Characterization of natural carious lesions by fluorescence spectroscopy at 405-nm excitation wavelength

Denise Maria Zezell
Adriana Costa Ribeiro
Instituto de Pesquisas Energéticas e Nucleares (IPEN/CNEN-SP)
São Paulo, Brazil

Luciano Bachmann
Faculdade de Filosofia
Ciências e Letras de Ribeirão Preto (FFCLRP/USP)
Departamento de Física e Matemática
Ribeirão Preto, Brazil

Anderson Stevens Leonidas Gomes
Universidade Faderal de Pernambuco
Departamento de Física
Recife, Brazil

Christel Rousseau
John Girkin
University of Strathclyde
Institute of Photonics
Glasgow, United Kingdom

Abstract. We aim to characterize natural caries enamel lesions by fluorescence spectroscopy. Sixty human samples with natural noncavitated caries lesions on smooth surfaces were selected and classified into three groups: dull, shiny, and brown lesions. All the samples were analyzed externally at the natural surface and after hemisectionig internally at the center of the lesion. The lesions were excited with a 405-nm InGaN diode laser and the fluorescence was collected with a single grating spectrometer. Four emission bands (455, 500, 582, and 622 nm) are identified in both sound and carious regions. The area under each emission band is correlated with the total area of the four bands for the sound and carious regions. The detected fluorescence from natural and cut surfaces through the caries lesions is not statistically different for the shiny and dull lesion, but is different [analysis of variance (ANOVA) ($p<0.05$)] for brown lesion at all emission bands. At the 405-nm excitation wavelength, the area of the fluorescence bands at 455 and 500 nm differ statistically for natural carious lesions and sound tissue. [DOI: 10.1117/1.2821192]

Keywords: caries; teeth; diagnosis; spectroscopy; laser.

Paper 07148R received Apr. 19, 2007; revised manuscript received Aug. 16, 2007; accepted for publication Aug. 30, 2007; published online Dec. 18, 2007.

1 Introduction

Since the first differentiation between sound and carious dentine[1] or enamel[2] by fluorescence spectroscopy, the nature of emission has not been clearly understood. Several emission and excitation bands have been identified that provide a method of distinguishing caries from natural tissue by fluorescence spectroscopy.[3] The different fluorescence characteristic between healthy tissue and lesions enabled the development the Diagnodent (Kavo GmbH) device for clinical caries diagnosis.[4] More recently, new emission bands have been identified in carious enamel[5] and potentially provide a new method to distinguish caries from natural tissue[6,7] with the longer term aim of diagnosing the state of the caries lesion.

Previous work showed that the preferred excitation wavelength is[3,5] around 400 nm, with light around this wavelength providing the optimal excitation for high emission, thus improving caries detection by fluorescence spectroscopy. A diode laser with emission around 400 nm is thus well matched to the indicated wavelengths for fluorescence spectroscopy of hard dental tissues and the inherent narrow spectral emission is preferred over the broad emission bands produced by nonlaser excitation sources.

The aim of this study was to characterize noncavitated natural enamel caries lesions by fluorescence spectroscopy with a diode laser (405 nm) as the excitation source; and to compare fluorescence intensity recorded from external natural surfaces with that from the internal surfaces after the samples had been cut through the center of the lesion and with intact enamel.

2 Materials and Methods

Sixty teeth with natural noncavitated enamel carious lesions on smooth surfaces were selected from a pool of extracted human teeth stored in a saturated thymol solution (0.12%) at 4 °C. The lesions were classified clinically, by one examiner according clinical judgement into classes as dull, shiny, and brown caries lesions[8,9] with twenty samples for each group. The dull lesions refer to white spot active lesions and shiny to white spot inactive lesions.

The lesions were excited with a 30-mW, 405-nm InGaN diode laser (Nichia, Inc., Japan). The experimental setup was previously described.[7] The fiber used formed part of the handpiece from a Diagnodent device (KaVo, GmbH., Germany). It had previously been disconnected from the Diagnodent control box to exposure the input end of the excitation fiber. The Diagnodent "A" tip was fitted to the end of the handpiece. The light output from the tip was measured using a photodiode power meter (Melles Griot, Inc., USA) at 1 mW. The light was directed onto the sample under investigation and the resulting fluorescence collected back through the "A" tip and

Address all correspondence to Denise M. Zezell, Laboratório de Biofotônica, Centro de Lasers e Aplicações IPEN/CNEN-SP, Av. Lineu, Prestes, 2242, Cidade Universitária–USP, CEP 05508-900 São Paulo-SP, Brazil; Tel: (55) 11 31339370; Fax: (55) 11 31339374; E-mail: zezell@usp.br

1083-3668/2007/12(6)/064013/5/$25.00 © 2007 SPIE

handpiece via the Diagnodent collection fiber bundle (six, 400-μm fibers arranged around the central excitation fiber). All spectra were measured in a dark laboratory. The collected fluorescence light was then sent into a single-grating spectrometer Avantes S2000/PC2000 and an analog-to-digital (A/D) converter ADC1000/PC2000, (Avantes, Inc., USA). This instrument consisted of a 10-μm entrance slit and 1200 lines/mm grating, giving a stated optical resolution of around 0.4 nm in the visible portion of the spectrum. Spectra were recorded with a 3-s integration time using the background subtraction provided in the software, and then three spectra averaged to produce a final spectrum.

Initially, the probe was directed into an empty sample holder in a darkened room and a background spectrum was recorded, which was subsequently subtracted from each reading. These tests were undertaken to ensure that the spectra recorded from dental samples were due to the sample and not due to materials used in the fabrication of the optical fibers used in the probe. The laser was activated and the output from the probe tip directed onto a sample of barium sulfate (Macam Instruments Ltd., Livingston, UK) and the returned light was analyzed in the spectrometer as already described. Some fluorescence was seen below 460 nm and several peaks were noticed but as these peaks were outside the spectral region of interest used for the later analysis and were therefore ignored. We were thus certain that the origin of the fluorescence we were examining was from the dental tissue under investigation. The risk of potential complications in interpreting the spectra as a result of "ghost images" was also removed using this method. We also monitored what happened if the spectrometer was saturated due to excessive laser light, and in this case, a second spectra peak appeared at around 810 nm, caused by the second-order spectra of the laser line. We were thus certain that any spectral features observed were due to components present in the dental samples. After the dental spectra were recorded, we also measured the spectra of the thymol storage agent and again no significant features were observed in the region of interest.

Normalization was undertaken by first subtracting from each spectral point the average (background) fluorescence within the range 740 to 750 nm. Each point was then normalized such that the maximum fluorescence value a nominal unit by dividing the fluorescence at each point by the maximum fluorescence value.

The samples were all stored under thymol and then dried using tissues just prior to fluorescence examination. Initially, the samples were examined on the external surface and were subsequently sectioned along the center of the lesion, and the fluorescence was measured again perpendicular to the freshly cut surface.

The spectra were stored on a PC and analyzed using computer software (Origin, Origin Lab. Corp., USA, version 7). Four fluorescence bands were identified: 455, 500, 582, and 622 nm. For each analyzed band, the recorded background was subtracted before the area calculation. The total area under the curve of all four bands was taken as the total fluorescence of the sample, and the area under the curve of each band was divided by the total fluorescence of the sample and multiplied by 100. This mathematical procedure provided the percentage value of each band, and by evaluating the percentage emission we eliminated the variance of intensity originated from the lesion size. The percentage value for each emission band, between sound and caries regions was determined for all groups. A comparison of the percentage area of the emission bands between sound and carious regions was made for each lesion.

3 Results

In the evaluated spectral range (400 to 1000 nm) four emission bands were identified, as shown in Fig. 1. The inset of the figure shows an enlarged spectrum of each of the two bands at 582 and 622 nm. This figure illustrates typical spectra recorded from the brown lesion group when the fluorescence was analyzed internally at the center of the lesion. All spectra recorded from sound or carious enamel evaluated at external or internal surfaces consist of a broad band with a maximum at 500 nm and three others bands at 455, 582, and 622 nm. The only differences in the fluorescence intensity were observed between different lesions and sound enamel; the band positions remained unchanged.

The area under each emission band was correlated with the total area of the four bands, in sound and carious regions. The percentage area values of all the groups are shown at Fig. 2.

Tables 1 and 2 show the statistical analyses of the comparison between sound and carious tissue when the fluorescence was analyzed externally and internally for a lesion. The variance analysis indicates a significant ($p < 0.05$) difference between sound and carious tissues for each band areas.

4 Discussion

The most important factor for caries detection, and subsequent diagnosis and treatment planning, is to determine the extent of a lesion and to monitor the course of the disease in a quantitative and noninvasive manner. Most of the optical methods are based on the differences in light scattering or fluorescence between the sound enamel and the lesion, though even in the case of fluorescence measurements, differences in the detected fluorescence recorded can be related to the scattering properties of the tooth.

Scattering is more pronounced in white spot lesions than sound enamel as the structure that normally helps to guide the light into the tooth is disturbed by the disruption of the enamel prisms. The incident light thus changes direction many times in demineralized tissue, so a higher proportion of the photons are backscattered before reaching deeper layers, such as dentine.[10]

Enamel is neither chemically nor structurally homogeneous. The variations in the chemical composition, including local gradient concentration of ions, endogenous organic material, and organic acids, have strict implications with the kinetics of the de- and remineralization process. The microstructure of enamel is not uniform, the dynamic process of de- and remineralization determines mineral lost and changes in pore structure, while, the redeposition of certain ions (i.e., fluoride) promotes pore reduction in birefringent and surface zones.[11]

The inhomogeneities must to be taken into consideration in caries monitoring, as they contribute to the variation in demineralization. Variations within and between teeth analyzed by microradiography and by an optical caries monitor

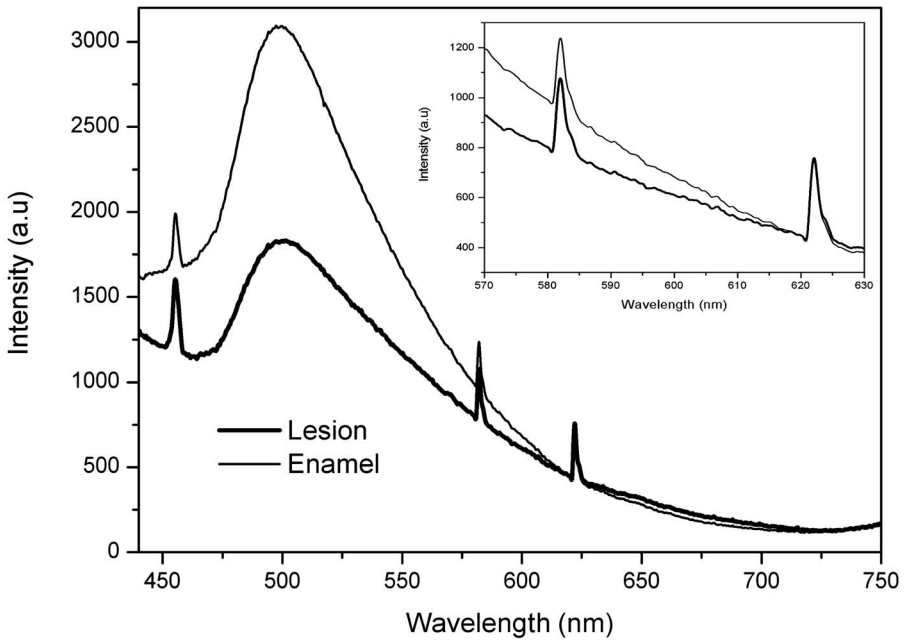

Fig. 1 Emission spectra of sound and carious tissue (brown characteristic) with 405-nm blue diode laser excitation. These spectra were recorded at the internal region of cut samples; the bands positions (455, 500, 582, and 622 nm) are the same for both internal and external irradiation and for all analyzed lesions (dull, shiny, and brown).

demonstrate a large spatial inhomogenity in calcium loss ($\cong 25\%$) within a softening, or subsurface, type lesion.[12] We suggest that the intertooth variation could be explained as a consequence of intratooth variation with position and depth of the lesions and the specific chemical composition of each tooth because it was not known whether the samples had experienced fluoridated dentifrices before our examination. These physical variations in combination with the different thicknesses of the ground off enamel layer are likely to lead to differences between any two dental spectra. The strongly linear relation between the extent of the lesion and the mineral loss from the lesion body was demonstrated by Birkman et al., specially for carious lesions *in vitro*.[13]

The origin of the natural enamel and carious enamel fluorescence is still not well understood. Benedict suggested that the organic matter is responsible for tooth fluorescence.[14] The fluorescence bands we report at about 590, 625, and 635 nm are assigned to endogenous porphyrins and the broadband near 500 nm to the natural enamel.[3] More recently, the broadband near 500 nm was assigned to Raman scattering of the tissue.[5] The other emission bands were not identified and further research is necessary to determine the origin of these bands. According our results, we would suggest that the three narrow bands can be classified as originated from the mineral matrix because of its narrow characteristic, while the broadband can be assigned to an organic structure.

All observed bands occur in natural and carious enamel; the position of the bands is unaltered by a lesion; only its intensity is changed. This observation indicates that the nature of the fluorophore is not affected by the lesion formation and may be due to changes in the relative concentration with the lesion presence, or a change in structure altering the detected level of fluorescence.

Table 1 Differences between natural tissue and lesions determined with laser irradiation at an external surface; the results were achieved by analysis of variance (ANOVA) ($p<0.05$). Only two bands (582 nm and 622 nm) do not show difference between natural and carious tissue (dull and shiny lesions).

	Lesions		
Bands	Dull	Shiny	Brown
455	Yes	Yes	Yes
500	Yes	Yes	Yes
582	Yes	No	Yes
622	No	No	Yes

Table 2 Differences between natural tissue and lesions determined with laser irradiation at an internal surface; the results were achieved by ANOVA analysis ($p<0.05$). Only one band (622 nm) does not show difference between natural and carious tissue (dull lesions).

	Lesions		
Bands	Dull	Shiny	Brown
455	Yes	Yes	Yes
500	Yes	Yes	Yes
582	Yes	Yes	Yes
622	No	Yes	Yes

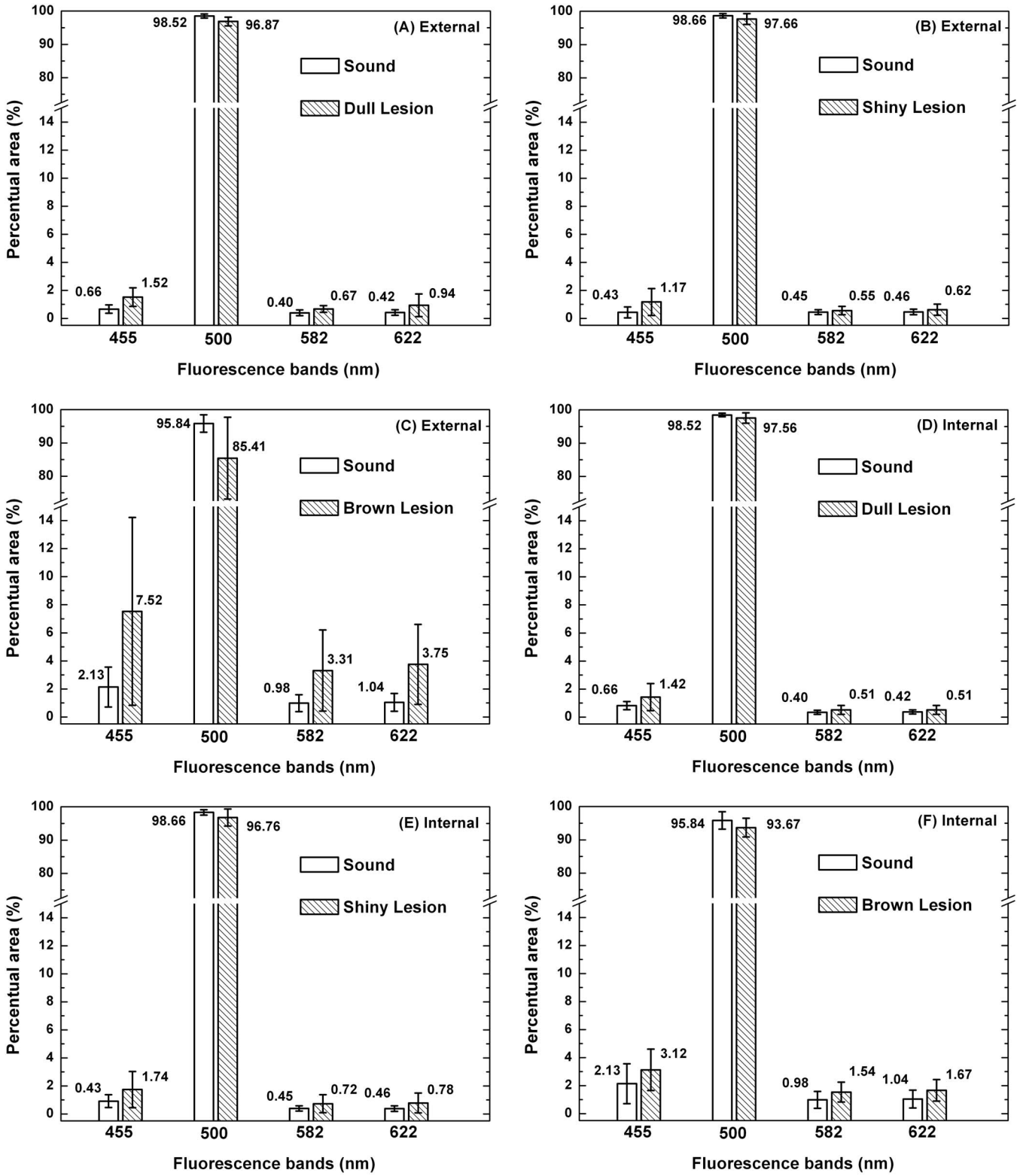

Fig. 2 Percentual fluorescence of all the four bands visualized for dull, shiny, and brown natural lesions: (A), (B), and (C) results for external irradiation and (D), (E), and (F) fluorescence obtained with internal irradiation. While the broad emission band (500 nm) intensity reduced after lesion installation, the other three narrow bands (455, 582, and 622 nm) increased.

Table 3 Fluorescence bands observed in carious lesions excited with 405 nm (blue diode laser), 400 nm (white source), and 407 nm (krypton ion laser).

	Excitation Wavelength		
	405 nm (This Work)	400 nm (Ref. 5)	407 nm (Ref. 3)
Fluorescence wavelength	455	—	—
	500	500 to 520	—
	582	—	590
	622	624, 650	625, 635
	—	690	670

Our results agree with the results observed in the literature; in the Table 3 we can observe the fluorescence bands of lesions excited with a filtered white light source[5] (400 nm) and a krypton ion laser[3] (407 nm) compared with our results recorded with a 405-nm excitation wavelength.

The band at 455 nm was observed in enamel and suspension of organic components by Spitzer[15] and is related to different levels of chromophores in enamel and in suspension. The broadband at 500 nm and the two narrow bands at 582 and 622 nm were detected. The broadband observed in this work and the literature shows a similar shape and was assigned to the natural tissue.[5] The two narrow bands at 582 and 622 nm are not exactly coincident with the bands observed in the literature. Different carious lesions show difference band intensities,[3] and this may be one of the reasons for the differences between our results and those in the literature. An additional band at 670 to 690 nm was observed in the literature but was not detected in this work, probably because the low intensity of the band.

This work for the first time used a coherent source with a better resolution than that in previous publications, which may be a reason why the new emission bands were detected. The percentual fluorescence of the three narrow band increases after lesion formation confirms their assignment to fluorophores presents in caries, while the broadband near 500 nm, which is assigned to sound enamel, decreases after caries formation.

The brown lesions show higher fluorescence for the narrow bands than the other two groups composed of dull and shiny lesions. A brown lesion is pigmented carious enamel and its higher fluorescence can be assigned to a higher absorption of the excitation wavelength at the lesion site, reducing the deep fluorescence from the sound enamel.

According the Table 1 and 2 it is possible to distinguish the lesions from sound enamel by fluorescence spectroscopy throughout two bands: 455 and 500 nm. The first band is weaker than the second and narrow, while the band at 500 nm is broader and easier to detect by a low-cost photodiode in any clinical instrument that might be developed.

5 Conclusion

We showed that it is possible to differentiate carious from natural tissue, indicating that the diode laser (405 nm) can be applied clinically to carious diagnosis. The presence of three narrow emission bands (455, 582, and 622 nm) indicates that the origin is from mineral matrix, while the broadband near 500 nm can be assigned to organic matrix.

Acknowledgments

This study was supported by the financial assistance of the Engineering and Physical Sciences Research Council (EPSRC), the British Council, Capes, FAPESP, and CNPq (Brazil).

References

1. W. G. Armstrong, "Fluorescence characteristics of sound and carious human dentine preparations," *Arch. Oral Biol.* **8**, 79–90 (1963).
2. R. R. Alfano and S. S. Yao, "Human teeth with and without dental caries studied by visible luminescent spectroscopy," *J. Dent. Res.* **60**, 120–122 (1981).
3. K. Konig, G. Flemming, and R. Hibst, "Laser-induced autofluorescence spectroscopy of dental caries," *Cell. Mol. Biol. (Paris)* **44**, 1293–1300 (1998).
4. R. Hibst and R. Paulus, "A new approach on fluorescence spectroscopy for caries detection," *Proc. SPIE* **3593**, 141–147 (1999).
5. W. Buchalla, "Comparative fluorescence spectroscopy shows differences in noncavitated enamel lesions," *Caries Res.* **39**, 150–156 (2005).
6. A. Hall and J. M. Girkin, "A review of potential new diagnostic modalities for caries lesions," *J. Dent. Res.* **83**, C89–94 (2004).
7. A. C. Ribeiro, C. Rousseau, J. Girkin, A. Hall, R. Strang, C. J. Whitters, S. Creanor, and A. S. Gomes, "A preliminary investigation of a spectroscopic technique for the diagnosis of natural caries lesions," *J. Dent.* **33**, 73–78 (2005).
8. A. Thylstrup, C. Bruun, and L. Holmen, "In vivo caries models-mechanisms for initiation ans assessment," *Adv. Dent. Res.* **8**, 144–157 (1994).
9. B. Nyvad, V. Machiulskiene, and V. Baelum, "Reability of a new caries diagnostic system differentiation between active and inactive caries lesions," *Caries Res.* **33**, 252–260 (1999).
10. J. J. ten Bosch, "Light scattering and related methods in caries diagnosis," in *Proc., Indiana Conf.*, pp. 81–88 (1996).
11. C. Robinson, R. C. Shore, S. J. Brookes, S. Strafford, S. R. Wood, and J. Kirkham, "The chemistry of enamel caries," *Crit. Rev. Oral Biol. Med.* **11**, 481–495 (2000).
12. J. J. ten Bosch, H. C. van der Mei, and P. C. Borsboom, "Optical monitor of in vitro caries. A comparison with chemical and microradiographic determination of mineral loss in early lesions," *Caries Res.* **18**, 540–547 (1984).
13. J. Brinkman, J. J. ten Bosch, and P. C. Borsboom, "Optical quantitation of natural caries in smooth surfaces of extracted teeth," *Caries Res.* **22**, 257–262 (1988).
14. H. C. Benedict, "A note on the fluorescence of teeth in ultra-violet rays," *Science* **LXVII**, 442 (1928).
15. D. Spitzer and J. J. ten Bosch, "Total luminescense of bovine and human dental enamel," *Calc. Fissure Res.* **20**, 201–208 (1976).