etadata, citation and similar papers at core.ac.uk

Accepted Manuscript

Title: Forensic applications: Fluorescence properties of tooth-coloured restorative materials using a fluorescence DSLR camera.

Authors: Ramya Kiran, James Chapman, Alexander Forrest, Marc Tennant, Laurence J. Walsh

PII: S0379-0738(17)30036-1
DOI: http://dx.doi.org/doi:10.1016/j.forsciint.2017.01.022
Reference: FSI 8740

To appear in: *FSI*

Received date: 15-8-2016
Revised date: 15-12-2016
Accepted date: 21-1-2017

Please cite this article as: Ramya Kiran, James Chapman, Alexander Forrest, Marc Tennant, Laurence J.Walsh, Forensic applications: Fluorescence properties of tooth-coloured restorative materials using a fluorescence DSLR camera., Forensic Science International http://dx.doi.org/10.1016/j.forsciint.2017.01.022

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Forensic applications: Fluorescence properties of tooth-coloured restorative materials using a fluorescence DSLR camera.

Running title: Fluorescence properties of tooth-coloured restorative materials

Authors:

Ramya Kiran[a]
James Chapman[a]
Alexander Forrest[b]
Marc Tennant[c]
Laurence J Walsh[d]

[a] School of Medical and Applied Sciences, Central Queensland University, Bruce Highway, Rockhampton, 4701 Australia
[b] School of Natural Sciences, Nathan Campus, Griffith University, 170 Kessels Road, 4111 Australia
[c] International Research Collaborative, Oral Health and Equity, School of Anatomy, Physiology and Human Biology. The University of Western Australia, 35 Stirling Highway, Crawley, 6009 Australia
[d] School of Dentistry, The University of Queensland, UQ Oral Health Centre, 288 Herston Road, Herston 4006 Australia

Corresponding author:
Professor Laurence J Walsh,
School of Dentistry, The University of Queensland, Oral health Centre, 288 Herston Road, Herston 4006 Australia

Email: l.walsh@uq.edu.au
Phone: 0733658160

fax +61-7-33658199

ACCEPTED MANUSCRIPT

Highlights

- Fluorescence-based photography is an adjunct to recognise tooth restorations
- The method could be used in dental examination & forensic identification purposes
- VitaEnamic, ormocers and glass-ionomer cements exhibit unique emission patterns
- The intensity of fluorescence is influenced by hydration properties of the material

Abstract: The objective of this study was to compare the fluorescence properties of dry and wet samples of contemporary tooth-coloured restorative materials using a fluorescence based DSLR camera and a variety of LEDs emitting different wavelengths of visible light as excitation sources. The materials examined included resin composites; ceramics and hybrid restorative materials such as ormocers, Vita Enamic™ and resin reinforced glass-ionomer cements. The levels of fluorescence for each sample under different combinations of incident light wavelengths and filters was analysed by using histogram data for colour channels from Adobe Photoshop software. Fluorescence patterns were influenced by water sorption of the materials. UV-A/Violet light (405±nm) produced the greatest range of luminosity values (10 to 204) amongst the tooth-coloured restorative materials, and showed the greatest differences between restorations and tooth structure. The best filter combinations with violet light were orange or yellow filters. Under ultraviolet excitation, Fuji VIII A2 exhibited a unique bright pink fluorescence emission, while VitaEnamic™, ormocer and glass-ionomer cements emitted bluish-pink fluorescence emissions. In conclusion, restorative materials exhibited varied emission pattern under UV-A (405nm) light, which enables their detection and differentiation from natural tooth structure.

ACCEPTED MANUSCRIPT

Keywords: Fluorescence, photography, composite, ceramics, LED lights, UV light

Introduction

Demand for aesthetic materials in restorative dentistry has led to the evolution of new types of tooth-coloured restorative materials, which attempt to accurately mimic the optical nuances of natural tooth structure. Various combinations of tooth-coloured materials now exist including resin modified and reinforced glass-ionomer cements (GIC), ormocers (organically modified ceramics) and hybrid ceramic materials, which are combinations of polymers and ceramic materials.

In forensic odontology, victim identification using dental records is an efficient and well-established method and may be used in combination with other means of identification. Information recorded on dental charts of restored, non-restored, missing and decayed surfaces of teeth can be used for comparison with post-mortem dental features. Additional individuation may be possible if the details of the brand and type of aesthetic restorative materials are accurately recorded in the treatment notes [1-3].

Metallic restorations are easily distinguished from sound tooth structure both by direct vision and by radiographic appearance. When restorative materials mimic the appearance of tooth structure very closely, however, this raises the challenge as to how they can be detected reliably both clinically and during radiographic examination [4,5]. Not all tooth-coloured restorations have sufficient radiographic contrast with tooth structure to allow detection on dental radiographs [6].

ACCEPTED MANUSCRIPT

Quick, accurate methods to detect their presence and to correctly classify their type and brand would be an asset for both routine clinical examination and forensic identification purposes [7,8].

Comparing the different fluorescence properties of sound and decayed teeth is a well-established method for finding early dental carious lesions, and the same method has been applied to distinguish tooth-coloured restorations from adjacent normal tooth structure [4,8 -10].

Aesthetic dental restorative materials vary considerably in their fluorescence properties, as it is difficult for them to replicate all the fluorescence properties of natural tooth structure at all wavelengths of light. Light in the ultraviolet and visible violet range has been found useful for detection of resin restorations [4, 11]. However historically, light sources used for eliciting such fluorescence were high-intensity fluorescent light sources and lasers. The latest generation of UV-emitting LEDS (Light-Emitting Diodes) have several advantages over these, including high electrical efficiency, small size and low cost, as well as long operating life. Given the potential for fluorescence to aid in recognising tooth coloured restorations, the objective of this study was to compare the fluorescence properties of dry and wet samples of contemporary tooth-coloured restorative materials when exposed to different wavelengths of visible light from LED sources. To remove the light used to excite the fluorescence, samples were viewed through coloured filters.

ACCEPTED MANUSCRIPT

The study tested two 2 hypotheses: (1) that short wavelength (UV-A/violet) light will give the greatest differentiation between different materials and between the materials and the adjacent tooth structure; and (2) that hybrid restorative materials would exhibit a recognisably unique emission spectrum different from that of all other classes of tooth-coloured materials.

Materials and methods:

In this study, a series of 27 selected tooth-coloured restorative materials and three human permanent and three human deciduous extracted teeth were included (Table 1). The restorative materials were all prepared according to the manufacturers instructions, and each was formed into the shape of a disc 2 mm thick and 10 mm in diameter, using a rigid plastic matrix. The samples were coded to de-identify them, and then stored in sealed containers at room temperature. An LED curing light (Mini LED, Acteon Satelec, Merignac, France), which emitted visible blue light over the wavelength range of 420 to 480 nm, was used in pulsed mode at a power density of 1250mW/cm^2 for curing the composite restorative materials. The prepared samples were photographed in a standardized manner with a digital single-lens reflex camera (Canon model EOS Rebel T2i/EOS 550D, Tokyo, Japan) fitted with a 60 mm macro lens in a dark environment illuminated by the various LEDs, in combination with clear, orange and yellow filters. Both the light source and the camera lens were kept at a fixed distance of 200 mm and an angle of 85 degrees from the surface of the samples. The camera was used with the following settings (speed ISO 400, aperture F2.8, and shutter speed 1/30 second). The camera was set to use the Adobe RGB1998 colour space.

ACCEPTED MANUSCRIPT

Images were recorded using 8 bits per colour channel (16,777,216 colours) with an image size of 18 megapixels, using a constant white balance setting of white fluorescent light with white balance correction set to zero. The samples were imaged with the incident light rays being perpendicular to the surface of samples (Fig. 1).

A programmable multi-colour LED array was used with a remote controller (model 1R-1627S, Tristar, Colour Stars Inc, Irvine, CA) to deliver the chosen wavelengths to illuminate the samples. From longest to shortest, the wavelengths were red (670 nm), orange (635 nm), yellow (585 nm), green (535 nm), cyan (470 nm), royal blue (450 nm), blue-violet (430 nm) and violet/UV-A (405 nm). Each light source had a spectral bandwidth of 30 nm. Samples were photographed with and without the use of filters (clear, yellow, or orange).

In order to assess the effect of water sorption on the fluorescence emissions of the various tooth-coloured restorative materials, each sample was first photographed in the dry state, then stored in distilled water for eight weeks at room temperature, and photographed again. Additional readings were then made after the samples had been returned to dry storage for a further 60 days. Extracted human permanent teeth were included as positive controls so that fluorescence patterns could be compared with the natural enamel of human teeth. The use of extracted teeth for this study was approved by the institutional human research ethics committee (approval number H15/03-035).

ACCEPTED MANUSCRIPT

Statistical analysis

Digital image analysis was undertaken using Adobe Photoshop™ Creative Cloud 2014 software, applying the histogram tool to collect colour channel data for each sample. The software was set to the RGB 1998 colour space to match the camera setting. Values varied from 0 to 255 for each colour channel data as the images were recorded in 8-bit colour.

The differences in fluorescence for each sample under different combinations of incident light wavelengths and filters was analysed using the mean and standard deviation values for a constant sample area of 40,000 pixels, which corresponded to a sample area of 53 mm^2. The mean values for luminosity were used for statistical analysis. Luminosity refers to the brightness of the object, which is the result of both fluorescence and reflectance phenomena. Appropriate filters can remove the reflected light and hence all the luminosity measured arises from fluorescence emissions.

Analyses were undertaken to show the influence of material type, variations due to differences in shades for the same material, and differences from natural tooth enamel. The statistical analysis for a given material compared the influence of moisture (dry versus wet samples), the choice of wavelengths of light used for excitation, and the effects of filters. Analyses were undertaken using ANOVA or repeated measures ANOVA as appropriate, with post-hoc Bonferroni tests. The threshold for significance was set at $P < 0.05$.

ACCEPTED MANUSCRIPT

Results:

*Effect of excitation wavelength and the effect of applying filters***:**

After imaging all the samples and extracted teeth selected for this study, using the complete array of LEDs ranging from 405nm to 670nm with and without filters, it was found that the samples exhibited within a narrow range of emission spectra for a given light and filter combination with few exceptions. Yellow light excitation with orange filter showed the maximum emission ranging from 210 to 255 for all samples, while characteristically under blue light excitation (450± nm) and with orange filter, all the materials exhibited smaller luminance values in the range of 20 to 75. In general when imaged under the orange filter most of the materials exhibited less emission spectra in comparison to without filter and the yellow filter for a given excitation light. It was also observed that the UV-A/Violet light (405 ± nm) produced the greatest range of emission spectra (10 to 204) among the tooth-coloured restorative materials and in comparison with tooth structure (Fig-1b). The analysis of variance test also showed statistically significant variation in fluorescence emission pattern by the restorative materials, a variance value of 6605 for UV-A/Violet light + the orange filter and 5560 for UV-A/Violet+ the yellow filter when compared to other combinations of light and filter which ranged from 149 to 1130 (Table 2). In comparison with UV-A/Violet + the yellow filter, UV-A/Violet + the orange filter showed the maximum differentiation visibly (Fig 2a, 2b, 2c and 2d) and statistically, which confirms the first of the study hypotheses: that short wavelength (UV-A/Violet) light will give the greatest differentiation between different materials and between the materials and the adjacent tooth structure. The fluorescence emission spectra plotted for each

ACCEPTED MANUSCRIPT

light wavelength and filter clearly demonstrated greater variation for UV-A/Violet light as compared to other wavelengths (Fig 3a and b). The repetitive data analysis for mean luminosity values of dry samples, which were re-recorded after 2 months, statistically did not show much variation ($p>0.05$) see (Table 3).

Material type by classification:

The restorative materials included in this study can be grouped into three categories of material type: resin composites, hybrids, and ceramics. Ceramic restorative materials very characteristically exhibited the lowest luminosity intensities under UV-A/Violet + orange filter (15) and blue + orange filter combination (44 to 63) Fig 4c. Interestingly, when illuminated with UV-A/Violet light and imaged with the orange filter, the hybrid materials such as VitaEnamic™, ormocers and resin modified glass-ionomer cements exhibited very low luminance values (Fig 4a). This addresses one of the hypotheses of this study, which was to determine whether hybrid restorative materials would exhibit a recognisably unique emission spectrum different from that of all other classes of tooth-coloured materials. VocoAdmira™, an ormocer, had low emission where as Admira Fusion™ which is a newer ormocer from the same brand showed high fluorescence emission i.e. around 234.63±. Among the GICs, Fuji-VIII A2 had the highest emission peak and Fuji-II the least. When illuminated with UV-A/Violet light and imaged with a yellow filter, Fuji VIII A2 exhibited unique bright pink fluorescence visibly, and other hybrid materials such as VitaEnamic™, ormocer and glass-ionomer cements exhibit bluish-pink emission (Figure 2a). Among resin composites, Herculite brand materials showed fluorescence emission (112 to 150), which was closer to tooth

ACCEPTED MANUSCRIPT

structure (108 to 162) when imaged under UV-A light and orange filter combination. Where as rest of the composite materials showed emission peak in a higher range (i.e. above 200) without much variation.

Shade distribution:

Considering that there are numerous shades for each material type, the study focused on selecting enamel/dentine shades and opaque/translucent shades based on the options available for that particular material. The number of available shades per brand ranged from 1- 4. Among the brands, which had both enamel and dentine shades, dentine shades exhibited reduced luminance peaks to enamel shades both in dry and wet states except for 3M Filtek, in which the dentine shade exhibited highest peak of emission under all combinations of light wavelength and filter with exception of UV-A/violet light illumination where it had the lowest emission peak (Figure 5a). Peak emission for all 6 materials under the enamel and dentine group was with yellow light + without filter combination. For brands, which had opaque shades, these demonstrated greater luminance values in both dry and wet samples, except for Vitabloc, which exhibited reduced emission spectra for dry sample of opaque shade (Figure 5c). Gradia XWT, which is an extra white product, very clearly had greater emission spectra under all combinations of light and filter in comparison to other shades of this brand.

Dry versus wet samples:

In general, all wet samples of restorative materials demonstrated reduced levels of fluorescence emission in comparison to dry samples when

ACCEPTED MANUSCRIPT

illuminated with red, orange and yellow light (670± nm to 585± nm). This is in contrast to illumination with light ranging from 535± nm to 405±nm (i.e. from green to UV-A/Violet), where the emission pattern exhibited some unique features in that, with orange filter, the wet samples showed greater emission in comparison to dry samples. For whole teeth samples this reverse pattern of emission among wet and dry samples was seen only with UV-A + orange filter and UV-A with a blue + orange filter combination (Figure 6a -6b). It was observed, however, that 3MFiltek Supreme XTE^{TM} composite material exhibited the highest level of fluorescence emission when the samples were wet, but exhibited the second lowest level of fluorescence emission when dry, in comparison to rest of the restorative materials.

Permanent Vs deciduous teeth:

Among the deciduous and permanent teeth the peak fluorescence emission did not differ significantly from one tooth to another, although an observed slight variation between posterior teeth and anterior teeth. Under all of the excitation light and filter combinations used in this study, the wet samples of teeth showed significantly reduced emission spectra in comparison to dry tooth samples with the exception of UV-A light + orange filter and blue light + orange filter combinations (see Figure 7).

Discussion

The results of this study show the usefulness of fluorescence for identification of different types of tooth coloured restorative materials. Fluorescence emissions have a longer wavelength than the excitation light source, which allows filters to be used to remove the excitation component reflecting from

ACCEPTED MANUSCRIPT

the sample surface, leaving only the fluorescence component to pass to the camera.

Natural human teeth exhibit fluorescence when illuminated under both broadband and narrow band light, with varying intensities depending on the excitation wavelengths. Ultraviolet light elicits green fluorescence while blue light elicits yellow fluorescence from healthy tooth enamel. Alterations in these patterns can be used to detect missing or decalcified tooth structure (in the case of dental caries), as well as the presence of a restorative material [11-13]. Human enamel exhibits three distinct luminescence peaks in the regions of 350-360, 405-410 and 440-450nm [14,15]. While fluorescence using excitation with ultraviolet light has been the most extensively examined, other wavelengths (including visible green and red) have also been used [16-22]. The present study indicates that there are variations in fluorescence patterns with all the wavelengths used to compare restorative materials and natural tooth structure, but the greatest differences occur for excitation at 405 nm, which is on the boundary of the UV-A and visible (violet) light spectra. Thus, the first hypothesis of this study was confirmed, since the best wavelength for discrimination of tooth-coloured restorative materials, including composite resins, ceramics and hybrid restorative materials was found to be 405 nm. This aligns with results of previous studies analyzing composite resin materials [11,23] which showed that optimal excitation wavelengths for the composite resin materials used in their studies were in the range of 365 - 380nm and 398 ± 5 nm, respectively.

ACCEPTED MANUSCRIPT

Fluorescence assessments of teeth and restorative materials are facilitated when filters are used. These remove specific wavelengths of light, blocking shorter wavelength excitation light reflected from the sample surface, but allowing longer wavelength fluorescence emissions to pass. The use of filters enables selective detection of the various colours (24). In the present study, the combinations of excitation light and three different filters were assessed. The orange filter gave the maximum variance in emission patterns for any given excitation light source. This filter blocks ultraviolet, violet, blue and cyan light. By making some materials appear darker and others brighter, such filters aid in identifying different types of tooth-coloured restorative materials. Similar benefits but of lesser scale were seen with the yellow filter. The hybrid restorative materials included in this study exhibited bright pink fluorescence when illuminated with UV-A/violet light and viewed through the yellow filter.

Fluorescence properties of materials in the mouth can be affected by the ingress of moisture as well as by degradation over time. Some studies have reported that the fluorescence properties of composite resin materials can alter as the material ages [25,26]. When the fluorescence properties of dry and hydrated samples were compared in the present study, storage in distilled water for 8 weeks at room temperature gave lower emissions. This difference could be due to several factors including greater scatter of fluorescence emissions (due to refraction by water), and quenching of fluorescence (from dissolved atmospheric oxygen in the water). There was no evidence of drift in the properties of materials when kept in the dry state over 60 days after

ACCEPTED MANUSCRIPT

having been previously immersed in water, which indicates that the effects caused by sorption of water are reversible.

Conclusion:

The present study suggests that fluorescence-based photography may be a useful adjunct for recognizing types of tooth-coloured restorations restorative materials. A fluorescence technique could be employed in routine dental examination as well as for forensic identification purposes. Certain restorative materials such as VITAEnamic, ormocers and glass-ionomer cements exhibit unique emission patterns, which makes their presence readily apparent. The intensity of fluorescence is influenced by hydration, since in general wet samples have less intense peak fluorescence emissions than those in the dry state. Further studies are needed to assess the accuracy of the fluorescence-based inspection approaches for detecting tooth-coloured restorative materials in the clinical post mortem forensic settings.

Acknowledgements:

This study was supported by grant 83-2015 from the Australian Dental Research Foundation. The authors thank 3M ESPE, Voco, One Dental and Herculite for providing some of the dental materials used in the study.

ACCEPTED MANUSCRIPT

References:

1) Sweet D, DiZinno JA. Personal identification through dental evidence: tooth fragments to DNA. J Calif Dent Assoc. 1996 May;24(5):35-42.
2) Ubelaker DH, Taphonomic applications in forensic anthropology, In: Haglund WD, Sorg MH, editors. Forensic Taphonomy: The post-mortem Fate of Human Remains. Boca Raton: CRC press Dec 13 1996.
3) Sweet DJ, Kashani S, Leavitt D, Manji T, Miao-Wan V, Peters N. Forensic dentistry: a review of its scope and application. Canadian Society of Forensic Science Journal. 1996 Jan 1;29 (3):143-53.
4) Pretty JA, Smith PW, Edgar WM, Higham SM. The use of quantitatve light-induced fluorescence (QLF) to identify composite restorations in forensic examinations. J Forensic Science. 2002 Jul; 47(4):831- 36.
5) Miller RG, Bush MA, Bush PJ. Implications of composite resin radio-opacity during dental inspection. Proceedings of the American Academy of Forensic Science, 2007 Feb 19-24;San Antonio, TX, Colorado springs, Co: American Academy of Forensic Sciences 2007
6) Chesne AD, Benthaus S, Brinkmann B. Forensic identification value of roentgen images in determining tooth-coloured dental filling material. Arch Kriminol. 1999 Mar-Apr; 203(3-4): 86-90.
7) Bush MA, Miller RG, Prutsman- Pfeiffer J, Bush PJ. Identification through X-ray fluorescence analysis of dental restorative resin materials: a comprehensive study of non-cremated, cremated, and processed-cremated individuals. J Forensic Sci. 2007 Jan; 52(1): 157-65
8) Senn DR, Weems RA, Manual of forensic odontology. Fifth ed. Taylor and Francis Group LLC: CRC press; 2013.
9) Nassau K. Color for science, Art and Technology. 1st ed. North Holland: Elsevier; 15 Dec 1997.
10) Angmar--Mansson B, ten Bosch JJ. Optical methods for the detection and quantification of caries. Adv Dent Res. 1987 Oct;1(1):14-20(Lim and Lee 2007)
11) Hermanson AS, Bush MA, Miller RG, Bush PJ. Ultraviolet illumination as an adjunctive aid in dental inspection. J Forensic Sci. 2008 Mar; 53(2):408-11
12) Lee Y-K. Fluorescence properties of human teeth and dental calculus for clinical applications. J Biomedical Opt. 2015 Apr; 20(4):040901.
13) Lefever D, Mayoral JR, Mercade M, Basilio J, Roig M. Optical integration and fluorescence: a comparison among restorative materials with spectrophotometric analysis. Quintessence Int 2010 Nov-Dec; 41(10): 837-44.
14) Matsumoto H, Kitamura S, Araki T. Autofluorescence in human dentine in relation to age, tooth type and temperature measured by nanosecond time-resolved fluorescence microscopy. Arch Oral Biol. 1999 Apr; 44(4):309-18.
15) Spitzer D, Bosch JJ. The total luminescence of bovine and human dental enamel. Calcif Tissue Res. 1976 Apr; 20:201-8.

ACCEPTED MANUSCRIPT

16) McLAREN E.A. Luminescent veneers. Journal of Esthetic and Restorative Dentistry. 1997 Jan; 9(1):3-12.
17) Zena R. Evolution of dental ceramics. Compendium of Continuing Education in Dentistry. 2001;22(12 suppl):12-4.
18) Wilson HJ. Restorative materials and ultraviolet radiation. BDJ 1969 Apr 15; 126 (8): 345
19) Benedict HC. A note on the fluorescence of teeth in ultraviolet rays. Science (New york NW) 1928 Apr 27;67(1739): 442
20) Shakibaie F, Rubinzstein- Dunlop H, Forrest AS, Meyers IA, Walsh LJ. Fluorescence as an aid to recognition of tooth coloured restorations in forensic odontology. Aust Dent J. 2012 Dec; 57(4):S34.
21) Shakibaie F, Roy G, Walsh LJ.Applications of laser induced fluorescence in dentistry.Int J of Dental Clinics 2011:3(3); 38-44
22) Walsh LJ, Shakibaie F. Ultraviolet-induced fluorescence: shedding new light on dental biofilms and dental caries. ADP 2007;18 :(6):56-60
23) C.Meller, C.Klien. Fluorescence properties of commercial composite resin restorative materials in dentistry, Dent.Mater.J. 31(2012) 916-923.
24) Borlinghaus R. Colours Count: how the challenge of fluorescence was solved in confocal microscopy. Formatex2007
25) Takahashi MK, Vieira S, Rached RN, Almeida JD, Aguiar M, Souza ED. Fluorescence intensity of resin composites and dental tissues before and after accelerated aging: a comparative study. Operative dentistry. 2008 Mar;33(2):189-95.
26) Lee YK, Lu H, Powers JM. Changes in opalescence and fluorescence properties of resin composites after accelerated aging. Dental Materials. 2006 Jul 31;22 (7):653-60

ACCEPTED MANUSCRIPT

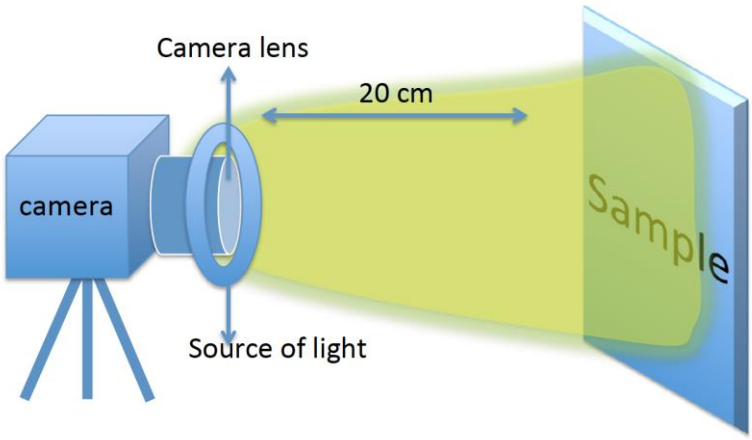


Figure 1: Schematic representation of Camera, light source and samples in laboratory

Figure 1: Schematic representation of camera, light source and samples in the laboratory

ACCEPTED MANUSCRIPT

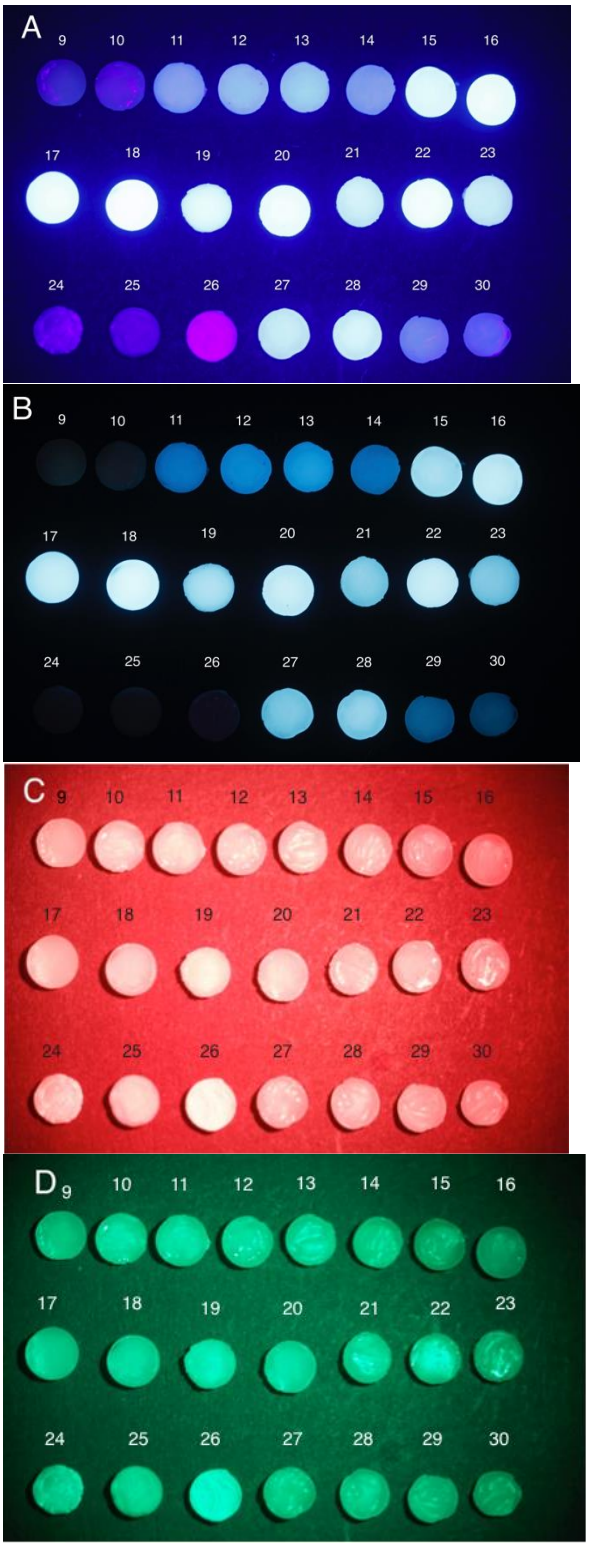


Figure 2
A: Images obtained using Canon EOS camera, following irradiation with UV-A/violet light (405nm) and using yellow filter (Shorter wavelength fluorescence emissions), **B:** UV-A/violet (405nm) light with orange filter (longer wavelength fluorescence emissions), **C:** Orange light-635nm with clear filter, **D:** Green light-535nm with orange filter. The numbers in the images represent the materials in the Table 1.

ACCEPTED MANUSCRIPT

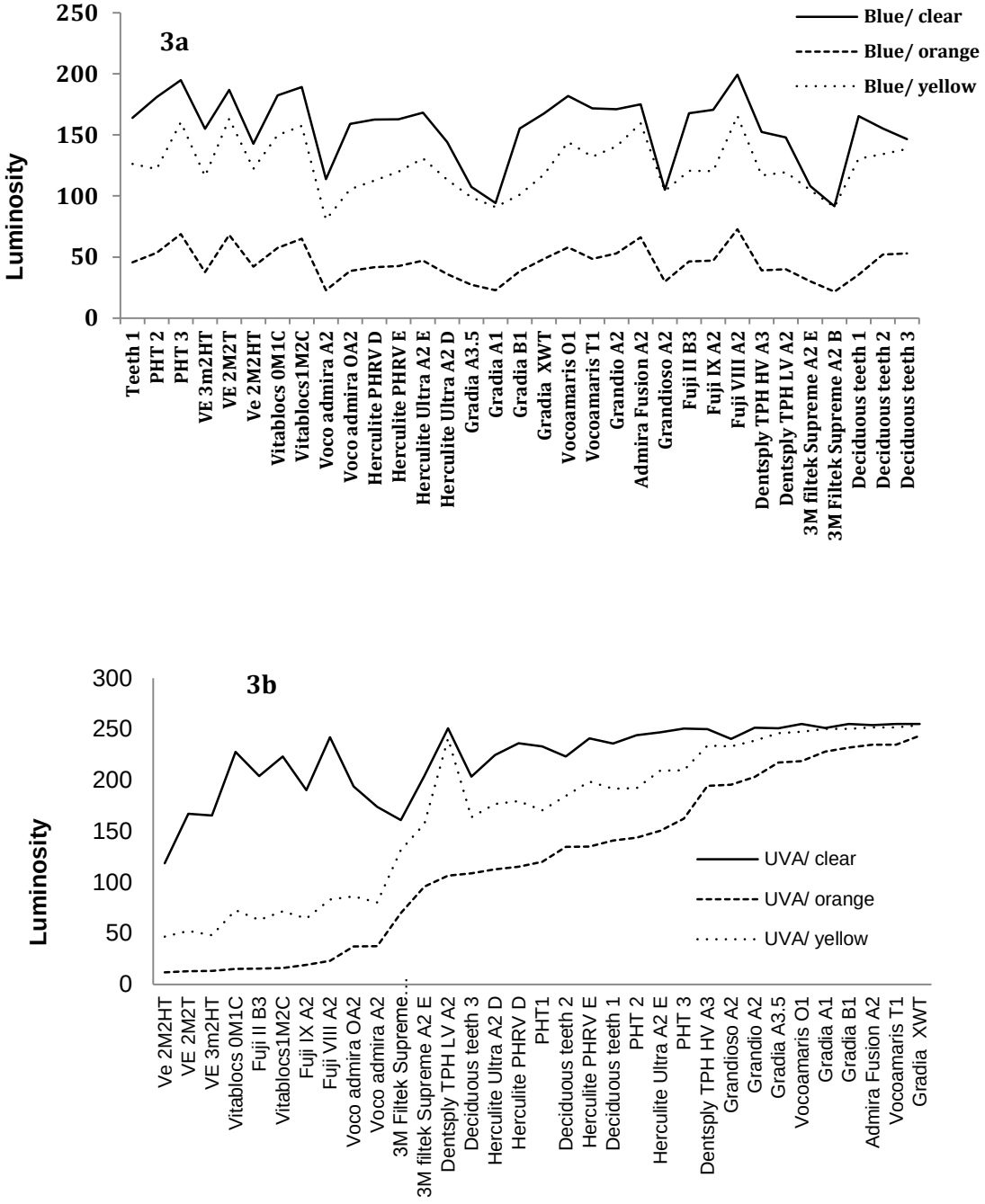
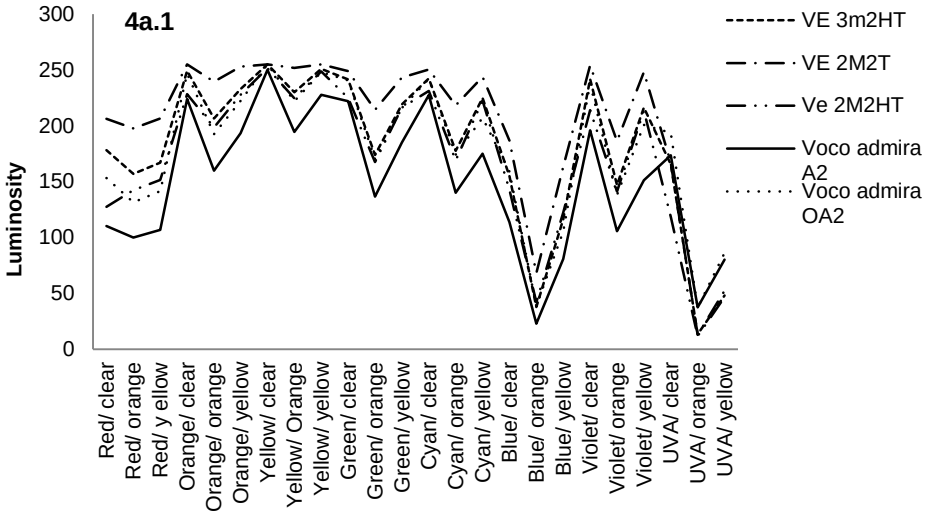
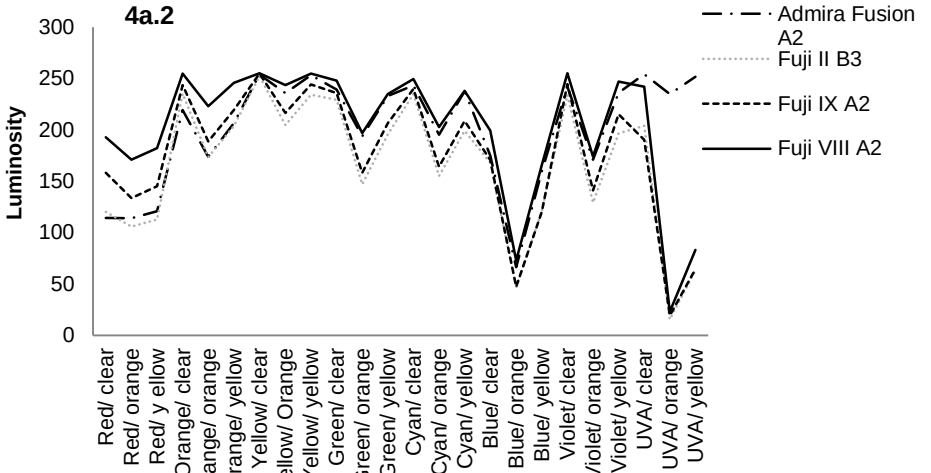
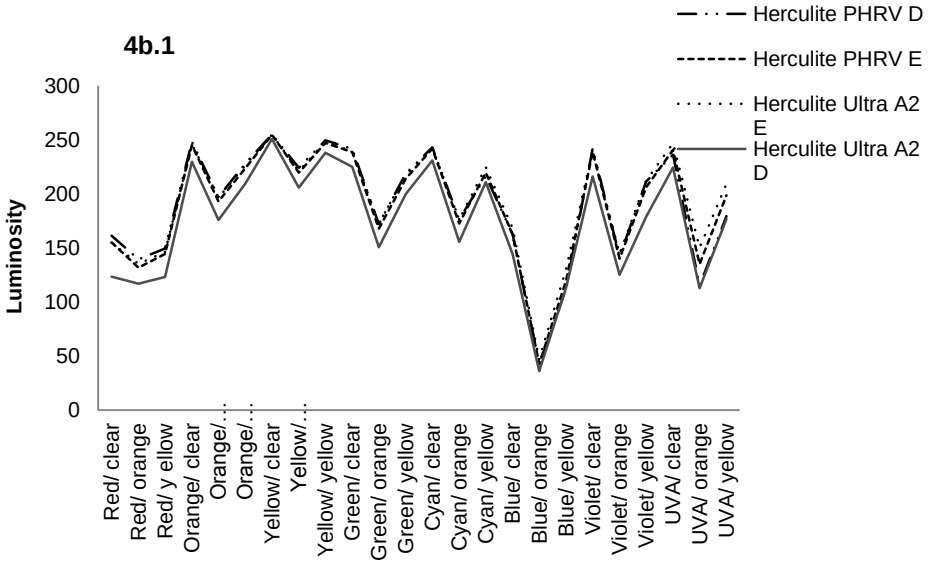


Fig.3 a Fluorescence emission of restorative materials and teeth samples when excited with blue (450 nm) light and imaged with clear filter (reflectance of light), orange (longer wavelength fluorescence emissions) and yellow (shorter wavelength fluorescence emissions) filters. **b** Peak fluorescence emission of restorative materials and teeth samples plotted in ascending order when irradiated with Ultraviolet-A light (405) nm and imaged with clear, orange and yellow filters.

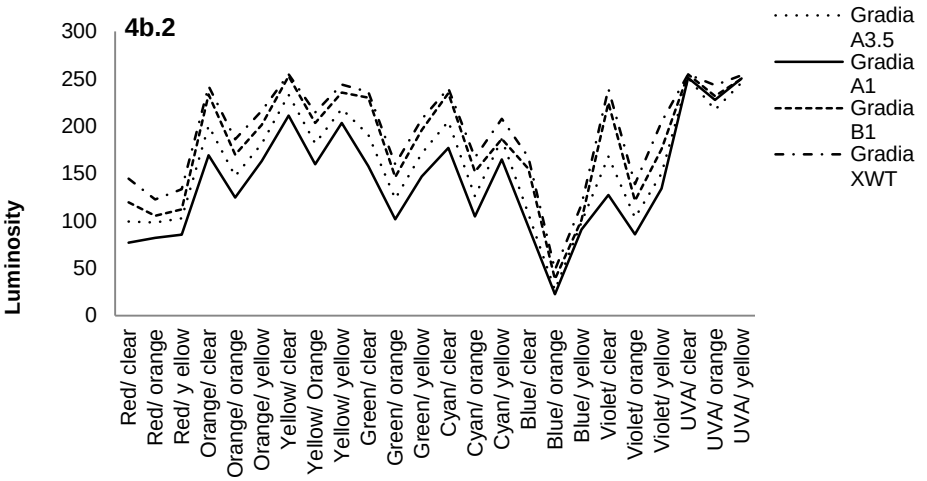
ACCEPTED MANUSCRIPT

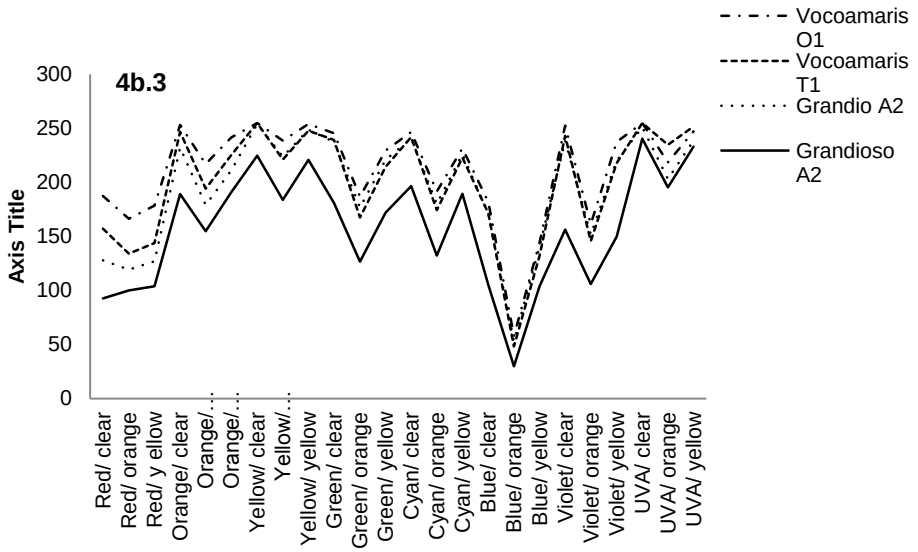


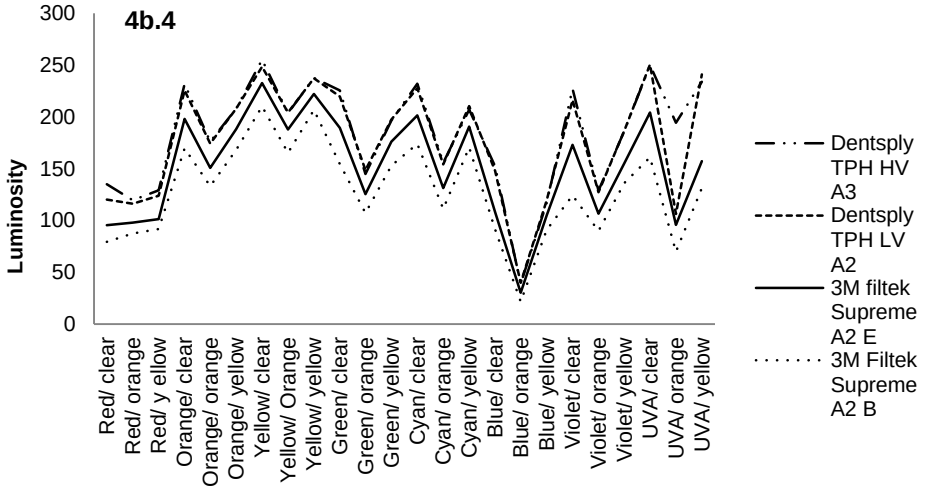




ACCEPTED MANUSCRIPT







ACCEPTED MANUSCRIPT

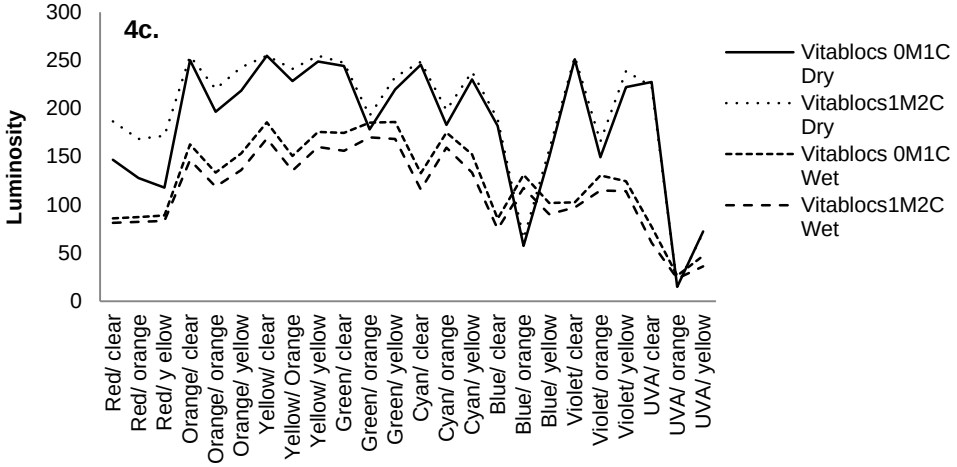
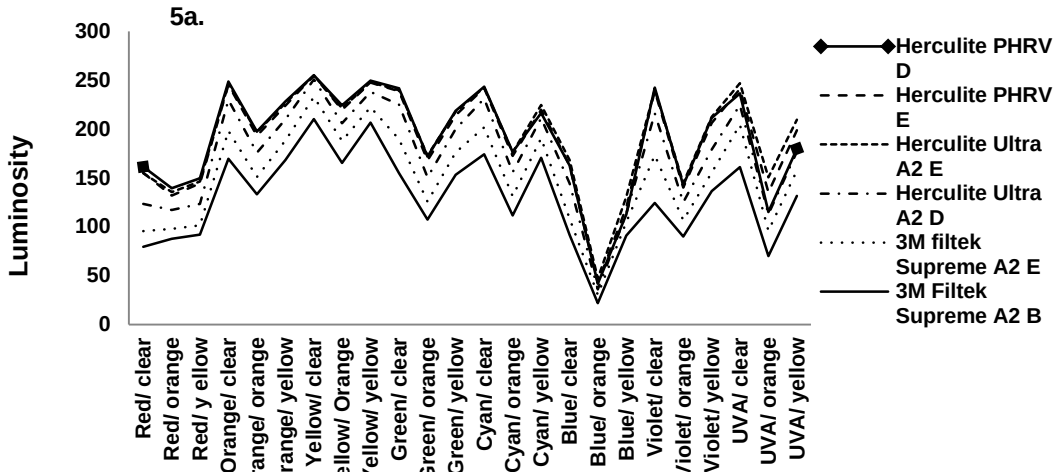
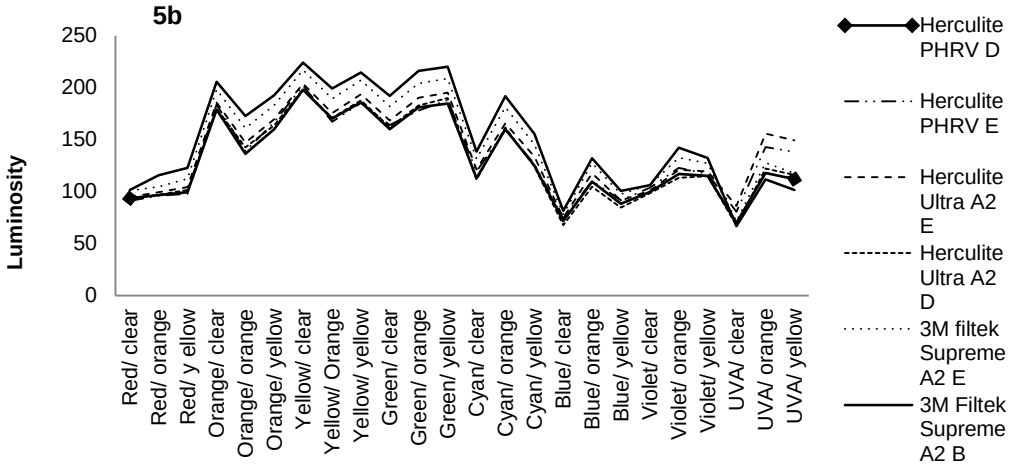
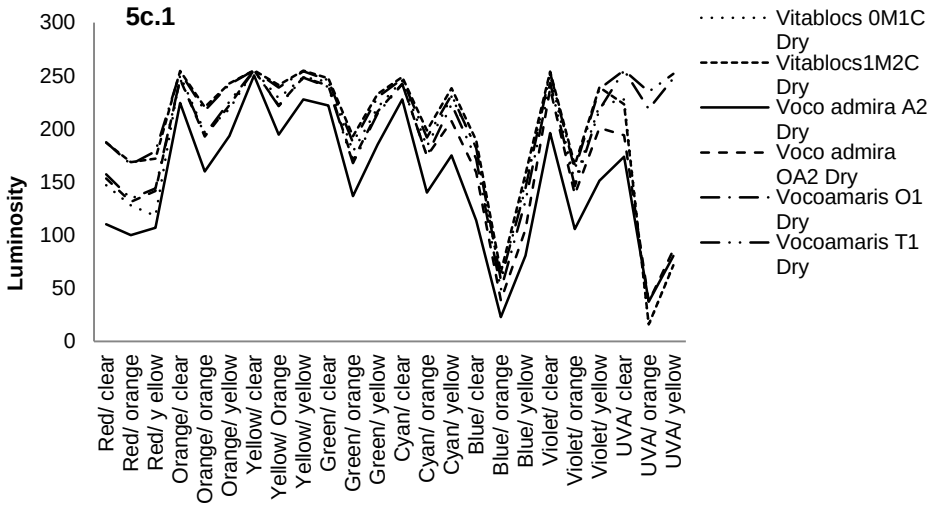


Figure 4: Distribution of peak emission according to material type when excited with red (670 nm), orange (635 nm), yellow (585 nm), green (535 nm), cyan (470 nm), royal blue (450 nm), blue-violet (430 nm) and violet/UV-A (405 nm) light and under clear, orange and yellow filters. 4a: Hybrid restorative materials, 4b: Resin composite materials, 4c: Ceramics

ACCEPTED MANUSCRIPT







ACCEPTED MANUSCRIPT

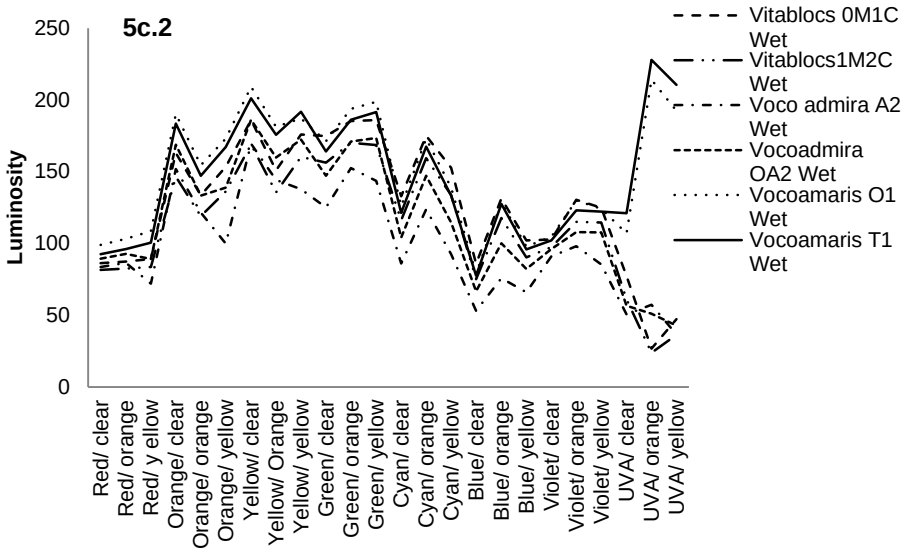


Figure 5: Distribution of peak emission according to materials shade **a:** Dry and wet samples of materials with dentine and enamel shades, **b:** Dry and wet samples of materials with opaque and translucent shades.

ACCEPTED MANUSCRIPT

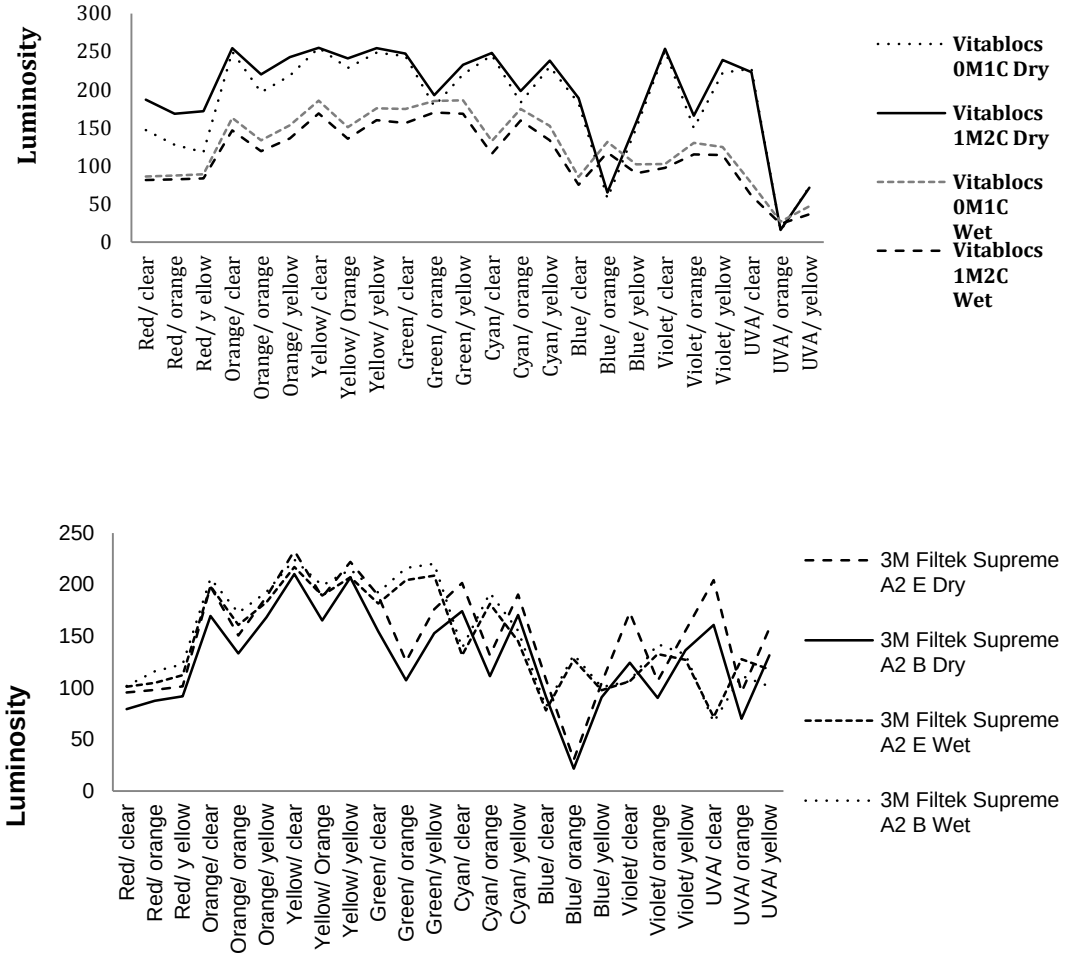


Figure 6: Fluorescence emission of dry and wet samples of Vitablocs and 3M Filtek Supreme when excited with red (670 nm), orange (635 nm), yellow (585 nm), green (535 nm), cyan (470 nm), royal blue (450 nm), blue-violet (430 nm) and violet/UV-A (405 nm) light and viewed under clear, orange and yellow filters..

ACCEPTED MANUSCRIPT

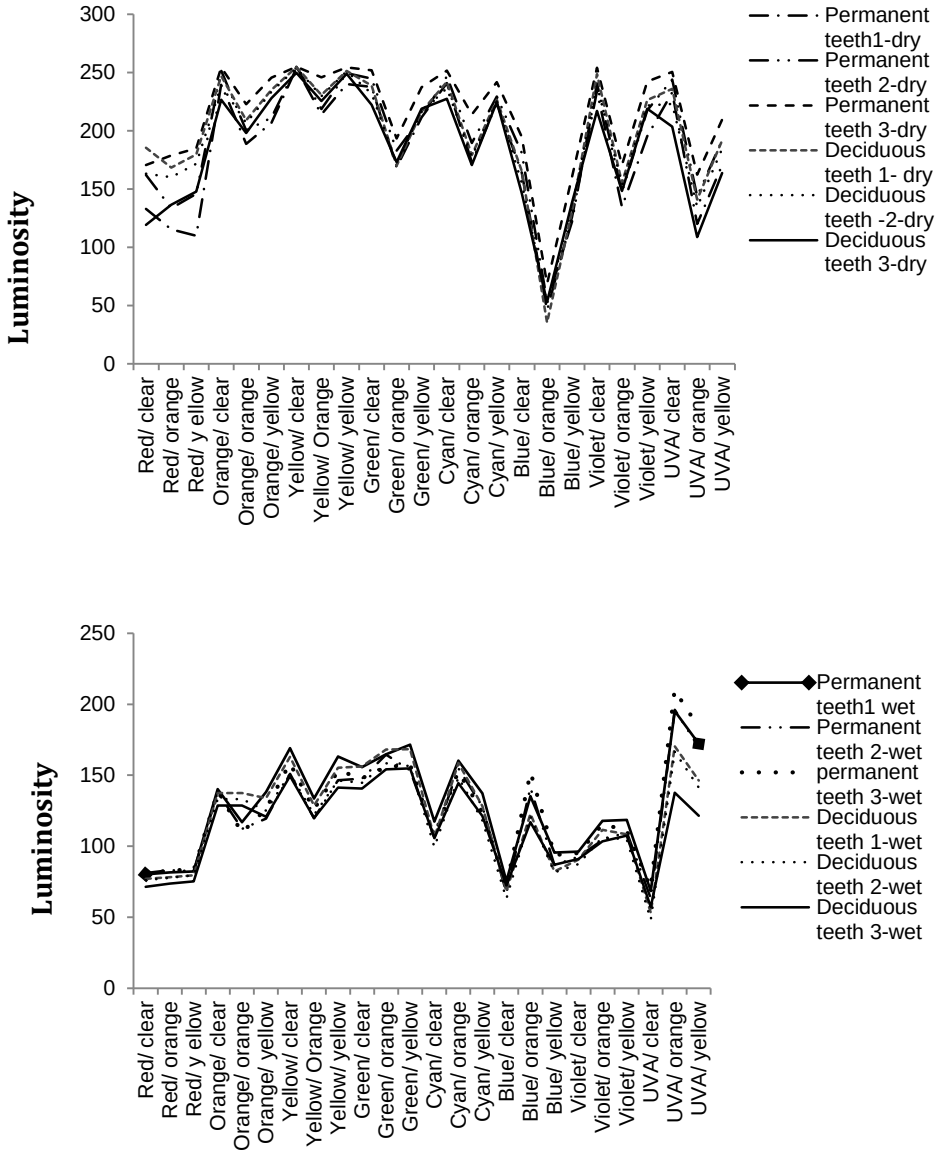


Figure 7: Peak fluorescence emission of wet and dry samples of permanent and deciduous teeth when excited with red (670 nm), orange (635 nm), yellow (585 nm), green (535 nm), cyan (470 nm), royal blue (450 nm), blue-violet (430 nm) and violet/UV-A (405 nm) light and viewed with and without filters

ACCEPTED MANUSCRIPT

Table 1: Brand names, shade and generic type of tooth coloured restorative materials used in this study

Sl No	Sample material	Shade	Generic type of material
1	Permanent Teeth-1		
2	Permanent Teeth -2		
3	Permanent Teeth-3		
4	VitaEnamic	3M2HT	Hybrid: ceramic reinforced with polymer network
5	VitaEnamic	2M2T	Hybrid: ceramic reinforced with polymer network
6	VitaEnamic	2M2HT	Hybrid: ceramic reinforced with polymer network
7	Vitablocs	OM1C	Feldspar ceramic blocks
8	Vitablocs	1M2C	Feldspar ceramic blocks
9	Vocoadmira	A2	Hybrid: Ormocers-organic modified ceramic
10	Vocoadmira	OA2	Hybrid: Ormocers-organic modified ceramic
11	Herculite XRV	D	Composite: Microhybrid filled
12	Herculite XRV	E	Composite: Microhybrid filled
13	Herculite Ultra	A2E	Composite: Nanohybrid filled
14	Herculite Ultra	A2D	Composite: Nanohybrid filled
15	Gradia	A3.5	Composite: Microfilled
16	Gradia	A1	Composite: Microfilled
17	Gradia	B1	Composite: Microfilled
18	Gradia	XWT	Composite: Microfilled
19	Vocoamaris	O1	Compoiste: Nanoreinforced hybrid
20	Vocoamaris	T1	Compoiste: Nanoreinforced hybrid
21	Grandio	A2	Composite: Nanohybrid filled
22	Admira Fusion	A2	Hybrid: Nanohybrid Ormocer
23	Grandioso	A2	Composite: Nanohybrid
24	Fuji II	A1	Hybrid: Resin modified Glass -Ionomer cement (light cured)
25	Fuji VIII	A2	Hybrid: Resin modified Glass -Ionomer cement (self cured)
26	Fuji IX	A2	Conventional Glass-Ionomer cement
27	Spectrum TPH Dentsply	A2	Composite: sub-micron filled
28	Spectrum TPH Dentsply	A3	Composite: sub-micron filled
29	3M Filtek Supreme XTE	A2E	Composite: Nanofilled
30	3M Filtek Supreme XTE	A2B	Composite: Nanofilled
31	Deciduous teeth-1		
32	Deciduous teeth-2		
33	Deciduous teeth- 3		

ACCEPTED MANUSCRIPT

Table 2

Overview of the ANOVA analysis for variance in peak fluorescence emission spectra of dry samples when illuminated with different combinations of light and filter

Light and filter combination	*Sum*	*Average*	*Variance*
Red with clear filter	4659.1	141.2	1129.5
Red with orange filter	4314.6	130.7	784.9
Red with yellow filter	4561.3	138.2	907.2
Orange with clear filter	7680.9	232.8	540.1
Orange with orange filter	6169.9	187.0	687.3
Orange with yellow filter	7118.3	215.7	473.8
Yellow with clear filter	8217.5	249.0	149.2
Yellow with orange filter	7116.8	215.7	484.5
Yellow with yellow filter	7975.4	241.7	191.7
Green with clear filter	7502.3	227.3	613.4
Green with orange filter	5369.2	162.7	676.9
Green with yellow filter	6849.0	207.5	536.7
Cyan with clear filter	7678.0	232.7	387.4
Cyan with orange filter	5544.0	168.0	712.9
Cyan with yellow filter	7063.5	214.0	442.5
Royal blue with clear filter	5140.0	155.8	831.5
Royal blue with orange filter	1497.0	45.4	187.9
Royal blue with yellow filter	4110.4	124.6	487.2
Bluish violet with clear filter	7387.6	223.9	1251.7
Bluish violet with orange filter	4578.1	138.7	574.6
Bluish violet with yellow filter	6672.6	202.2	1050.6
Ultra violet A with clear filter	7178.6	217.5	2268.4
Ultra violet A with orange filter	3998.8	121.2	6605.4
Ultra violet A with yellow filter	5532.1	167.6	5560.8

ACCEPTED MANUSCRIPT

Table 3: ANOVA repeated measures test results of two repetitive data of dry samples.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	211.2321394	1	211.2321394	0.181999588	0.669722811	3.847783877
Columns	3685300.221	22	167513.6464	144.3313252	0	1.549313996
Interaction	1692.048576	22	76.9112989	0.066267495	1	1.549313996
Within	1708430.843	1472	1160.618779			
Total	5395634.345	1517				