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The *in vitro* and *in vivo* validation of a mobile non-contact camera-based digital imaging system for tooth colour measurement

Richard N. Smith [a], *Luisa Z. Collins* [b,*], *Mojgan Naeeni* [b], *Andrew Joiner* [b], *Carole J. Philpotts* [b], *Ian Hopkinson* [b], *Clare Jones* [b], *Darren L. Lath* [c], *Thomas Coxon* [a], *James Hibbard* [a], *Alan H. Brook* [a]

[a] *International Collaborating Centre in Oro-facial Genetics and Development, Liverpool University Dental Hospital & School of Dentistry, Edwards Building, Daulby Street, Liverpool L69 3GN, UK*
[b] *Unilever Oral Care, Quarry Road East, Bebington, Wirral CH63 3JW, UK*
[c] *Department of Oral Health and Development, School of Clinical Dentistry, University of Sheffield, 1st floor, Claremont Crescent, Sheffield S10 2TA, UK*

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ABSTRACT

Objective: To assess the reproducibility of a mobile non-contact camera-based digital imaging system (DIS) for measuring tooth colour under *in vitro* and *in vivo* conditions.
Methods: One *in vitro* and two *in vivo* studies were performed using a mobile non-contact camera-based digital imaging system. *In vitro* study: two operators used the DIS to image 10 dry tooth specimens in a randomised order on three occasions. *In vivo* study 1: 25 subjects with two natural, normally aligned, upper central incisors had their teeth imaged using the DIS on four consecutive days by one operator to measure day-to-day variability. On one of the four test days, duplicate images were collected by three different operators to measure inter- and intra-operator variability. *In vivo* study 2: 11 subjects with two natural, normally aligned, upper central incisors had their teeth imaged using the DIS twice daily over three days within the same week to assess day-to-day variability. Three operators collected images from subjects in a randomised order to measure inter- and intra-operator variability.
Results: Subject-to-subject variability was the largest source of variation within the data. Pairwise correlations and concordance coefficients were >0.7 for each operator, demonstrating good precision and excellent operator agreement in each of the studies. Intraclass correlation coefficients (ICCs) for each operator indicate that day-to-day reliability was good to excellent, where all ICC's where >0.75 for each operator.
Conclusion: The mobile non-contact camera-based digital imaging system was shown to be a reproducible means of measuring tooth colour in both *in vitro* and *in vivo* experiments.

1. Introduction

Tooth whiteness is routinely measured subjectively by a dental practitioner or other suitably qualified person to match the colour of a tooth with a corresponding shade from a commercially available colour tooth shade guide. This method of evaluating tooth colour is quick and cost-effective and is frequently used to evaluate the effectiveness of tooth

* *Corresponding author*. Tel.: +44 151 641 3980; fax: +44 151 641 1800.
E-mail address: luisa.z.collins@unilever.com (L.Z. Collins).
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whitening cosmetic products.[1–5] There are however several drawbacks associated with this subjective method, namely that the shade guides do not cover the complete range of natural tooth colour and that when ordered, the shade guides are non-linear, i.e. the perceptual whiteness between adjacent tabs is not a consistent interval.[6,7] Also, researchers have shown disagreement between dental professionals shade matching the same tooth as well as day-to-day inconsistencies with the same assessor,[8,9] although intra-assessor reproducibility can be improved with training and through experience.[10]

Instrumental analysis of tooth colour provides an objective measure. It can be attempted using spectrophotometers or colorimeters. However these instruments are not ideal for use *in vivo* as they require contact with the tooth surface, making cross-infection difficult to control. They are also complex to use *in vivo* and require the fabrication of custom positioning jigs, which can be costly and time consuming, to ensure reliable repositioning intra-orally when measuring longitudinal changes in tooth colour.[11,12] These issues have been overcome through the development of non-contact camera-based digital imaging systems.[13–19] Such systems have been successfully used to demonstrate the bleaching effects of peroxide-containing products, over time.[19,20] This paper describes the *in vitro* and *in vivo* validation studies performed to evaluate the reproducibility of a new mobile non-contact camera-based digital imaging system for measuring tooth colour.

2. Materials and methods

For increased versatility and flexibility of use, a mobile version of a non-contact camera-based digital imaging system (DIS) has been developed.[13–16,21] It comprises four 50 W SoLux D65 daylight halogen lamps (Outside-in Ltd., Cambridge, UK) with 105 mm polarising filters, positioned at 90° intervals on a purpose built metal frame. The lights are an integral part of the frame and adjustable for direction. The glass circular polarising filters are also engineered to be a single unit with the lights. The frame is fitted with eight, 4 W UV (blacklight-blue) fluorescent tube lights (Lighting Technology, Manchester, UK), at 30° angles to the neighbouring halogen lamp. The four SoLux lamps and the four UV lamps (positioned on the vertical sides of the frame) emit light which closely matches D65, daylight at noon. The number and location of lights required can be manually set using switches and then all operated together using one command switch. The camera is a Professional Kodak SLR/N 14 Mpixel digital camera (Eastman Kodak Company, Rochester, NY, USA), fitted with a 90 mm macro Nikon lens (Nikon Corporation, Japan) that is positioned in the frame, a set distance from a height adjustable chin rest and forehead guide. The camera is connected via a Belkin IEEE 1394 Fire-Wire PCI card (Belkin International Inc., CA, USA), to a Sony Viao lap-top computer (Sony Corp, Japan). Although the frame is very stable and rigid, Kodak Camera Manager software (Eastman Kodak Company, Rochester, NY, USA), is used remotely to capture images in order to avoid any possible camera shake from the operator. Collected raw data files are analysed in Adobe Photoshop CS2 version 9 (Adobe System Inc., Seattle, WA, USA). The upper central incisors are highlighted using the magnetic lasso tool to obtain values for red, green and blue (RGB) from within the highlighted region. The RGB values are converted to CIELAB and CIEWI values, using a modified version of the method described by Guan et al.,[17] or WIO whiteness index as previously described.[22,23] The algorithm described by Guan et al. accounts for the small difference measured between absolute D65 lighting values and those created by this apparatus. All values are standardised against the known CIEWI value of a ceramic white tile colour standard (Ceram, Staffordshire, UK) positioned in a tile holder which is an integral part of the chin rest. This tile is imaged at the start of each imaging session and the RGB values transformed into CIEWI values and compared to the standard within the algorithm.

This mobile unit has been fully engineered to meet the requirements of the European Union Declaration of Conformity for safety under the Laboratory Directives. The relevant harmonised (EN) standards have been applied to allow the mobile non-contact camera-based DIS to carry the ''Conformité Européene'' (CE) safety mark. The unit is designed to separate into easily transportable pieces.

2.1. *In vitro assessment*

Ten extracted incisors/canines, selected to represent a range of different tooth colours, were mounted into the headrest of the DIS using a clamp and positioned in front of a black felt background. Teeth were dry when imaged to reduce the variation in colour which occurs as teeth dry out. Images of each tooth were collected in triplicate by two different operators (operator 1: CP and operator 2: JH). The order in which the tooth specimens (samples) were imaged by each operator was randomised.

2.2. *In vivo study 1*

Male and female students and staff (aged 18–65) from the University of Liverpool Dental School who voluntarily consented to participate and signed a consent form were enrolled onto the study. The 25 suitable subjects were in good general health and possessed normally aligned upper anterior teeth without restorations. Women who were pregnant or nursing were not included. Liverpool Paediatric Research Ethics Committee reviewed and approved the protocol, including the information sheet and informed consent. By consenting to participate, subjects agreed to return to the study site on four consecutive days and have their teeth imaged by one operator in order to measure subject day-to-day variation. On one of the four test days subjects remained seated in front of the DIS while three different operators (operator 2: JH, operator 3: TC and operator 4: CJ) collected duplicate images of the subjects teeth to measure inter-operator reproducibility and intra-operator repeatability. To collect the images subjects wore sterile cheek retractors, protective goggles and placed their chin on the DIS chin rest and forehead against the forehead rest. The operator manually focussed the camera before collecting the images.

2.3. *In vivo study 2*

The protocol, information sheet and informed consent for this study were reviewed and approved by Unilever Research

and Development Research Ethics Committee. Adult males and females aged 18 years or older from the Wirral area, UK, were invited to participate in this study. To be considered suitable, all subjects had to be in good general health. Women who were pregnant or nursing were not included. All subjects had an oral examination and were required to have healthy oral soft and hard tissues and two normally aligned natural upper central incisors, free from restorations visible from the labial surface.

The 11 suitable subjects returned to the test site twice a day (once in the morning and once in the afternoon) on three separate test days within the same week. Subjects arrived at the test site in groups of three and were given sterile plastic cheek retractors and protective eye goggles to wear. Each of the three subjects were imaged by three different operators; operator 1: CP (who participated in the *in vitro* test), operator 4: CJ (who also participated in the *in vivo* test 1) and operator 5: LC. The order in which the operators imaged each subject was randomised and subjects swapped between operators to obtain a measure of reproducibility on the repositioning of the subjects using the head and chin rest.

To collect an image, the subject placed their chin on the DIS chin rest and forehead against the forehead rest. The operator manually focussed the camera before collecting the images. The DIS operator collected one digital image of the subject's teeth after the lights had been illuminated for a fixed time. The image was checked for quality, and saved. If the first image was out of focus, due to patient movement or operator error, additional images were captured.

2.4. Statistical analysis

All data was processed in JMP statistical software version 6 (SAS Institute Inc., NC, USA). In order to assess operator precision, '*u*' and '*v*' were calculated where: '*u*' is the sum of the averages of the readings for each pair of operators and '*v*' is the difference between the averages of the readings for each pair of operators. Operator precision is classed as good if there is no correlation between u and v. To assess operator agreement and measurement reliability, pairwise correlations were first calculated and tested for significance (where $p < 0.05$). Concordance correlation coefficients (ρ_c) were then used to evaluate the degree with which the pair of operators fitted on the 45° line through the origin. Perfect operator agreement is achieved when measurements are not only highly correlated (generating significantly high pairwise correlation coefficient) but lie along the line of equality (indicated by ρ_c, that ranges from '0' to '1'. The larger the deviation from the line of equality, the smaller the value of ρ_c).[24]

To assess day-to-day variability within the *in vivo* data sets, a one-way analysis of variance (ANOVA) was performed for each operator in which subject was included as a random effect and day was viewed as measurement error. From the ANOVA results the intraclass correlation coefficient (ICC) was calculated. The ICC assesses the rating of reliability by comparing the variability of different ratings of the same subject to the total variation across all ratings and all subjects.[25]

3. Results

3.1. Inter-subject/sample variability

Within all data sets the largest source of variation was inter-subject/sample variability. The mean CIELAB and WIO whiteness index values are shown in Table 1.

3.2. Inter-operator variability

All calculations for operator precision showed no correlations between u and v values, demonstrating good operator precision. Pairwise correlations and ρ_c values were all >0.7 (Table 2) for operator comparisons. All comparisons for *in vitro* data and *in vivo* study 1 were greater than 0.9. All values indicate excellent operator agreement and show the DIS to be reproducible for different operators.

3.3. Day-to-day variability

From *in vivo* test 1, operator 3 measured subjects on three of the four test days and operator 2 measured subjects on the other test day. Since operator precision and agreement was excellent between operators 2 and 3, the one-way ANOVA assumed the same operator measured subjects over the four test days. Calculated ICCs are displayed in Table 3 and show that day-to-day reliability ranged from good to excellent, where all ICC's where >0.75 for each operator.

4. Discussion

The subject-to-subject (inter-sample) variability was the largest source of variation within all data sets. For the *in vitro* study this was investigated by selecting extremes of tooth shades from a spectrum of specimens. For the *in vivo* studies, a natural variation in tooth colour is expected between different people. From the *in vivo* data, the mean CIELAB values recorded per operator using the DIS ranged from 64.81 to 67.16 for L^*, 6.56 to 6.99 for a^* and 26.30 to 29.72 for b^*. The mean CIELAB values recorded using other non-contact camera-based systems range from 54.9 to 75.63 for L^*, 4.4 to 10.57 for a^* and 16.23 to 24.68 for b^*.[26–34] The mean L^* and a^* values are within the range of those previously reported, with the mean b^* being slightly higher. This difference in the range of mean b^* is most likely due to differences in designs between the other non-contact camera-based digital imaging systems, which will lead to variations in lighting.[35] The difference can also be attributed to the natural variations in the subject populations. When the mobile DIS is used in the future to measure product effects, the inter-subject variation will be accounted for in power calculations of cell size and design of the experiment.

Pairwise correlations and ρ_c values showed excellent agreement between operators from the *in vitro* and *in vivo* 1 data, where all values were >0.9. On day 1 of *in vivo* 2 study, operators 4 and 5 show excellent agreement for all measures. However the agreement between operators 1 versus 5 for a^* and 1 versus 4 for L^* and a^* is slightly lower (pairwise

Table 1 – Mean CIELAB and WIO whiteness index per operator

Study identity	Operator	Mean colour indices (S.E.)			
		L^*	a^*	b^*	WIO
In vitro	1	74.92 (1.46)	3.52 (0.33)	25.44 (2.04)	−24.46 (8.94)
	2	75.57 (1.42)	3.59 (0.33)	25.19 (2.04)	−22.40 (8.83)
In vivo 1	2	65.69 (1.11)	6.72 (0.18)	26.30 (0.51)	−53.93 (3.42)
	3	65.95 (1.00)	6.69 (0.19)	26.62 (0.54)	−54.12 (3.26)
	4	65.22 (1.09)	6.73 (0.18)	26.37 (0.47)	−55.12 (3.43)
In vivo 2 (Day 1)	1	64.82 (0.90)	6.83 (0.27)	28.20 (0.74)	−64.47 (3.63)
	4	64.91 (0.93)	6.99 (0.21)	28.52 (0.64)	−62.47 (3.54)
	5	64.81 (0.79)	6.89 (0.25)	28.40 (0.70)	−62.16 (3.26)
In vivo 2 (Day 2)	1	66.89 (0.74)	6.96 (0.27)	29.09 (0.67)	−59.21 (3.44)
	4	67.10 (0.77)	6.76 (0.27)	29.33 (0.73)	−58.99 (3.77)
	5	67.16 (0.69)	6.69 (0.28)	30.01 (0.68)	−60.49 (3.50)
In vivo 2 (Day 3)	1	64.84 (0.84)	6.56 (0.24)	29.53 (0.67)	−64.56 (3.44)
	4	65.28 (0.69)	6.58 (0.25)	29.50 (0.67)	−63.49 (3.30)
	5	65.83 (0.75)	6.65 (0.24)	29.72 (0.66)	−62.87 (3.33)

correlations and ρ_c values are <0.9, but >0.7). This could be attributed to operator 1 being unfamiliar with participating in an *in vivo* study, as their previous experience involved using the DIS to measure the tooth colour of *in vitro* specimens. On day 2 of *in vivo* 2 study, operators 1 and 4 show excellent agreement for all measures. However the agreement between operators 1 versus 5 and 4 versus 5 for a^* is slightly lower (pairwise correlations and ρ_c values are <0.9, but >0.7). Again this could be attributed to operator inexperience as this was only the second day that operator 5 had used the DIS. On day 3 of *in vivo* 2 study, all operators show excellent agreement for all measures (pairwise correlations and ρ_c values are ≥0.9).

The inter- and intra-operator variability was not a statistically significant source of error amongst the five operators used in these studies. However each time a new

Table 2 – Measure of operator agreement

Study Identity	Operator comparisons	Analysis	L^*	a^*	b^*	WIO
In vitro	1 versus 2	Pairwise	0.98	0.99	1.00	0.99
		ρ_c	0.97	0.98	1.00	0.99
In vivo 1	2 versus 3	Pairwise	0.96	0.96	0.96	0.99
		ρ_c	0.95	0.96	0.95	0.98
	2 versus 4	Pairwise	0.98	0.96	0.97	0.99
		ρ_c	0.95	0.99	0.96	0.98
	3 versus 4	Pairwise	0.97	0.99	0.98	0.99
		ρ_c	0.98	0.96	0.97	0.96
In vivo 2 (Day 1)	1 versus 4	Pairwise	0.93	0.79	0.96	0.98
		ρ_c	0.93	0.75	0.94	0.97
	1 versus 5	Pairwise	0.88	0.82	0.97	0.96
		ρ_c	0.87	0.81	0.95	0.95
	4 versus 5	Pairwise	0.96	0.93	0.99	0.98
		ρ_c	0.95	0.91	0.97	0.98
In vivo 2 (Day 2)	1 versus 4	Pairwise	0.96	0.93	0.99	0.99
		ρ_c	0.95	0.90	0.98	0.98
	1 versus 5	Pairwise	0.94	0.76	0.98	0.98
		ρ_c	0.93	0.72	0.90	0.97
	4 versus 5	Pairwise	0.98	0.85	0.99	0.99
		ρ_c	0.97	0.84	0.93	0.98
In vivo 2 (Day 3)	1 versus 4	Pairwise	0.98	0.96	0.99	0.99
		ρ_c	0.95	0.96	0.98	0.98
	1 versus 5	Pairwise	0.92	0.97	0.98	0.99
		ρ_c	0.85	0.96	0.98	0.98
	4 versus 5	Pairwise	0.93	0.98	0.99	0.98
		ρ_c	0.92	0.97	0.98	0.98

Table 3 – *In vivo* day-to-day variability expressed as ICCs per operator

Operator	Intraclass correlation coefficients			
	L^*	a^*	b^*	WIO
1	0.82	0.77	0.92	0.94
3	0.77	0.87	0.93	0.86
4	0.86	0.90	0.96	0.95
5	0.75	0.84	0.92	0.94

operator is introduced, the reproducibility of their measurements should be assessed against a previously trained and validated operator. Any inconsistencies can be reduced with further training/experience. Although inter-operator agreement was excellent, it is recommended that the operator remains constant within an experiment to remove operator variability.

The day-to-day variability was not significant and all operators showed excellent day-to-day reproducibility. For future *in vivo* studies, where product effects are assessed in a cross-over design study, day-to-day variability can be controlled by adjusting for baseline measurements on each test day.

The mobile non-contact camera-based DIS has proven reliable and easy to use benefiting greatly from the degree of engineering required to obtain CE approval. Further automation has now been achieved by using a custom built Adobe Photoshop macro which automatically calibrates the algorithm using a white tile input and rapidly provides an Excel spreadsheet output with RGB, CIELAB and whiteness values from any selected area of interest. For more detailed analysis of tooth colour a tooth surface may be automatically split into gingival, middle and incisal thirds providing independent data output values for each.

5. Conclusion

The mobile non-contact camera-based digital imaging system was shown to be a reproducible means of measuring tooth colour in both *in vitro* and *in vivo* experiments.

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Conflict of interest

Richard N. Smith, Thomas Coxon, James Hibbard and Alan H. Brook received funding support from Unilever Oral Care for the reported work. Alan H. Brook has also been a speaker for Unilever Oral Care. Luisa Z. Collins, Mojgan Naeeni, Andrew Joiner, Carole J. Philpotts, Ian Hopkinson and Clare Jones are employees of Unilever Plc.

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