DL Lath
C Johnson
RN Smith
AH Brook

Measurement of stain removal *in vitro*: a comparison of two instrumental methods

Authors' affiliations:
D. L. Lath, *R. N. Smith*, *A. H. Brook*, Department of Oral Health and Development, School of Clinical Dentistry, University of Sheffield, Sheffield, UK
C. Johnson, Department of Engineering Materials, University of Sheffield, Sheffield, UK

Correspondence to:
Darren Lath
Department of Oral Health and Development
School of Clinical Dentistry
Claremont Crescent
Sheffield S10 2TA
UK
Tel.: +44 (0) 114 271 7891
Fax: +44 (0) 114 271 7843
E-mail: d.lath@sheffield.ac.uk

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Lath DL, Johnson C, Smith RN, Brook AH. Measurement of stain removal *in vitro*: a comparison of two instrumental methods

Abstract *Aim:* The aim of this study was to compare an established spectrophotometrical approach for the measurement of stain removal *in vitro* with a new digital image analysis system. *Method:* Eighteen acrylic blocks were stained by cycling them through human saliva (2 min), chlorhexidine (2 min) and tea (1 h), rinsed with deionized water and left to air dry. The absorbance of each block was then measured at 395 nm using a single-beam spectrophotometer. The lightness (*L*-value) of the stained blocks (after a baseline correction) was measured using digital image analysis. Image acquisition and *L*-values were obtained using Adobe Photoshop software. The stain removal ability of two whitening toothpastes and deionized water was tested by immersing each stained block in a test slurry (15 g paste/60 ml deionized water) for 1 min, rinsing and finally left to air dry. This cycle was repeated until the blocks had 5 min exposure to the slurry. Absorbance values from spectrophotometry and *L*-values by image analysis were obtained after each cycle. *Results:* Fleiss' coefficient of reliability for intra-operator repeatability of the image analysis system and spectrophotometry was 0.999 for both methods which shows excellent reliability. Pearson's correlation coefficients for the two methods (stain build-up) were 0.976. Test products A, B and C gave correlations of 0.962, 0.998 and 0.817 respectively (stain removal), significant at the 0.01 level. *Conclusion:* The image system is a reliable alternative measurement method validated here against spectrophotometry for stain removal *in vitro*, and can provide full colour measurement.

Key words: absorbance; image analysis; lightness; spectrophotometry; staining

Introduction

The removal of extrinsic staining on teeth caused mainly by food and drink is desirable aesthetically (1) and it is important to be able to quantify the stain removing ability of dentifrices *in vitro* and *in vivo*.

The most frequently used technique for assessing tooth stain *in vivo* is visual scoring by trained operators using stain indices such as those of Lobene (2) and Shaw and Murray (3). This approach relies on the subjective estimation of stain area and intensity on a tooth. Although it is convenient to use, it can give rise to substantial inter-operator differences.

Instrumental methods such as colorimetry and absorbance spectrophotometry have been used for *in vitro* studies (4). While these instrumental methods overcome some of the subjectivity problems associated with visual stain assessment, their disadvantages include the following: (a) contact with specimens is necessary when using colorimetry, which could disrupt the surface stain on a specimen; (b) only a small area of a sample can be analysed at a time, with multiple measurements necessary to obtain a total area assessment of the specimen; and (c) the widely used CIE Lightness values are often not given.

Digital image analysis can be utilized for assessing stain on specimens and overcomes some of the problems associated with the current subjective and instrumental approaches. The digital image analysis system, first developed for plaque quantification (5–7), has been modified for measuring the whiteness of flat surfaces and extracted teeth *in vitro* (8).

The aim of this study was to assess the suitability of this image analysis system for measuring stain build-up and removal on acrylic blocks and to compare it with absorbance spectrophotometry using a protocol utilized in previous *in vitro* staining studies (9–11).

Materials and methods

This method incorporated the original *in vitro* staining model first described by Prayitno and Addy (10).

Preparation of acrylic blocks

Acrylic blocks were cut to a size of 30 mm × 10 mm × 5 mm so they would fit into the chamber of a Cecil 1000 single beam spectrophotometer (Cecil Instruments Ltd, Cambridge, UK). Stain was built up on 18 of these blocks by cycling them through human saliva (pooled from three volunteers) for 2 min, then rinsing in deionized water for 2 min; immersion in chlorhexidine for 2 min and rinsing in deionized water for 2 min; immersion in a tea solution (1 g in 100 ml) for 1 h at room temperature, and rinsing in deionized water for 2 min before being left to air dry. The absorbance of each block was then measured using the spectrophotometer at 395 nm. The stain cycle was repeated until the blocks had an absorbance of one or more.

Stain removal

Stain was removed from the blocks by immersing them in slurries (15 g toothpaste/60 ml deionized water) of two whitening toothpastes (A and B) or a deionized water (C) control for 1 min, rinsing them in deionized water and leaving it to air dry. Six blocks were allocated to each of the three test regimes. The absorbance and lightness of each block was measured using the two measurement techniques. The soaking cycle was repeated until the blocks had been exposed for 5 min to the test slurries or water.

The image analysis system

In addition to spectrophotometry, the *L*-value of each block was assessed using the digital image analysis system.

The image analysis system consisted of a 1.5-mega-pixel 32-bit professional digital camera (Kodak Nikon DCS410, with an aperture setting of F/11 and a shutter speed of 1/10 s), giving an ISO of 100. The camera was fitted with a 90-mm high-quality Elicar macro single-reflex lens. The camera and lights were mounted on a rigid adjustable copy stand (Kaiser Fototechnik, Buchen, Germany Fig. 1). The lighting was comprised of four natural colour fluorescent tubes (Philps 'TL'20 W/05, Koninklijke Philips Electronics, Eindhoven, The Netherlands).

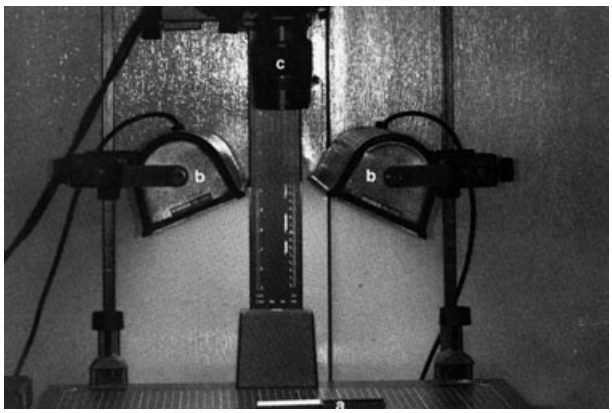

Fig. 1. The digital image analysis system showing (a) specimen position, (b) standardized lighting and (c) camera position.

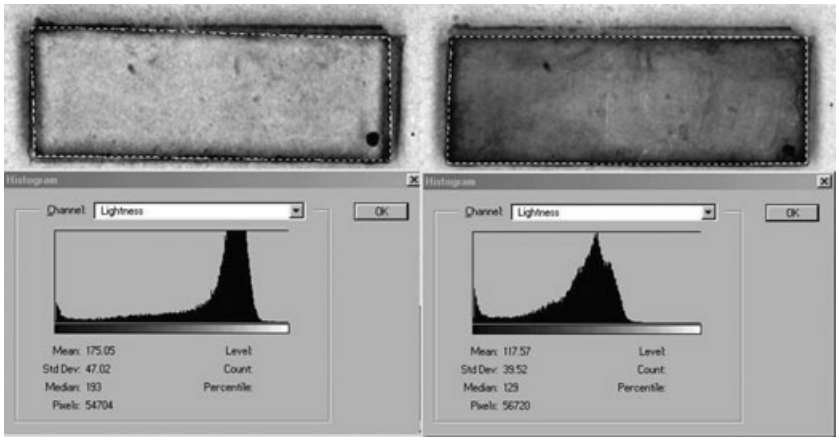

Fig. 2. Adobe Photoshop Histograms showing lightness value of a stained and unstained acrylic block.

Image acquisition

Each block, to be measured was placed on a standard grey background, ensuring the block was in the middle of the viewfinder. An image was taken of the block and transferred from the camera using a twain driver and displayed using Adobe Photoshop version 5, software (Adobe Systems, Uxbridge, UK). The images were saved as tagged image format files (TIFFs). A control block was measured with every test block giving *L*-values.

Image analysis

Each TIFF file image was examined using Adobe Photoshop software. The blocks were outlined using the marquee tool within the toolbar option of Adobe Photoshop. A histogram is then provided that gives the mean *L*-value of all the pixels within the area of interest (Fig. 2). All *L*-values were recorded on a microsoft Excel spreadsheet for further statistical manipulation.

System validation

The reliabilty and reproducibilty of the image system was tested by taking 10-l value measurements of an unstained acrylic block on two consecutive days. Ten absorbance measurements were also taken of the same block using the sepctrophotometer.

Analysis of data

Fliess' coefficient of reliability (12) was used to calculate the differences between the 20 repeat measurements of the unstained block to assess intra-operator repeatability of the image system and the spectrophotometer. Pearson's correlation coefficient was used to give evidence of validation of the image system.

Results

Fleiss' coefficient of reliability for inter-operator reproducubility for the 10 repeat measurements of the control block using the image system was 0.999. This value is in the excellent range. The same value was obtained for spectrophotometry.

Pearson's correlation coefficient for the 10 stain build-up cycles of the 12 acrylic blocks for the two methods was 0.976. This indicates a strong negative correlation (Fig. 3) significant at the 0.001 level. Pearson's correlation coefficient for the five stain removing cycles of the three sets of six blocks, comparing the two methods, and the three stain removal ability of whitening paste A and B and water was 0.984, 0.865 and 0.798 respectively. These strong or moderate negative correlations (Fig. 4) were significant at the 0.01 level.

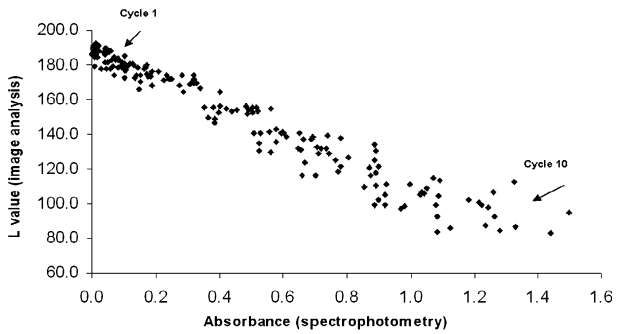

Fig. 3. Graph showing *L*-values of the image analysis system against absorbance values obtained from spectrophotometry for the 10 staining cycles of 18 acrylic blocks. As stain is built up on the blocks, *L*-value decreases, whilst absorbance increases.

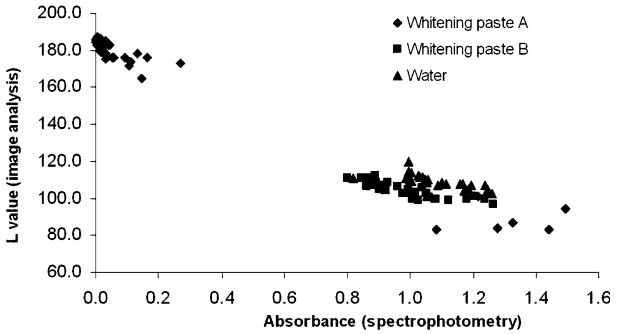

Fig. 4. Graph showing *L*-values of the image analysis system against absorbance values obtained by spectrophotometry for the five stain removal cycles of three groups of six acrylic blocks using two whitening toothpastes (a, b) and water (c). As stain is removed from the blocks, *L*-value increases, whilst absorbance decreases.

Discussion

The image analysis system has been developed to measure stain build-up and removal on acrylic blocks. The calibrated frame, type of lighting and camera positioning provided a high level of standardization of images for *in vitro* measurement.

The inter-operator result for the image analysis system (0.999) demonstrates the high level of reproducibilty this approach achieves. This supports the results from a previous study of whiteness measurement (8).

Pearson's correlation coefficient for the build-up of stain on acrylic blocks (Fig. 3) showed a strong correlation when the image analysis system was compared with the established spectrophotometrical approach, although some scattering was present towards the latter staining cycles. This was probably caused by the heterogeneous build-up of stain. The correlation for stain removal by two whitening pastes and a water control also gave comparable results, with moderate to strong correlations. A possible reason for the lower correlation for stain removal is that, in some cases, more stain is removed towards the centre of the blocks than at the edges and the spectrophotometrical approach only assesses a small area at the centre of the block whilst the image system assesses the whole area of each block and gives a mean *L*-value.

The results show that the image system is a valuable instrumental approach for *in vitro* stain removal work. The procedures are highly reproducible and provide a permanent database of images. This fascilitates analysis at a later date and other research using the same images. This method analyses the whole surface of a specimen rather than a small section and *L*, *a*, *b* colour output is still provided. Although this study used acrylic blocks for the staining model, the image system could also assess stain build-up or removal on extracted teeth and could be adapted for *in vivo* clinical use.

Conclusion

In conclusion, the results show that digital image analysis is a valid method for assessing stain build-up and removal on acrylic blocks. Further adaptation for *in vivo* studies is possible.

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References

1 Watts A, Addy M. Tooth discoloration and staining: a review of the literature. *Br Dent J* 2001; **190:** 309–316.
2 Lobene RR. Effect of dentifrices on tooth stains with controlled brushing. *J Am Dent Assoc* 1968; **77:** 849–855.
3 Shaw L, Murray JJ. A new index for measuring stain in clinical trials. *Community Dent Oral Epidemiol* 1977; **5:** 116–120.
4 Pontefract H, Courtney M, Smith S, Newcombe RG, Addy M. Development of methods to enhance extrinsic tooth discoloration for comparison of toothpastes. 1. Studies *in vitro*. *J Clin Periodontol* 2004; **31:** 1–6.
5 Brook AH, Smith RN, Elcock C, Al-Sharood M, Shah A, Karmo M. Developmental and validation of a new analysis system. In: Mayall J, Heikkinen T, eds. *Dental Morphology 1998*. Oulu, Finland: Oulu University Press, 1999, 380–397.
6 Smith RN, Brook AH, Elcock C. The quantification of dental plaque using an image analysis system: reliability and validation. *J Clin Periodontol* 2001; **28:** 1158–1162.
7 Smith RN, Rawlinson A, Lath DL, Elcock C, Walsh TF, Brook AH. The quantification of dental plaque on lingual tooth surfaces using image analysis: reliability and validation. *J Clin Periodontol* 2004; **31:** 569–573.
8 Guan YH, Lath DL, Lilley TH, Willmot DR, Marlow I, Brook AH. The measurement of tooth whiteness by image analysis and spectrophotometry; a comparison. *J Oral Rehabil* 2005; **32:** 7–15.
9 Sharif N, Macdonald E, Hughes J, Newcombe RG, Addy M. The chemical stain removal properties of 'whitening toothpaste' products: studies *in vitro*. *Br Dent J* 2000; **188:** 620–624.
10 Prayitno S, Addy M. An *in vitro* study of factors affecting the development of tooth staining associated with the use of chlorhexidine. *J Periodontal Res* 1979; **14:** 397–402.
11 Addy M, Prayitno S, Taylor L, Cadogan S. An *in vitro* study of the role of dietary factors in the aetiology of tooth staining associated with the use of chlorhexidine. *J Periodontal Res* 1979; **14:** 403–410.
12 Fliess JL. *The Design and Analysis of Clinical Experiments*. Chichester, NY, Wiley, 1986, 1–32.