

Investigation of Dental Caries Using Laser and Light-Induced Autofluorescence Methods

E. Borisova¹, Tz. Uzunov², L. Avramov¹

¹Institute of Electronics, Bulgarian Academy of Sciences,
BG-1784 Sofia, Bulgaria

²Medical University, Dentistry Department, Sofia, Bulgaria

Received 5 June 2006

Abstract. The aim of this study was to investigate the intrinsic fluorescence in human teeth *in vitro* and its correspondence to the stages of the carious lesions using different excitation sources. Fluorescence spectra of teeth illuminated with light with wavelengths of 337, 440 and 488 nm were recorded. The spectra were obtained from the healthy, pre-carious and carious stages of the teeth investigated. Fluorosa dentis and odontolithiasis lesions were also studied to determine the effect of other pathologies on the teeth fluorescence spectra. We observed a significant decrease of the autofluorescence signal intensity related to the carious stage. The carious samples also revealed characteristic emission with fluorescence bands in the red spectral region which relative peak intensity increases depending on the stage. Healthy hard dental tissue exhibited no emission bands in the long-wave region.

PACS number: 87.64.Ni, 42.62.Be, 42.62.Fi

1 Introduction

Painless, instant diagnoses using lasers, LEDs and photon detectors will soon be a reality. This is a fast moving branch of science. Fluorescence, absorption, and excitation spectroscopy have been widely used as probes to acquire fundamental knowledge about physical, chemical, and biological processes. Laser-induced autofluorescence spectroscopy (LIAFS) is applied to discriminate between normal and atherosclerotic tissues [1, 2], for early detection of cancer [3-5]. Fluorescence is used to obtain information about salt concentration, pH [6], Ca²⁺ concentration [7], dynamical conformation of molecules, and structure of the DNA and RNA [8, 9]. A hematoporphyrin derivative is now used as a fluorescent marker for early cancer detection [10, 11].

Optical spectroscopy offers many ways to detect and characterize biochemical and morphological changes occurring in teeth structures. Reflectance and light

Investigation of Dental Caries Using LIAF Methods

scattering spectroscopy are utilized for investigation of tooth decay [12-14]. Optical coherent tomography is an effective imaging method for investigating the optical and structural properties of dental tissues [15, 16].

Fluorescence detection may be an alternative to caries detection by dental probe or X-ray examination. Promising results have been demonstrated using fluorescence spectroscopy with excitation wavelengths in the violet and blue spectral regions. Most of the investigations in the field of caries research have been related with quantitative laser-induced fluorescence-QLF [17-20], which detects the decrease of the fluorescence radiance (F, %) without identifying the spectral shape changes. The data on the spectroscopy of teeth are limited. A few groups have reported results on the spectral changes in the fluorescence signal between intact and carious tooth [21-24].

The objectives of this study were to determine the feasibility of applying the laser-induced and light-induced fluorescence spectroscopy technique for detection of different carious stages. The cases of the fluorosa dentis and odontolithiasis were also studied, for better determination of the influence of other teeth pathologies on the teeth autofluorescence spectra.

We investigated the autofluorescence signal at different excitation wavelengths, namely, 337, 440 and 488 nm.

A problem with using a laser as a light source for early detection of caries is the expenses associated with the operation of these types of installations. That is why we compared the capability of a cheaper excitation source – blue light-emitting diode (LED) – with the most widely used laser sources in the caries fluorescence diagnosis – the nitrogen and argon-ion laser systems.

Our results suggested that the new diode excitation source is more promising for assessing the severity of early caries lesions than the argon-ion laser generally used for quantitative laser-induced fluorescence. Besides the decrease of the fluorescence signal intensity in the case of caries, which is observed with all excitation sources, the most significant spectral changes in the fluorescence signal were obtained when the excitation source was a diode with emission wavelength of 440 nm.

These results demonstrated the promising possibility to implement an inexpensive system for detection of the pre-carious stages that could have wide clinical applications. Non-invasive sensitive *in vivo* caries detection by means of appropriate excitation sources and fluorescence detectors should thus become possible.

2 Materials and Methods

2.1 Samples

We studied forty-five incisors, thirty two premolars and fifty eight molars (extracted for reasons including caries and periodontal problems). Immediately after extraction, the teeth were fixed in formalin and rinsed out in distilled water, and sampled independently by specialist-dentist. The stage of each pathological section in the samples was determined according to ICD-10 [25]. A special study of the influence of formalin traces on the fluorescence spectra was carried out. No formalin fluorescence signals from the samples were observed. The samples were coded and kept in a proper environment. Some of the teeth investigated exhibited more than one disorder. Each pathological section in the samples was determined and was investigated by laser- or light-induced fluorescence spectroscopy. Table 1 summarizes the number of sections and the different tooth disorders detected.

Table 1. Number of tooth sections with different disorders.

Tooth condition	Number of lesions
White spot lesion	40
Brown spot lesion	34
Superficial cavity	33
Medium depth cavity	31
Deep cavitation	36
Odontolithiasis	37
Fluorosa dentis	11

2.2 Detection system

The fluorescence spectra were detected with a miniature fiber optic spectrometer (PC2000, "Ocean Optics", Inc., Dunedin, FL, USA). A high-sensitivity, 2048-element linear CCD-array detector makes the system especially useful for fluorescence and other low-light-level applications. The CCD-array detects the fluorescence light dispersed by a grating with 600-lines/mm. The spectral resolution of the microspectrometer is approximately 8.5 nm per pixel.

A computer was used to control the system and to store and display data. The spectra were stored using the microspectrometer specialized software (OOI Base, "Ocean Optics", Inc. Dunedin, FL, USA) and were analyzed and graphically represented with another computer program (Microcal Origin 5.0, Microcal Software, Inc., Northampton, MA, USA).

2.3 Laser-induced fluorescence spectroscopy of teeth (LIF I)

Two excitation sources were used to obtain the autofluorescence spectra: a nitrogen laser “ILGI-503” (Russia) (337 nm, 14 J, 10-Hz repetition rate) and an argon-ion laser “ILA 120-1” (Russia) (0.1-watt, 488 nm, CW).

The excitation and fluorescence signals were delivered via optical fibres. Ten fluorescence spectra of each tooth section were detected and averaged, five from the disorder investigated and five from an intact area of the tooth. The averaged spectrum of the healthy tooth surface was used as an indicator of the spectral changes in the lesion areas.

Schematically the LIF I experimental setup is shown in Figure 1.

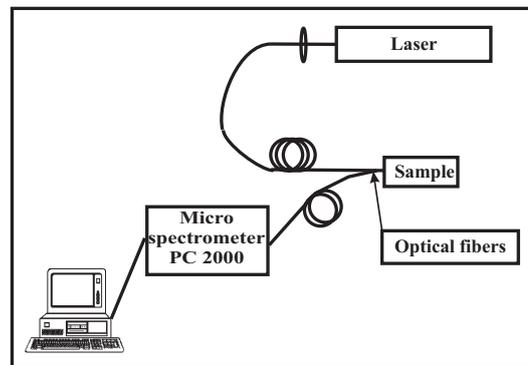


Figure 1. Schematic diagram of the LIF I system. Nitrogen and argon-ion lasers were used as excitation sources.

2.4 Light-induced fluorescence spectroscopy of teeth (LIF II)

The excitation source was an LED-matrix consisting of five blue-light-emitting diodes (max at 440 nm). The schematic diagram of the LIF II system is shown in Figure 2. A waveguide silica cone was used to deliver the excitation light to the sample; filter 1 was a 500-nm low-pass filter, rejecting the long-wave emission of the diodes.

Five fluorescence spectra were obtained and averaged for each lesion investigated through the 500-high-pass filter 2, transmitting only light with wavelengths higher than 500 nm. Other five spectra were detected and averaged originated from an intact area of the tooth. The averaged spectrum obtained from the healthy tooth surface was compared with the spectra detected from the lesion area.

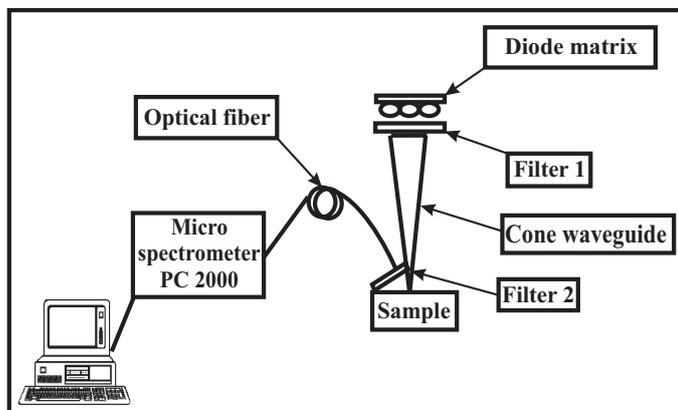


Figure 2. Schematic diagram of the LIF II system. An LED-matrix was used as an excitation source.

3 Results

3.1 337-nm excitation

Figure 3 shows averaged and normalized with respect to the maximum intensity peak fluorescence spectra for sound teeth and for teeth affected by different stages of caries, namely, white spot lesion, brown spot lesion, superficial cavity, medium-depth cavity and deep cavitation, using nitrogen laser as an excitation source. Normalization was used to obtain better view for the spectral shape changes. The sound tooth spectrum consisted of a broad band (480–490 nm maximum) of fluorescence with one secondary maximum at 440 nm; that of the carious tooth exhibited the same maxima at 490 and 440 nm, but another secondary maximum appeared at 550 nm. The fluorescence intensity at 490 nm of white spot lesion was lower than that of the sound tooth. This intensity peak decreased further for all carious areas depending on the stage, with deep cavitation displaying the weakest fluorescence. The secondary peak of the carious areas at 440 nm decreased for all carious areas depending on the carious stage too. However, the peak at 550 nm relatively increased depending on the stage of the caries. A direct relationship was observed between the changes of the fluorescence spectra shape and the caries condition.

3.2 488-nm excitation

Figure 4 shows averaged and normalized with respect to the maximum intensity peak fluorescence spectra for a sound tooth and different stages of caries, namely, white spot lesion, brown spot lesion, superficial cavity, medium-depth cavity and deep cavitation, excited with argon-ion laser. Normalization was used

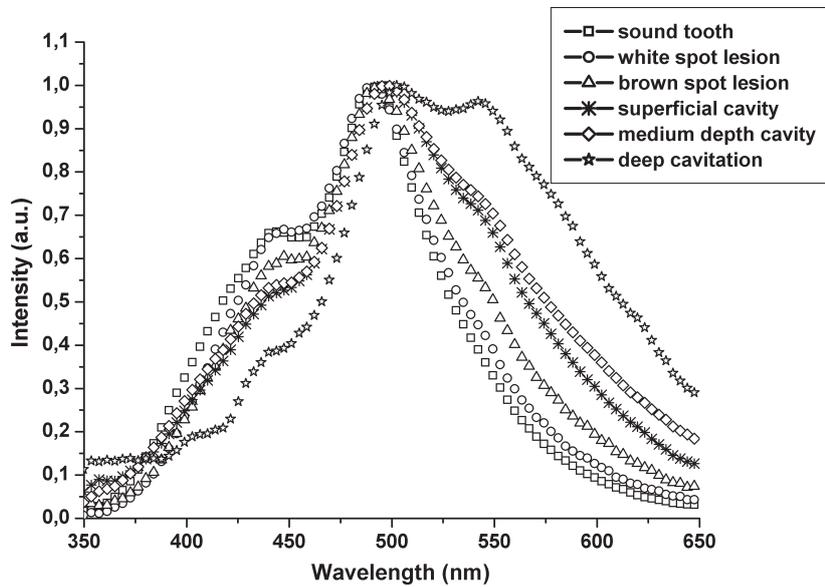


Figure 3. Comparison between spectra of a sound tooth and of different carious stages. Excitation source is nitrogen laser (337 nm). The spectra are normalized with respect to the maximum intensity peak. The relative fluorescence intensity at 550 nm increases depending on the stage of the caries.

to obtain better view for the spectral shape changes. The sound tooth spectrum consisted of a single broad band (525-530 nm maximum) of fluorescence. The spectra of the diseased tooth areas showed a slight shift to the long-wave spectral region.

In addition, the fluorescence signal intensity from the carious areas showed a significant decrease in comparison with that from the healthy tooth, thus proving that the argon-ion laser is the proper excitation source for quantitative laser-induced fluorescence detection of caries.

3.3 440-nm excitation

Figure 5 shows averaged and normalized with respect to the maximum intensity peak fluorescence spectra for a sound tooth and different stages of caries, excited with light-emitted diodes. Normalization was used to obtain better view for the spectral shape changes. The sound tooth spectrum consisted of a single broad band with a maximum at 525 nm. In contrast, the carious tooth spectra consisted of two broad bands, one at 525 nm like the sound tooth, and a secondary band in the red spectral region, with a maximum at 680 nm. The first band was sharp and strong, while the second band had lower intensity.

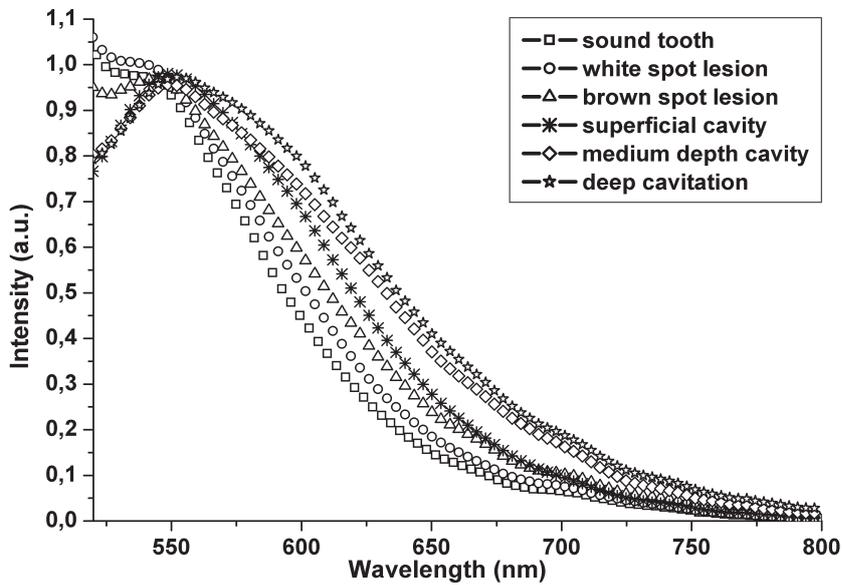


Figure 4. Comparison between spectra of a sound tooth and of different carious stages. Excitation source is argon-ion laser (488 nm). The spectra are normalized with respect to the maximum intensity peak. The spectra of the diseased tooth areas show a slight shift to the red.

The fluorescence signal intensity of the initial caries showed a decrease in comparison with that from the sound tooth. The intensity dropped further for all carious areas depending on the stage, with deep cavitation displaying the weakest fluorescence.

3.4 Non-carious lesions

The influence of other pathologies to the tooth fluorescence spectra was also investigated. In the case of the fluorosa dentis, dark orange-brown spots are visually observed, resulting from the hyperfluoridization of the teeth. These types of teeth usually are more resistant to caries, but dark spots are perceived as an embarrassing cosmetic defect. The fluorescence spectra obtained from the healthy and fluorosa tooth sections, exhibited no spectral-shape changes. The fluorescence intensity from the fluorosa dentis areas was significantly lower in comparison with the sound tooth. This is a result of the larger absorption coefficient of the enamel, related to the tooth coloration. These characteristics of the recorded spectra were universal for all excitation wavelengths.

Thirty-seven samples of odontolithiasis (tartar) were investigated by the LIF methods. In the most of the samples, no significant spectral changes were ob-

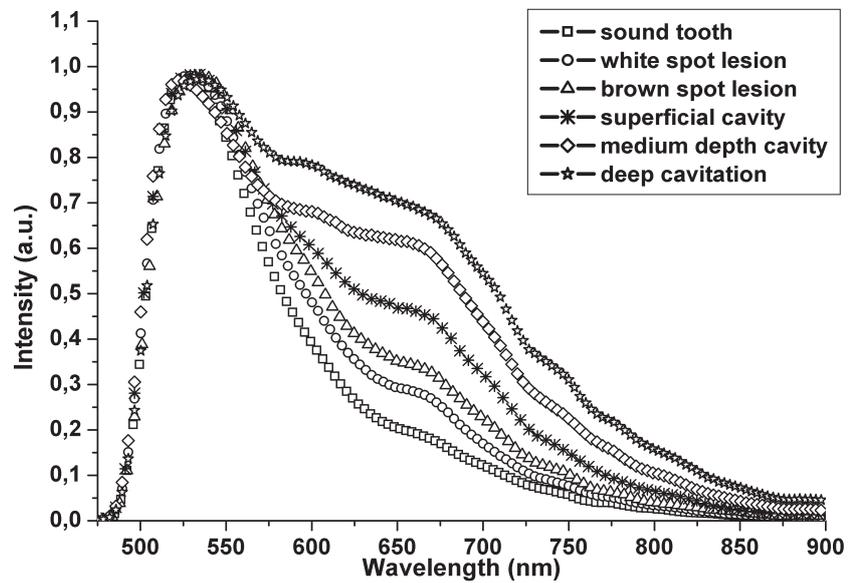


Figure 5. Comparison between spectra of a sound tooth and of different carious stages. Excitation source is light-emitting diode (440 nm). The spectra are normalized with respect to the maximum intensity peak. The peak at 680 nm is increasing depending on the stage of the caries.

served in comparison with the fluorescence signal of the sound tooth, except for a certain decrease of the fluorescence signal intensity.

3.5 Intensity ratios

The tendency of fluorescence signal intensity fall was observed with the three excitation sources – nitrogen laser (337 nm), argon-ion laser (488 nm), and light-emitting diodes. In Figure 6 are presented intensity decreases of the fluorescent peaks, depending on the stage of caries for different excitation sources. All three excitation sources could therefore be used for QLF – technique. However, the interesting results obtained were the variations in the shape of the fluorescence spectra.

Even in the cases of white spot lesion, which is the initial pre-carious stage, the intensity decreased for all three excitation sources. But the changes observed of the signal intensity could be misleading if one uses the intensity decrease technique only for screening for caries, because the similar intensity decrease was observed in the fluorescence spectra of other teeth pathologies (fluorosa dentis, odontolithiasis). The more informative changes of the spectral shape present the possibility to classify the different stages of caries. In the case of argon-ion laser

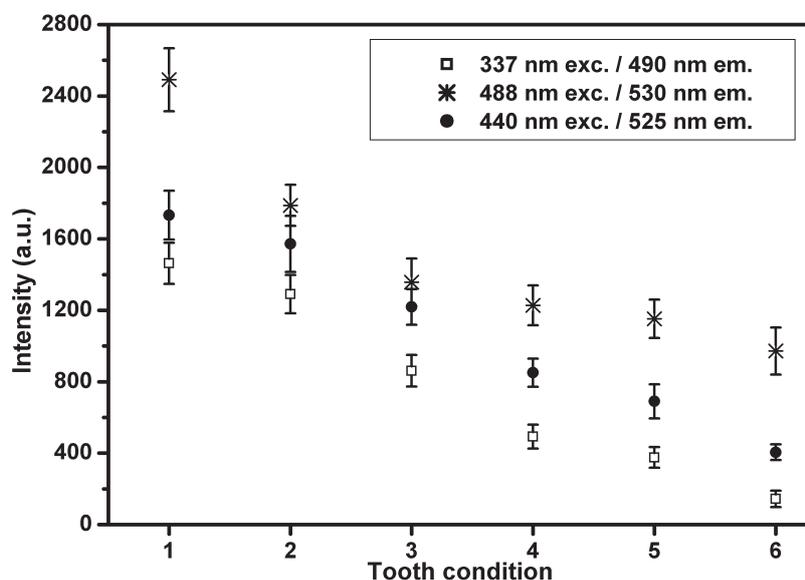


Figure 6. Intensity decrease, depending on the stage of caries. The points presented are the mean values of the averaged for each kind of lesion maxima of fluorescence spectra, namely 490, 525 and 530 nm with their standard deviation for corresponding different excitation wavelengths – 337, 440, and 488 nm. 1 – sound tooth fluorescence, 2 – white spot lesion, 3 – brown spot lesion, 4 – superficial cavity, 5 – medium-depth cavity, 6 – deep cavitation.

excitation source we observed decrease of the intensity at the main fluorescence maximum, but except the changes in this single maximum we did not observe any other significant changes in the shape of obtained fluorescence spectra.

When nitrogen laser and LEDs were used like excitation sources we observed more interesting changes in the spectral shape. This allowed us to use these results for creation of initial diagnostic valuation of caries stage. For nitrogen laser the most informative ratio was I_{550}/I_{490} .

In the values of this ratio was observed significant increase in depend on the carious stage.

When excitation source was LED matrix an increase of secondary maximum at 680 nm was observed in depend on carious stage, and the most appropriate ratio for diagnostic valuation was I_{680}/I_{525} .

The ratios also present the fluorescence differences related to the excitation source used, in table 2. These results demonstrated the possibility to implement LED system for detection of caries.

Investigation of Dental Caries Using LIAF Methods

Table 2. Fluorescence ratios mean values for each kind of carious lesion with their standard deviation using excitation at 337 nm and 440 nm.

Tooth condition	$R_1 = I_{550}/I_{490}$ exc. 337 nm	$R_2 = I_{680}/I_{525}$ exc. 440 nm
Sound tooth	0,37 ± 0,01	0,26 ± 0,02
White spot lesion	0,43 ± 0,02	0,32 ± 0,03
Brown spot lesion	0,54 ± 0,02	0,38 ± 0,04
Superficial cavity	0,69 ± 0,03	0,40 ± 0,03
Medium-depth cavity	0,73 ± 0,03	0,54 ± 0,02
Deep cavitation	0,95 ± 0,03	0,61 ± 0,05

4 Discussion

In this in vitro study, we observed significant differences between the autofluorescence spectra of sound tooth and those of different carious stages. These differences can be attributed to differences in fluorophore content, but absorption and scattering of excitation and fluorescent light by the carious substance must also be taken into account. When the tooth is illuminated by blue light, this light may be absorbed by the chromophores in the tooth. In a lesion, the light path lengths are different in comparison with the sound tooth so that more fluorescent photons are emitted in a sound tissue than in a lesion [17] – a possible explanation of this effect lies in the changes in light scattering. The normal enamel layer has a prism structure with waveguide properties and if one irradiates the tooth surface, the light will penetrate deeply [26]. When the tooth surface is damaged, the prism structure of the enamel layer is destroyed and its waveguide properties disappear so that the excitation light cannot penetrate as deeply as in the case of normally structured enamel and the fluorescent signal obtained has intensity decreased in comparison with sound tooth fluorescence [17].

The optical properties of the teeth, as well as pH change, chemical modification, and influence of exogenous chromophores, could be very important for the studies of fluorescence spectra. The pH is reduced in the lesion, but pH changes do not affect the autofluorescence signal and can be excluded as a cause of fluorescence decrease. The chemical modification is another less possible cause of fluorescence changes [27].

A good candidate for a source of fluorescence changes might be the import of exogenous fluorescent molecules during the carious process; this is supported by the progressive rise of the fluorescent signal in the red spectral region as the caries progresses. The fluorescent band with a maximum at 680 nm might be a manifestation of porphyrin fluorescence, which is a bacterial product. The red fluorescence signal is used in some caries detection techniques [18, 28, 29].

Despite the lack of spectral changes in the cases of odontolithiasis, apart from intensity decrease, it is important to emphasize the results of this part of our investigation. The shape of the fluorescence signal did not show changes, but again the signal intensity reduction observed could be misleading when the QLF method is applied for screening of caries. Since the QLF method cannot be expected to differentiate caries from odontolithiasis, it should probably be used as an addition to a clinical examination. Therefore, it is very important to take into consideration not only the changes of the intensity but also the change of the shape of the fluorescence signal. Bearing in mind the information about variations in the shape of the fluorescence signal, one will obtain a more complete picture of the pathologies investigated.

Early diagnosis of the caries lesion has assumed a particular importance because the ability to detect these reversible early lesions offers many advantages, including opportunities for research and shortening of the time for therapy. Any diagnostic procedure for the diagnosis of carious tooth structure must be specific, valid, reliable and clinically proven. Caries detection system should therefore permit proper discrimination between healthy and diseased tooth structures.

Non-invasive, sensitive, quantitative and qualitative methods for clinical caries diagnosis give new possibilities for research and for the clinical practice as well. The dentists obtain a powerful tool to monitor the changes in the tooth surface, and they are provided with a basis for decision-making on the appropriate choice of therapy [30].

5 Conclusions

We assessed the ability of laser- and light-induced fluorescence spectroscopy to distinguish between initial caries and sound tooth and to classify the different stages of caries. The present study demonstrated the potential of the LIF technique to distinguish sound tooth from initial caries, including white spot lesion. Three different excitation sources were used successively: 337 nm (nitrogen laser), 488 nm (argon-ion laser), and 440 nm (light-emitting diode). The light with wavelength 440 nm was found to be the most suitable of the three excitation wavelengths tested, as it provided the greatest amount of information related to intrinsic fluorophores and allowed an accurate diagnosis by the use of the fluorescence intensity changes at 525 and 680 nm. No absolute intensity determination was required in this situation, since a definite diagnosis could be established based on the fluorescence intensity ratio and these results could be useful in designing a simplified fluorescence-imaging device for detection of initial caries.

Acknowledgments

This work was supported by EU Framework V programme, IMPECABLE project (contract number – G5RD-CT-2000-00372) and by the Bulgarian Ministry of Education and Science under grant MU-F-03/05.

References

- [1] A. Gmitro, F. Cutruzzola, M. Stetz, L. Deckelbaum (1988) *Appl. Opt.* **27** 1844.
- [2] S. Andersson-Engels, J. Johansson, U. Stenram, K. Svanberg, S. Svanberg (1990) *IEEE J. Quantum Elect.* **26** 2207.
- [3] R. Alfano, A. Pradhan, G. Tang, S. Wahl (1989) *JOSA B* **6** 1015.
- [4] D. Goujon, M. Zellweger, H. Bergh, G. Wagnieres (2001) *Photodynamics News* **3** 11.
- [5] M. Zellweger, P. Grosjean, D. Goujon, P. Monnier, H. Bergh (2001) *J. Biomed. Opt.* **6** 41.
- [6] S. Jayaraman, Y. Song, L. Vetrivel, L. Shankar, A. Verkman (2001) *J. Clin. Invest.* **107** 317.
- [7] A. Takahashi, P. Camacho, J. Lechleiter, B. Herman (1999) *Physiol. Rev.* **79** 1089.
- [8] E. Speel (1999) *Histochem. Cell Biol.* **112** 89.
- [9] S. Santos, N. Singh, A. Natarajan (1997) *Exp. Cell. Res.* **232** 407.
- [10] R. Bonnett (1995) *Chem. Soc. Rev.* **75** 19.
- [11] S. Montan, K. Svanberg, S. Svanberg (1985) *Opt. Letters* **10** 56.
- [12] J. ten Bosch, J. Coops (1995) *Dent. Res.* **74** 374.
- [13] C. Ko, D. Tantbirojn, T. Wang, W. Douglas (2000) *J. Dent. Res.* **79** 1584.
- [14] J. Zijp, J. ten Bosch (1993) *Appl. Opt.* **32** 411.
- [15] X. Wang, T. Milner, J. Boer, Y. Zhang, D. Pashley, J. Stuart Nelson (1999) *Appl. Opt.* **38** 2092.
- [16] B. Colston, Jr., U. Sathyam, L. DaSilva, M. Everett, P. Stroeve, L. Otis (1998) *Opt. Exp.* **3** 230.
- [17] M. Ando, M. van der Veen, B. Schemehorn, G. Stookey (2001) *Caries Res.* **35** 464.
- [18] E. Sheehy, S. Brailsford, E. Kidd, D. Beighton, L. Zoitopoulos (2001) *Caries Res.* **35** 421.
- [19] E. de Josselin de Jong, F. Sundstrom, H. Westerling, S. Tranaeus, J. ten Bosch, B. Angmar-Mansson (1995) *Caries Res.* **29** 2.
- [20] A. Hall, E. DeSchepper, M. Ando, K. Stookey (1997) *Adv. Dent. Res.* **11** 507.
- [21] R. Alfano, S. Yao (1981) *J. Dent. Res.* **60** 120.
- [22] F. Sundstrom, K. Fredriksson, S. Montan, U. Hafstrom-Bjorkman, J. Strom (1985) *Swed. Dent. J.* **9** 71.
- [23] U. Hafstrom-Bjorkman, F. Sundstrom, J. ten Bosch (1991) *Acta Odontol. Scand.* **49** 133.
- [24] M. van der Veen, J. ten Bosch (1995) *Eur. J. Oral Sci.* **103** 375.
- [25] The International Statistical Classification of Diseases and Related Health Problems (1996), *Vol.1, 10th revision*, p.564 (in Russian)

E. Borisova, T. Uzunov, L. Avramov

- [26] T. Uzunov, A. Gisbrekht, M. Nenchev (1996) *Proc. SPIE* **3052** 405.
- [27] H. van der Veen, J. ten Bosch (1996) *Caries Res.* **30** 93.
- [28] A. Lussi, S. Imwinkelried, C. Longbottom, E. Reich (1998) *Caries Res.* **32** 297.
- [29] E. Reich, F. Al Marrawi, N. Pitts, A. Lussi (1998) *Caries Res.* **32** 298.
- [30] B. Angmar-Mansson, S. Al-Khateeb, S. Tranaeus (1996) *Eur. J. Oral Sci.* **104** 480.