Higher scores are given to more similar characters, and lower or negative scores for dissimilar characters. The similarity scores were plotted on similarity matrices, which express the similarity between two data points. The similarity scores given by the software were plotted on similarity matrixes. The similarity matrix was numbered from 1 to 262 (according to the tooth number) in the ordinate and in the abscissa ($N \times N$). The similarity scores were divided into four groups: group I, 2000–1001 bits (very high similarity); group II, 1000–101 bits (high similarity); group III, 100–10 bits (low similarity); group IV, 9–0 bits (very low similarity).

(b) *Analysis of HSB thickness*

The average thickness (micrometre) of HSB in the central portion of 70 human lower incisors was measured using the IMAGEMASTER TOTALLAB Software v. 2.00 (Amersham Pharmacia Biotech).

(c) *Effect of temperature*

To test the effect of tooth burning on the visualization of HSB teeth were incubated for 1 h on a heat oven at temperatures ranging from 100 to 500 °C.

3. RESULTS

HSB could be examined and photographed without special preparation directly from the mouth of 30 individuals (figure 1) and from 262 isolated central incisors. Incisors teeth were chosen because they are frontal, thereby easy to observe.

The similarity matrix shown on figure 2 represents the matching of each tooth with the teeth stored on database. The best match (i.e. the higher score) was always achieved when a tooth was compared with itself on the database. It indicates that toothprint analysis as fingerprint exhibits a high discrimination power.

The light and dark bands of a toothprint can be reversed, creating a negative image, by changing the direction of the light source. In order to assess the reliability and robustness of this biometrics in identification/verification procedures, the toothprints of 100 teeth were captured with the light source directed to the mesial side of the teeth and stored in a new database. The toothprints of these 100 teeth were also captured in a distinct occasion with the light directed to the distal side of the teeth. The toothprints of distally illuminated teeth were then compared with the database composed by the mesially illuminated teeth. In this case, the best match was also always achieved when a tooth was compared with its template (i.e. negative image) on the database (figure 3).

We have also performed an *in vivo* analysis directly from the mouth of 30 individuals. The comparison of right-left upper and lower central incisors showed that a toothprint is distinct from its homologous tooth on the opposite side (not shown). This indicates that a toothprint pattern is highly specific, been unique for each teeth of an individual.

HSB bands could not be observed in 4.5% of the teeth examined. Teeth without HSB could not be included in the database. Scanning electron microscope analysis

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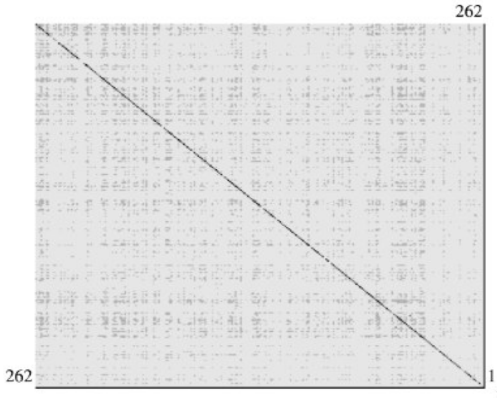

Figure 2. The similarity matrix: $N \times N$ (N: numbered teeth from 1 to 262). Toothprints were classified into four groups according to the similarity scores: group I, 2000–1001 bits (very high similarity) (black filled square); group II, 1000–101 bits (high similarity) (dark grey filled square); group III, 100–10 bits (low similarity) (light grey filled square); group IV, 9–0 bits (very low similarity) (open square). The darker line on the main diagonal shows that the higher scores (i.e. the best match), were always achieved when a tooth was compared with itself on the database.

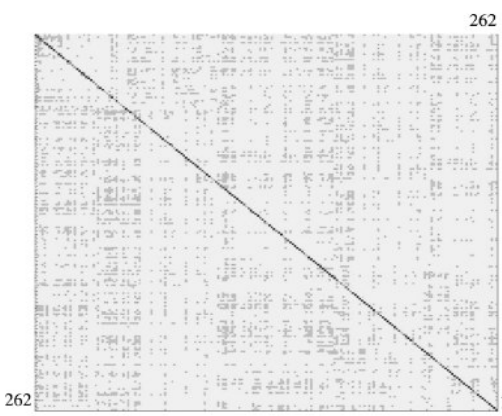

Figure 3. Similarity matrix comparing 100 toothprints. The images of each tooth were captured with light source directed to the mesial (abscissa) and distal (ordinate) sides. Toothprints were divided into four groups according to the similarity scores. Group I, 2000–1001 bits (very high similarity) (black filled square); group II, 1000–101 bits (high similarity) (dark grey filled square); group III, 100–10 bits (low similarity) (light grey filled square); group IV, 9–0 bits (very low similarity) (open square). The darker line on the main diagonal shows that the higher scores (i.e. the best match), were always achieved when a tooth was compared with its negative image on the database.

confirmed absence of HSB in these teeth. Although most teeth presented HSB, 3.3% of the teeth analysed had only 0 or 1 minutia. These teeth gave low similarity scores, thereby increasing the false acceptance and rejection rates in the identification process.

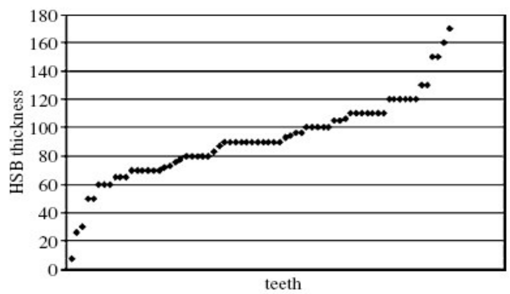

Figure 4. Average thickness (micrometre) of HSB in 70 human lower incisors.

Different from fingerprint, the average thickness of HSB are variable, and this feature can be used as an additional parameter for automated identification (figure 4). After the use of two automated systems the identification of the few remaining teeth with low similarity scores can be made by simple visual comparison. This work can be performed by individuals without much special training (see figure 5 of electronic supplementary material).

HSB were clearly observed in the enamel of isolated teeth heated at temperatures of up to 300 °C for 1 h (see figure 6 of electronic supplementary material). Above this temperature enamel becomes opaque, probably due to the incorporation of smoke residues resulting from the burn of organic matrix. This hinders the diffusion of the directed light throughout HSB.

4. DISCUSSION

Teeth have been extensively used as a source of information in human identification, especially when the soft tissues cannot provide reliable information (Stefen 2001; Holland *et al.* 2003). We show here that HSB can be observed and recorded directly from the mouth (figure 1) and from isolated teeth or even in tooth fragments collected in the field. Modern life is characterized by the concentration of large populations in urban areas with the gathering of people in huge office buildings, schools, theatres and mass transportation systems. Unfortunately, with that comes an increase in the necessity of new and diverse methods of forensic investigation to identify the victims of mass incidents and perpetrators of terrorist acts. The attacks on the World Trade Centre towers on September 11, 2001 set a dramatic example. This event represented the single largest terrorist-related mass fatality incident in the history of the United States. More than 2700 individuals of varied racial and ethnic background lost their lives that day. Through the efforts of thousands of citizens, including recovery workers, medical examiners and forensic scientists, the identification of only approximately 1500 victims had been accomplished through June 2003 (Holland *et al.* 2003). Toothprint is a highly robust biometric-based procedure for personal identification that could be useful in mass disasters. The analysis of HSB pattern or toothprint may complement fingerprint and DNA as well as others methods of identification. Accordingly, toothprint recording would be specially recommended for individuals working in dangerous occupations such as firefighters, soldiers, jet pilots, divers and people that live or travel to politically instable areas.

HSB withstand extreme environmental conditions and are preserved after skin decomposition. HSB could be clearly observed and recorded in teeth burned at temperatures of up to 300 °C for 1 h. Experimental evidences indicate that teeth can withstand much higher temperatures in burned corpses. The preservation is achieved due to the protective effect of the tongue, mastication muscles and other soft tissues in the face and mouth (Valenzuela *et al.* 2000). Furthermore, well-preserved HSB have been observed from the outside in fossil teeth dating from several hundred thousand to over 60 million years (Koenigswald 1994; Ferretti 1999; Stefen 2001; Line & Bergqvist in press). Toothprint analysis is non-invasive, accurate and can be readily performed in automated systems. Toothprint is, therefore, a suitable physiological biometric trait for human personal identification and verification.

5. UNCITED REFERENCES

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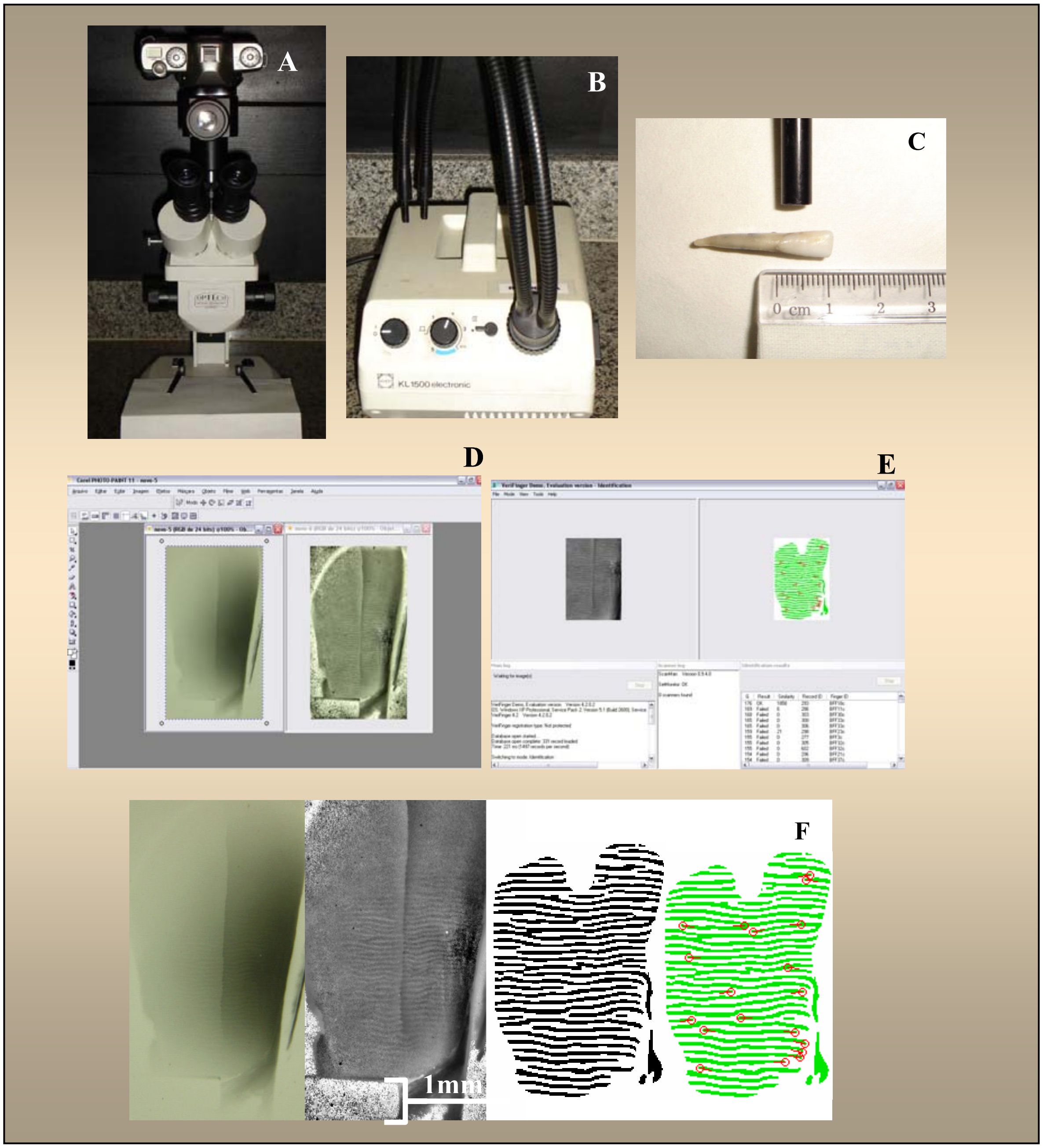
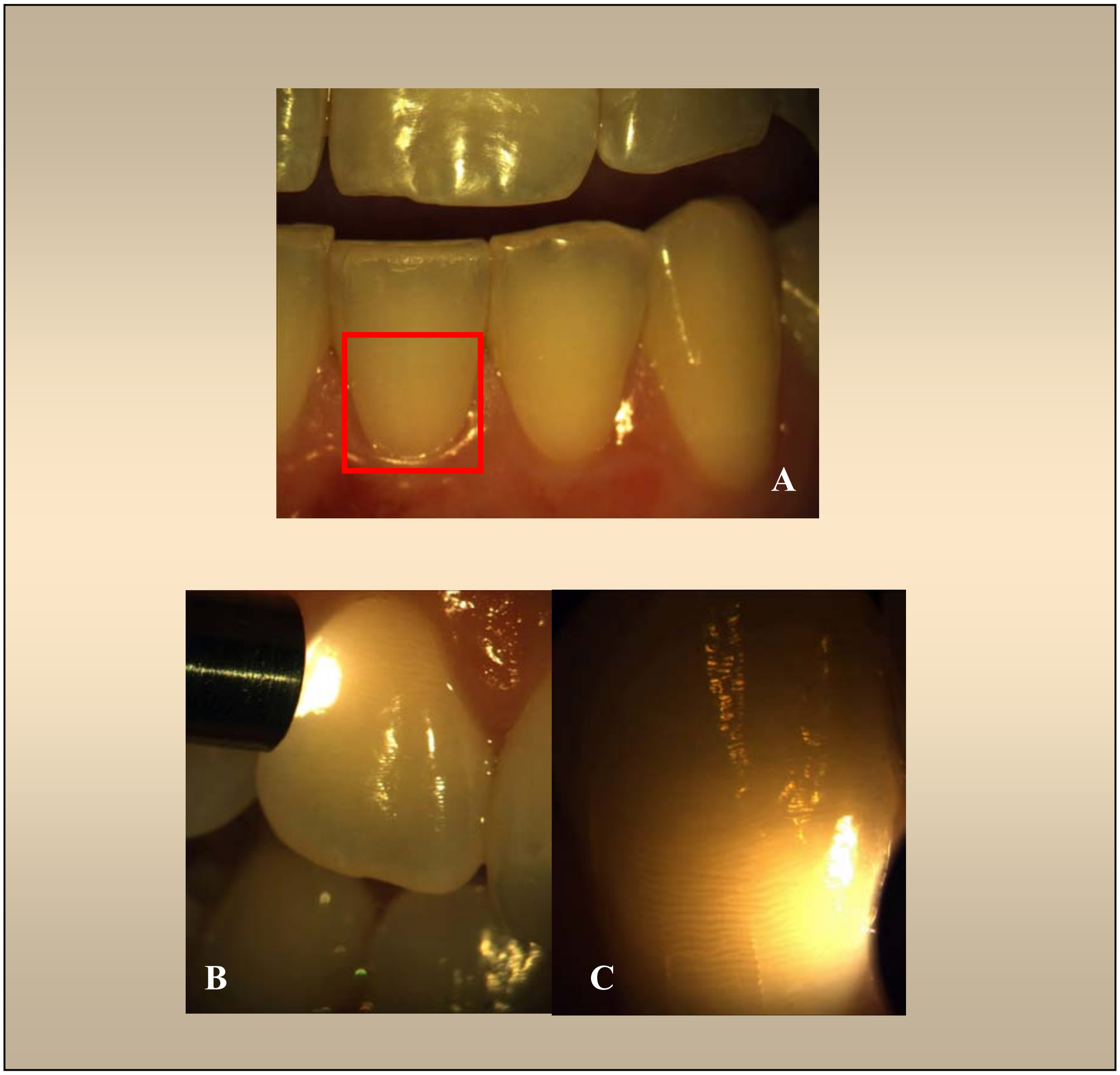
Anexo 3



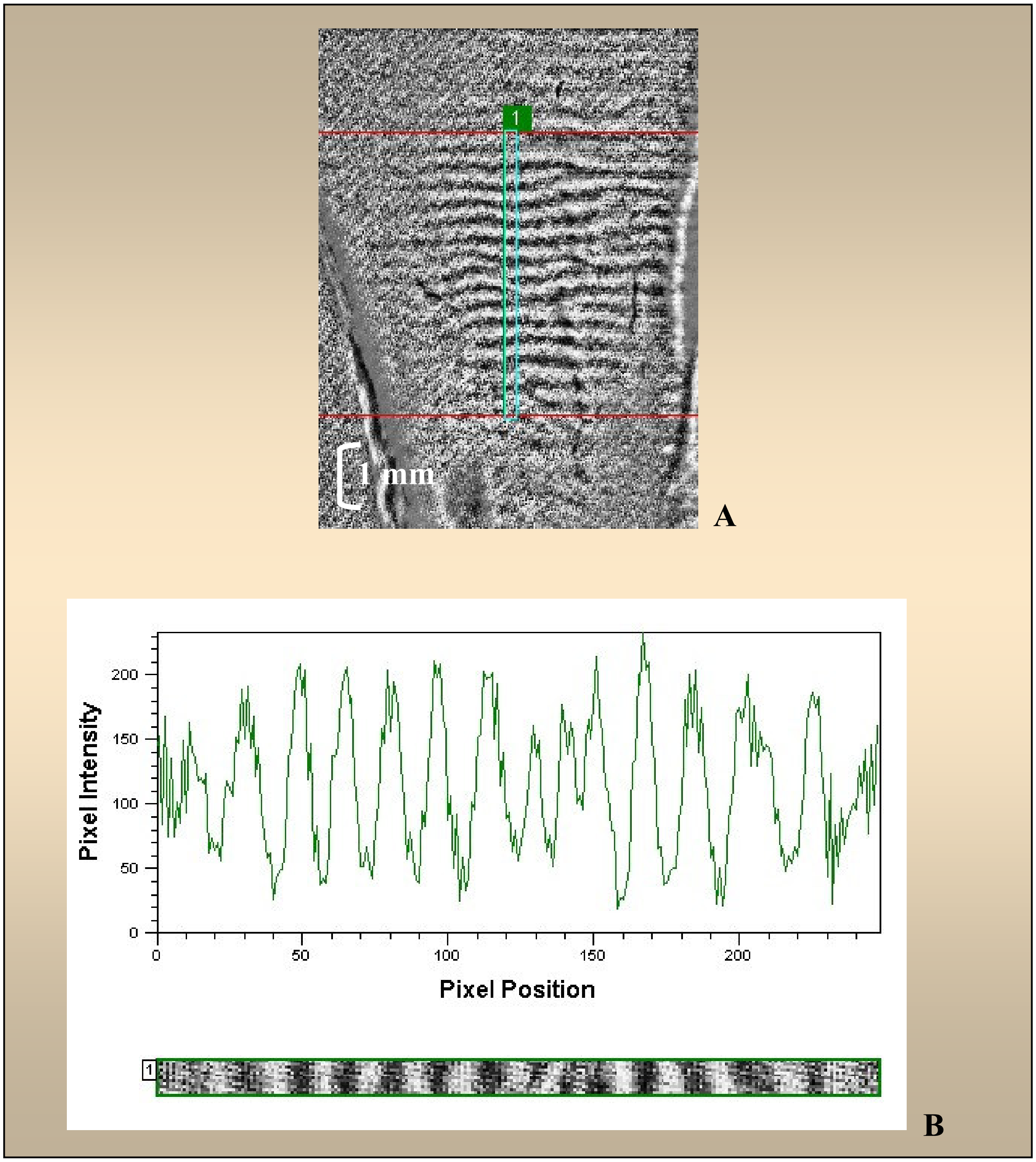
Obtenção das imagens em dentes isolados. **A e B.** Observação e captura de imagens das HSB com câmera Pentax MZ-M® e lupa esteroscópica (aumento de 10 a 20x). **C.** iluminação oblíqua mesial ou distal diretamente no dente (Fiber Light®), delimitação da área de observação (± 6mm^2) **D.** Aumento de contraste das imagens digitais com programa Corel Photo Paint 9 (Corel Corporation ®). **E.** Extração das imagens contrastadas no programa Verifinger Demo 4.2 SDK / Fingersec®. **F.** Da esquerda para direita: negativo, contraste, extração no programa Verifinger, visualização das minutias.

Anexo 4



Captura das imagens das HSB *in vivo*. **A.** Região mesial vestibular fotografada (± 6,0mm^2). **B.** Iluminação focalizada com a fibra óptica (Fiber Light® - diâmetro 1,0mm a 5,0mm) na área desejada em ambiente escuro. **C.** Visualização das bandas em maior aumento.

Anexo 5



Análise da espessura média (μm) da HSB, uso do software ImageMaster TotalLab version 2.00 (Amersham Pharmacia Biotech®). **A.** Delimitação da área das bandas (porção central do incisivo inferior - área 1). **B.** Área 1 analisada em gráfico, contagem dos picos de intensidade de pixels, mensuração das bandas.

Anexo 6

HSB preservadas e observadas em dente fóssil de mamífero (*Parastrapotherium*) datando cerca de 11 milhões de anos.