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LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Laser-induced fluorescence from sound and carious tooth substance: Spectroscopic studies

FOLKE SUNDSTRÖM¹⁾, KENT FREDRIKSSON²⁾, SUNE MONTÁN²⁾,
ULRIKA HAFSTRÖM-BJÖRKMAN¹⁾ AND JANE STRÖM¹⁾

Department of Cariology¹⁾, Karolinska Institute, Stockholm and
Department of Physics²⁾, Lunds Institute of Technology, Lund, Sweden

Key words: Enamel caries, fluorescence, laser

ABSTRACT

Fluorescence spectra of dentine and enamel illuminated with laser light of wavelengths of 337, 488, 515 and 633 nm respectively were recorded. The fluorescence obtained by illumination with UV laser light at 337 nm had a peak at about 400 nm in dentine as well as enamel. Compared to intact enamel the fluorescence from enamel with initial carious lesions was of lower intensity and had a slight red shift.

No fluorescence within the visible range was obtained by illumination with a low power He-Ne laser at 633 nm. Illumination at 488 nm produced fluorescence with a peak at about 540 nm in dentine as well as enamel. The difference in the intensity of fluorescence between sound and carious enamel was generally greater at this wavelength than at any of the others tried, and the red shift from the carious enamel was also more pronounced. Illumination at 515 nm produced fluorescence of similar wavelengths but with much less difference between intact and carious enamel. It was concluded that illumination at 488 nm was the most suitable wavelength of those investigated for the detection of initial carious lesions by the fluorescence technique.

SAMMANFATTNING

Fluorescensspektra från emalj och dentin i homotänder belysta med laserljus av respektive våglängderna 337, 488, 515 och 633 nm har upptagits. Den fluorescens som erhöles genom belysning med kvävelaser vid 337 nm visade en intensitetstopp vid ungefär 400 nm i såväl dentin som emalj. Jämförd med intakt emalj hade fluorescensen från emalj med initial karies lägre intensitet och var något förskjuten åt rött.

Ingen fluorescens inom det synliga området erhöles vid belysning med en lågeffekts He-Ne-laser vid 633 nm. Belysning med argonlaser vid 488 nm gav fluorescens med en intensitetstopp vid 540 nm i såväl dentin som emalj. Skillnaden i intensitet mellan fluorescensen i intakt och karierad emalj var genomgående större vid belysning med denna våglängd än vid någon av de andra testade, och rödförskjutningen av fluorescensen från karierad emalj var också tydligare. Belysning med argonlaser vid 515 nm gav en fluorescensstopp liknande den vid belysning med 488 nm men med avsevärt mindre skillnad mellan intakt och karierad emalj.

Av de prövade våglängderna befanns 488 nm vara den lämpligaste för diagnos av initialkaries med laserfluorescensstekniken.

INTRODUCTION

Fluorescence occurs as a result of the interaction of electromagnetic radiation with molecules. Molecules are transferred to higher energy states when irradiated by light of suitable wavelengths. This process is termed excitation, and the molecules are said to be in excited states. When the molecules return from these high energy states into lower states light can be emitted, the frequency of which corresponds to the energy differences between the involved states. The large-scale energy level structure of a molecule is given by the possible states for the electrons in the molecule. In addition, vibrational and rotational energies will cause a splitting of the electronic levels into a vast number of substates. The general appearance of a molecular energy level diagram and the corresponding fluorescence spectrum is shown in Fig. 1. For excitation to a higher electronic state a photon

energy generally corresponding to ultra-violet or visible light is necessary, whereas vibrational and rotational transitions occur from interaction with infrared photons.

From an excited state, a molecule with a certain probability decays radiatively to the ground level or one of the other levels in between. When this happens, a photon is emitted, the colour of the light corresponding to the energy given off. The light is referred to as fluorescence. However, emitting a quantum is not the only way for transferring a molecule from an excited state to a lower one. There are also important radiationless processes and usually the decay is a chain of different processes. The spectral distribution of the fluorescence light is often independent of the exact excitation wavelength and reflects the distribution of vibrational and rotational levels in the ground state. Due to energy loss by the non-radiative processes in the

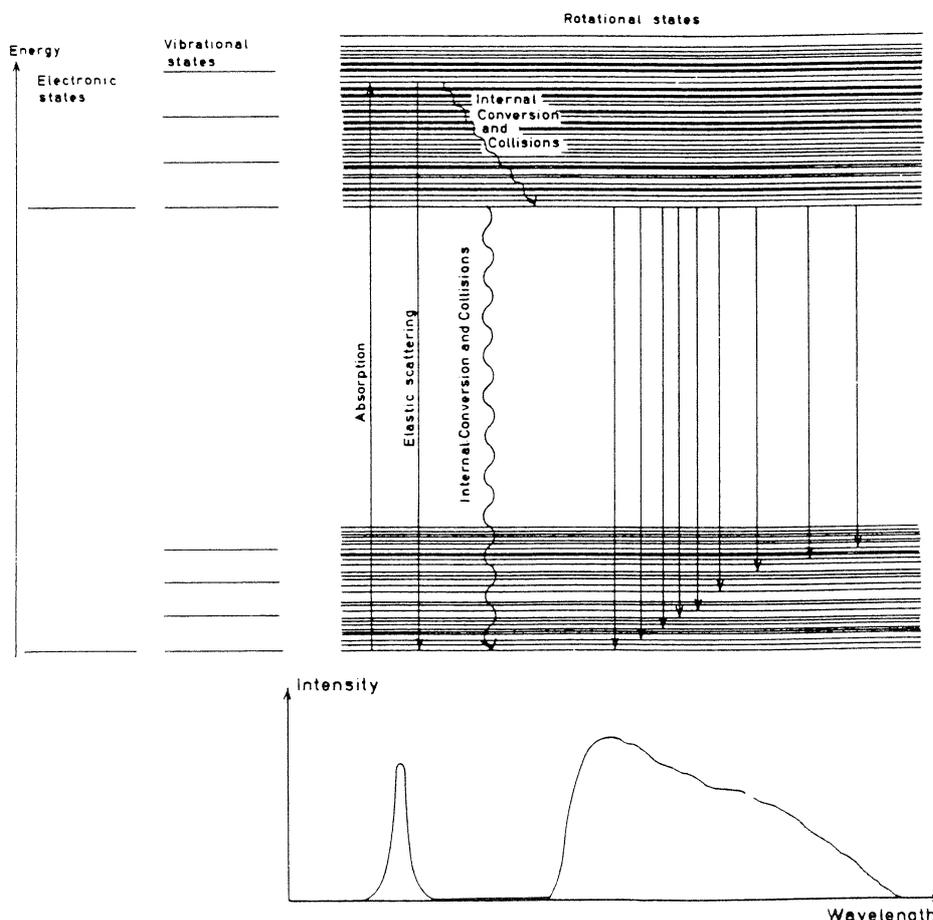


Fig. 1. Two energy bands in a molecule and a general fluorescence spectrum of a solid. The spacings between the rotational levels are enlarged. In solids, transitions between several bands contribute to the fluorescence light.

decay the fluorescence band is obtained at longer wavelengths than the excitation wavelength. In fact the fluorescence observed from a solid material is made up of several interfering fluorescence bands.

The occurrence of fluorescence in tooth substance under ultra-violet light irradiation has been known for long and has been the subject of several investigations. Most of the work has concerned the fluorescence in the dentine and attempts have been made to characterize the components responsible for the fluorescence in sound dentine as well as the suppression of fluorescence in carious dentine (review, see *Foreman* 1980).

Similar research has been carried out on dental enamel, but has so far been less extensive (review, see *Booij & ten Bosch* 1982). In enamel, as in dentine, carious lesions generally exhibit less fluorescence than the undamaged tissues and similar effects occur in artificial demineralizations (*Naleway et al.* 1979), *Alfano & Yao* 1981). It has further been found that fluorescence in the tooth substance can be obtained by light in the visible as well as in the ultra-violet range (*Alfano & Yao* 1981, *Bjelkhagen & Sundström* 1981, *Bjelkhagen et al.* 1982). When the enamel is illuminated with light in the blue-green range the observable fluorescence occurs in the green-yellow range. The difference in fluorescence between intact and carious dental enamel seems to be greater in this range than in the blue fluorescence obtained by ultra-violet excita-

tion (*Alfano & Yao* 1981). Using a monochromatic light source without any light output at the wavelengths of fluorescence, e.g. an argon-ion laser at 488 nm, the difference in fluorescence between intact and carious enamel is generally easy to observe and photograph with the aid of barrier filters. By this method incipient carious lesions can be observed, permitting detection of smooth surface and pit and fissure lesions at an earlier stage than by other clinically applicable methods (*Bjelkhagen & Sundström* 1981, *Bjelkhagen et al.* 1982).

To establish optimal conditions for the detection of carious lesions by this method, it was decided to record the spectral distribution of the fluorescence from intact and carious tooth substance illuminated with laser light at 488 nm. For comparison fluorescence spectra induced by laser light of certain other wavelengths were also obtained.

MATERIALS AND METHODS

Extracted permanent human premolars with initial enamel carious lesions of different sizes were used. Some of the teeth had been cut in halves through the carious lesions to enable the study of the fluorescence of dentine and intact as well as carious enamel in transverse sections. The teeth were kept in thymolized 0.9 percent NaCl when not subjected to measurements.

The set-up for the experiment is shown in Fig. 2. Three different light sources, with four

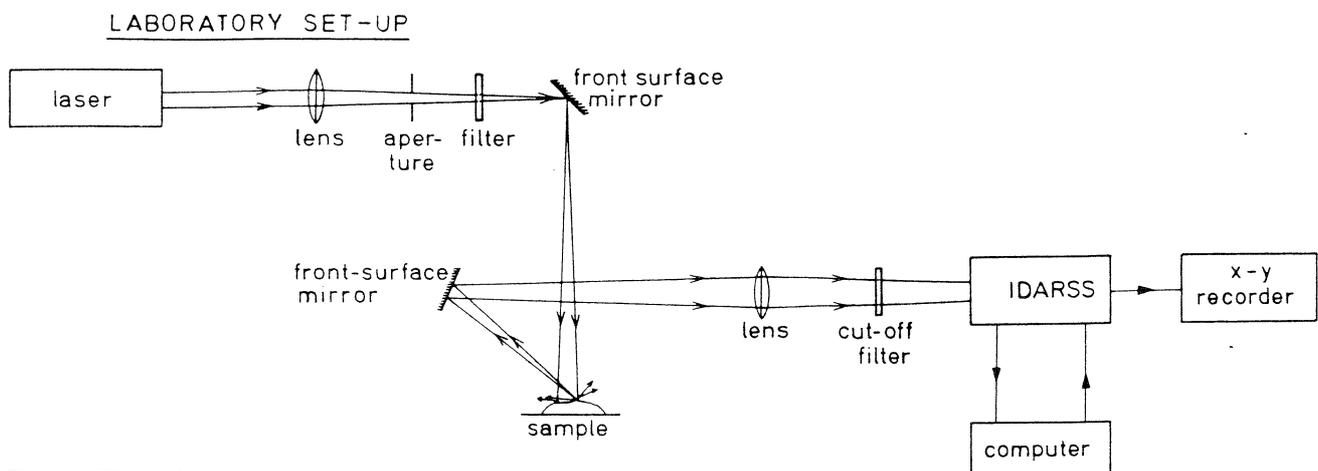


Fig. 2. The laboratory set-up.

different wavelengths were used: an argon-ion laser, working at 488 or 515 nm, a nitrogen laser working at 337 nm and a helium-neon laser at 633 nm. The argon-ion laser was run at about 50mW cw while the N₂-laser emitted pulses of 10 ns length with 0.5 mJ of energy. The laser light could be focused at the sample. An aperture was placed in the laser beam to vary the size of the illuminated spot from less than 1 mm² to several mm². The fluorescence light was deflected via a front-surface mirror through a lens to the entrance slit of the monochromator of the detection system. The region measured by the optical detection system was less than 1 mm². A cut-off filter (Fig. 3) was placed in front of the entrance slit to suppress elastically scattered laser light. The detection system was a Tracor Northern IDARSS (Intensified Diode Array Rapid Scan Spectrometer) model TN-1710. In this unit, the light was spread over a photo-diode array by means of a grating. The array contained 1024 diodes and covered the spectrum from 200 to 800 nm. The dark current of the detector was quite large, but since there were possibilities to treat the digitized signal mathematically, this background could be eliminated. The spectra were stored on floppy-

discs via a computer and could be plotted on a x-y-recorder. The spectra were also corrected for the varying spectral response of the detector. Measurements were made on several specimens of carious and non-carious teeth. The relative strength of the laser-induced fluorescence and the spectral signature were registered.

After the measurements had been completed contact microradiography of ground sections of the teeth was performed with Ni-filtered CuK α -radiation (1.54 Å) at 18 kV with a focal spot-to-film distance of 750 nm. The microradiographs were studied in a Leitz photomicroscope.

RESULTS

Some typical examples of fluorescence spectra using the argon-ion laser at 488 nm are shown in Figs. 4 and 5. Spectra from regions with initial carious lesions and from sound enamel are displayed. In these measurements the laser beam was focused to a spot with a diameter of a few tenths of a millimeter. An optical filter (Schott OG 515) which absorbed the scattered laser light and the fluorescence

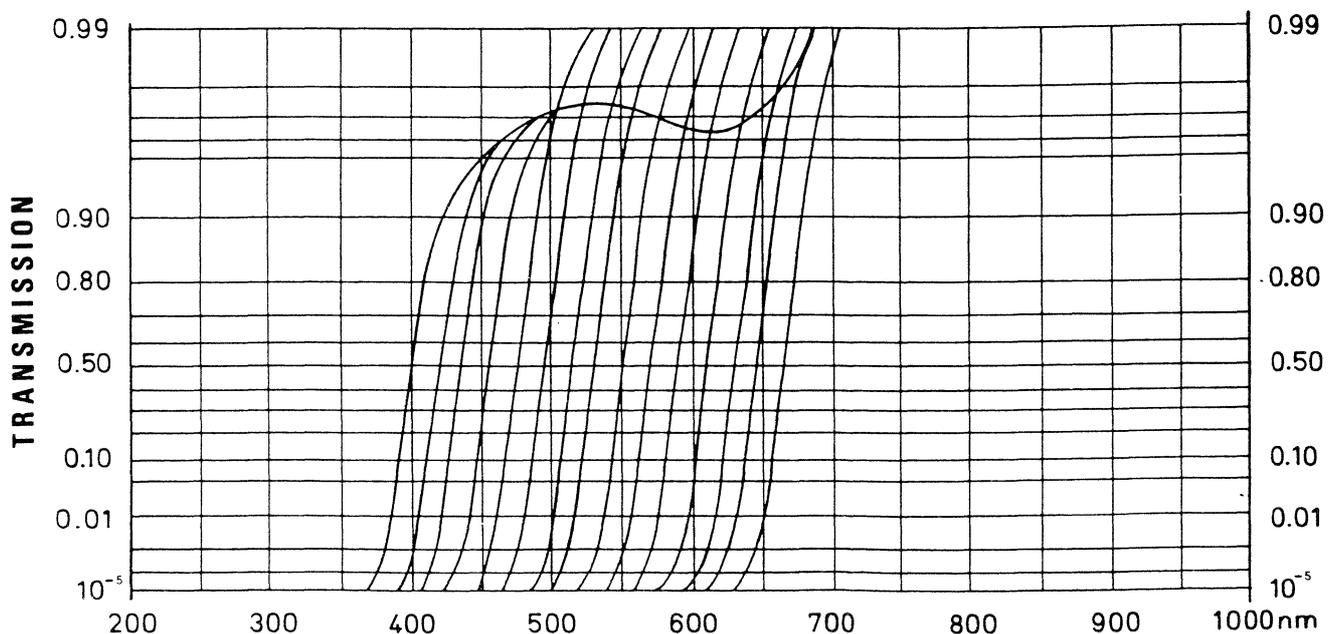


Fig. 3. Transmission of some Schott cut-off filters.

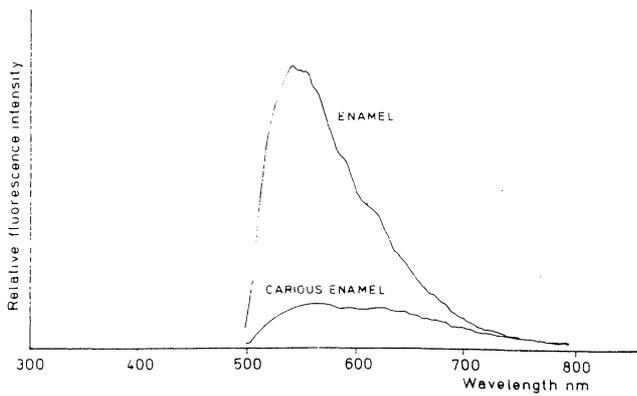


Fig. 4a

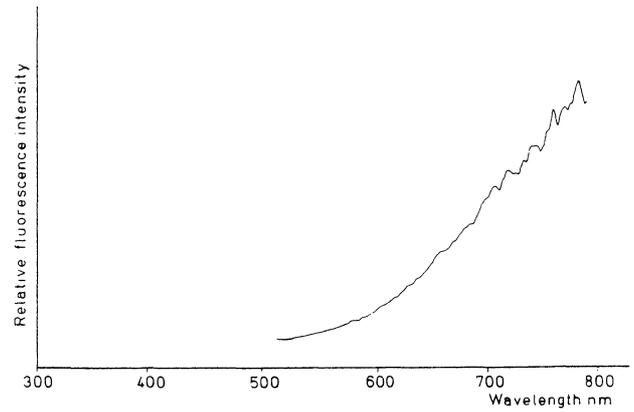


Fig. 4b

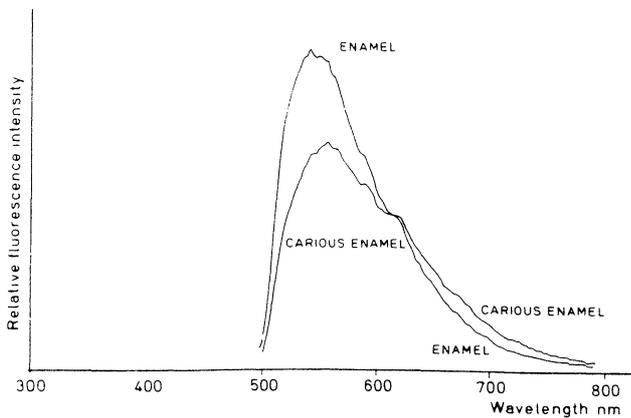


Fig. 4c

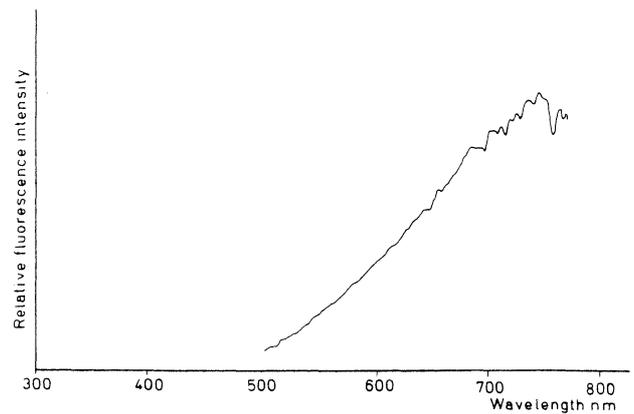


Fig. 4d

Fig. 4a. Fluorescence spectra from a region with initial carious lesion and from intact enamel. The fluorescence was induced by the 488 nm output of an argon-ion laser. The illuminated area and the measured region were a few tenths of a millimeter in diameter.

Fig. 4b. The divided quota of the fluorescence intensities, carious enamel/enamel, from the two recordings in Fig. 4a.

Fig. 4c. A similar measurement as in Fig. 4a of another tooth specimen.

Fig. 4d. The divided quota of the fluorescence intensities, carious enamel/enamel, from the two recordings in Fig. 4c.

below 500 nm was employed for the detection. The relative intensity of the fluorescence from the sound enamel was very high compared to that from the carious region. During the measurements the yellow and red fluorescence from the tooth could be viewed through a cut-off colour filter (Schott OG 550) and it was observed that the fluorescence induced by the focused laser was spread throughout the tooth when the sound enamel was illuminated. In the carious region the illuminated substance absorbed most of the laser light while the fluorescence from the rest of the tooth was much weaker. Thus, it was easy to

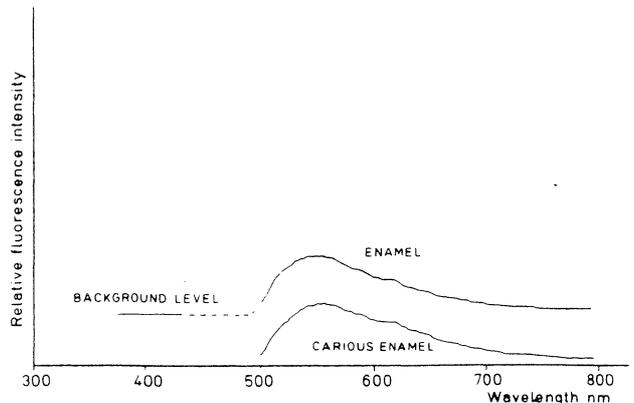


Fig. 5. Fluorescence spectra of carious and intact enamel induced by the 480 nm laser. The laser beam was spread to illuminate the whole tooth, whereas the measured region was of the same size as in 4a.

distinguish a carious region when illuminated by the laser spot.

The fluorescence from the tooth specimens peaked at 540 nm when induced by the 488 nm laser light, and there was a second minor peak at 610 nm. The relative strength of the carious enamel spectrum was shifted somewhat to the red compared to the intact enamel spectrum. This effect was more evident when the ratio of the intensities of the two spectra was calculated. Fig. 4b shows the intensity ratio carious enamel/enamel for each wavelength in the two spectra in Fig. 4a. The value at 700 nm was several times larger than the value at 540 nm. This means that the 540 nm fluorescence was more effectively suppressed in the carious lesion than the fluorescence at 700 nm. The relative red shift of the spectrum is another indication of a carious lesion beside the general suppression of the fluorescence.

Fig. 4c is an example of the fluorescence from a similar tooth specimen. The fluorescence was not suppressed as much as in the example above but the red shift, which is shown in Fig. 4d, was even more obvious. The carious lesion was very easy to observe also in this case, as the total fluorescence from the tooth was considerably weaker when the carious enamel was illuminated by the laser spot.

Fig. 5 shows fluorescence spectra from sound and carious enamel when the illuminating laser beam was spread to cover the entire tooth crown instead of being focused to a small spot. The area measured by the optical detection system was the same as in the previous measurements, i.e. less than 1 mm². The relative intensity of the spectra in Fig. 5 is several times weaker than that of the corresponding spectra in Fig. 4a and 4c, and the spectra from the sound and carious enamel are of similar appearance.

An example of the fluorescence from dentine and dentino-enamel junction is shown in Fig. 6. The intensity of the fluorescence from the dentino-enamel junction is higher than that from dentine or enamel. A focused laser spot was employed and therefore the spectra

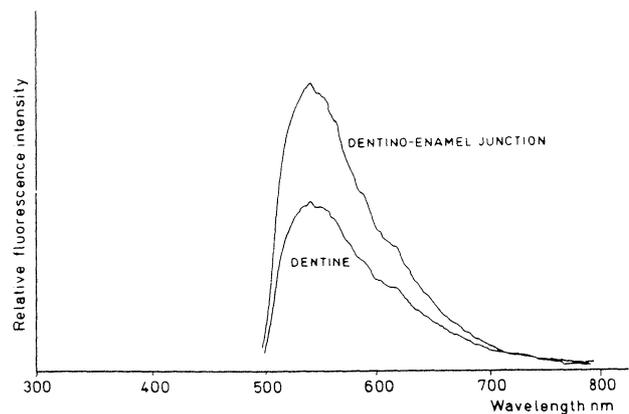


Fig. 6. Fluorescence spectra of dentine and a dentino-enamel junction. The 488 nm laser beam was employed and the experimental set-up is similar to the one used for Fig. 4, with the laser beam focused to a small spot.

originate from small regions.

Measurements were also made with the 515 nm output of the argon-ion laser. Fig. 7 is an example of the fluorescence spectra of sound enamel and carious enamel. The recording is not directly comparable to the examples above regarding intensity, but the observed fluorescence is much weaker in this measurement. A cut-off colour filter was used, which absorbs the fluorescence below 540 nm. The laser spot was focused. The ratio calculations revealed some red shift of the spectrum from the carious lesion as shown in Fig. 7b.

Fig. 8 is an example of measurement with the 515 nm laser in dentine and dentino-enamel junction. The induced fluorescence intensity from the dentino-enamel junction was much higher than the corresponding intensity from the enamel shown in Fig. 7a.

Examples of measurements with the nitrogen-laser at 337 nm are shown in Figs. 9a and 10. The laser beam was focused to a spot of the size of 1 mm², which was also the approximate size of the area viewed by the detection system. A cut-off filter (Schott WG 360), which absorbed the light below 340 nm, was employed for the detection. The measured fluorescence peaked at 400 nm and a second minor peak could be observed at 490 nm. A suppression of the fluorescence by the carious

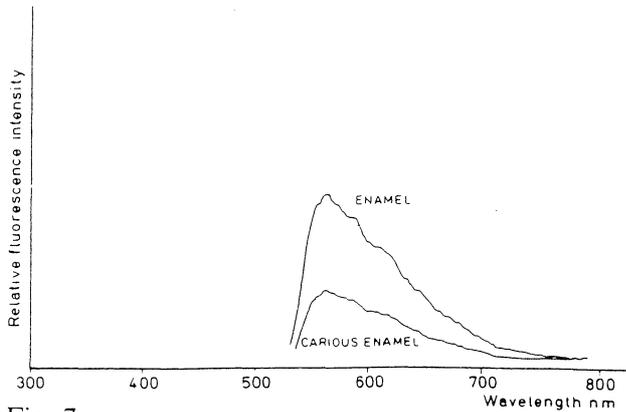


Fig. 7a

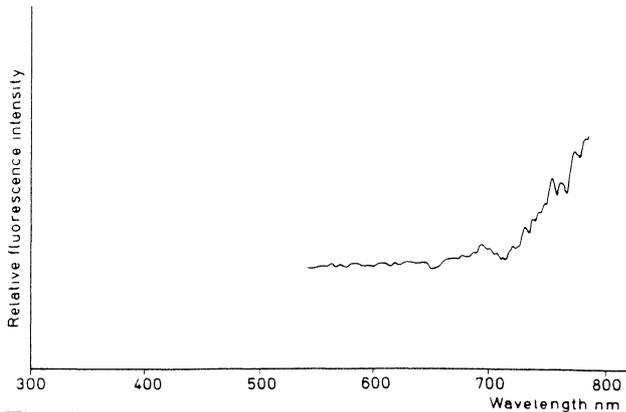


Fig. 7b

Fig. 7a. Fluorescence spectra induced by the 515 nm output of an argon-ion laser. Regions with initial enamel caries and intact enamel were studied. The laser beam was focused to the measured region.

Fig. 7b. The divided quota of the fluorescence intensities, carious enamel/enamel, from the two recordings in Fig. 7a.

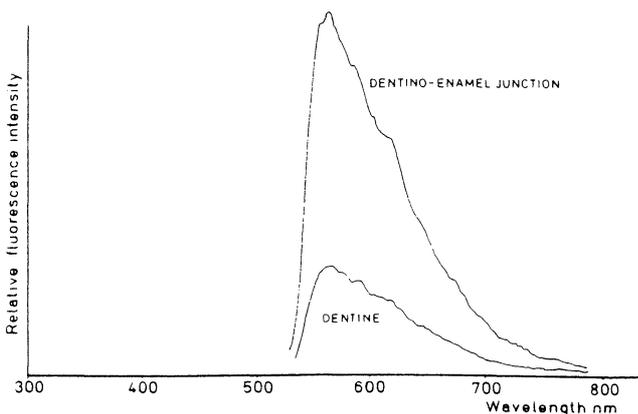


Fig. 8. Similar measurement with the 515 nm laser as in Fig. 7a but on dentine and dentino-enamel junction.

lesion was clearly seen in the recordings, and could also be seen by direct observation through a suitable filter.

Fig. 9b shows the intensity ratios carious enamel/enamel for the example given in Fig.

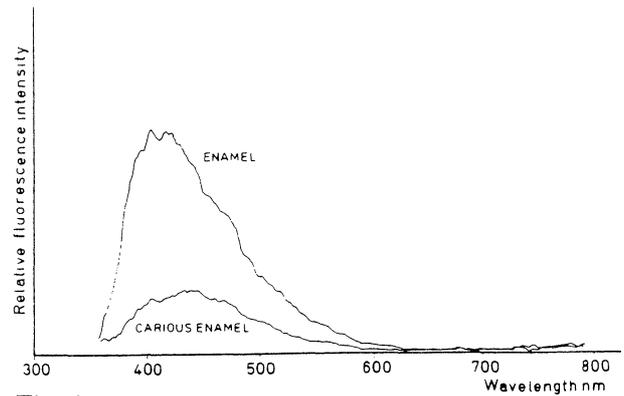


Fig. 9a

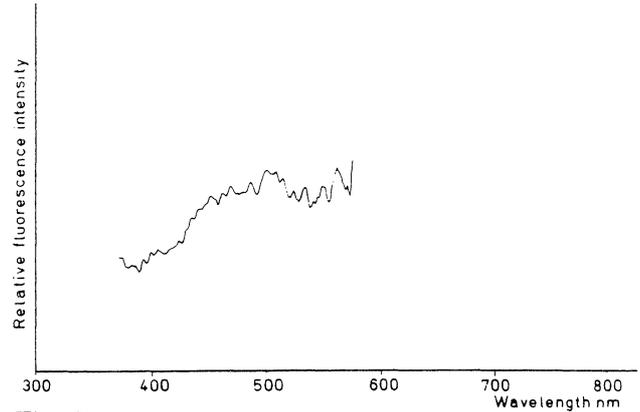


Fig. 9b

Fig. 9a. Fluorescence spectra from carious and intact enamel induced by the 337 nm output of a nitrogen laser. The laser spot and the measured region were of the same size, less than 1 mm².

Fig. 9b. The divided quota of the fluorescence intensities, carious enamel/enamel, from the recordings in Fig. 9a.

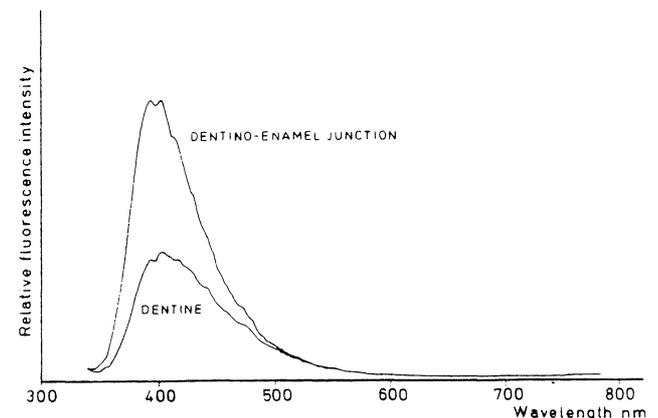


Fig. 10. The laser spot and the measured region were of the same size, less than 1 mm².

9a. There was an evident shift of the fluorescence to the longer wavelengths. The fluorescence at 400 nm was suppressed twice as much as the corresponding fluorescence at 500 nm.

The red light from the low power helium-neon laser at 633 nm, which was employed for studies of different tooth specimens, gave no fluorescence within the visible range and no clear contrast between sound and carious enamel.

The microradiographs confirmed the presence of initial carious lesions of various sizes in the areas where such lesions had been diagnosed by visual observation and the measurements in laser fluorescence had been made.

DISCUSSION

Measurements on several tooth specimens were made with lasers emitting at different wavelengths. As expected from earlier research with other types of UV-sources it was found that the nitrogen laser at 337 nm was applicable for the detection of carious lesions. There was even a red shift of the fluorescence from the regions with enamel caries, compared to that from sound enamel. This red shift was slight, however, and the main difference observed between carious and sound regions was the lower intensity of the fluorescence from the carious regions. In comparison with the argon-ion laser at 488 nm the contrast between sound and demineralized enamel was considerably lower with the nitrogen laser. Since the nitrogen laser is a pulsed laser source it may be suitable for some future applications, where the induced fluorescence is hard to distinguish from other light. With the human eye as the detector this is no advantage, however.

The helium-neon laser at 633 nm was evidently not suitable for the detection of enamel caries with the present technique.

Of the two investigated wavelengths from the argon-ion laser, the 488 nm output was found to be a better choice than the 515 nm output. At 488 nm there was an apparent red shift of the fluorescence from carious enamel regions compared to sound regions and there was also a pronounced difference in the intensity of the fluorescence.

The red shift is probably large enough to allow the construction of a relatively simple opto-electrical system for its detection. Since the difference in intensity is so pronounced, however, there seems to be no immediate need of such a detection system. In most cases, when a wide area of sound enamel is illuminated together with the carious lesion, the spectral signature of sound and carious enamel appears to be the same because the fluorescence from the sound enamel penetrates the lesion. Under such circumstances, there is a need of a two-dimensional recording of the spectra, since the carious lesion is only observed as a relative intensity change over the surface. The human eye is a perfect recorder in this respect, and a video system can be applied to improve the detection limits and make it possible to study also regions which are difficult to observe directly.

In the clinical studies made so far, ordinary laser safety goggles (*Laserguard*, Glendale Optical Co) for the argon-ion laser have been employed to suppress the scattered laser light and to enhance the fluorescence. These goggles absorb light below 550 nm. Since it can be seen in this study that the most drastic effect on the fluorescence by the carious lesion is just above 500 nm, it ought to be better to use goggles with a cut-off at a somewhat shorter wavelength. Such goggles, however, are not commercially available at present, but can be custom-made at considerably higher costs.

To explore the capacities of different filters an interference filter with a 10 nm window at 540 nm (Oriel 5388) was tried. This filter provided good contrast between sound and carious enamel but the visual observation of the resulting green-yellow, almost monochromatic picture was rather strenuous, and the laser goggles were generally preferred. With a narrow band filter at this wavelength the red shift from the carious enamel could not be seen, and although faint, this red shift may sometimes provide useful information for the observer.

In this study it was also noted that the only light qualities necessary for the detection of carious lesions are a rough monochromacity and adequate light intensity, whereas the other qualities of a laser source, e.g. the light wave coherence and the low beam divergence, are not employed. Therefore, it was considered that another light source could be used instead of a laser. A suitable source would be what is known as a curing light, a halogen lamp commonly used by dentists to initiate the polymerization of certain resin materials.

Curing lights in the blue range have been tried for diagnostic transillumination of teeth in a similar way as the conventional white light fiber optics (*O'Brien et al.* 1983). As regards detection of caries, it was found that the method was useful in anterior teeth.

The main difference between methods using transillumination with white or coloured light and the fluorescence method is evidently that in the latter the light observed originates in the tooth substance. The light output and the changes in this output associated with demineralizations are for that reason relatively independent of the direction of the illuminating light beam. Hence the detection possibilities are extended to tooth segments not accessible to transillumination, while at the same time the contrast between sound and demineralized enamel seems generally enhanced.

The possibility of detecting carious lesions with fluorescence induced by an ordinary halogen lamp seems promising and this technique can be applicable in the near future. Clinical tests suggests that the filtering of the light used must be very efficient, however. Since the light sources used for the curing of dental materials generally emitted light at the wavelengths where the fluorescence was studied, they had to be combined with additional optical filters to suppress the emission above 490 nm. Even the output in the region above 640 nm of the 480 nm short pass filter used (Ditric Optics Inc., Fig. 11) impaired the observation of demineralizations to a certain degree.

Because of the loss of light output in the filters and the relatively extensive optical arrangements required to direct the output from a halogen lamp into fiber optics, rather powerful and expensive devices are presently needed. Thereby the advantage in cost and convenience of the halogen light source in relation to the laser is greatly diminished, but further work to select the most suitable and least expensive light source is going on.

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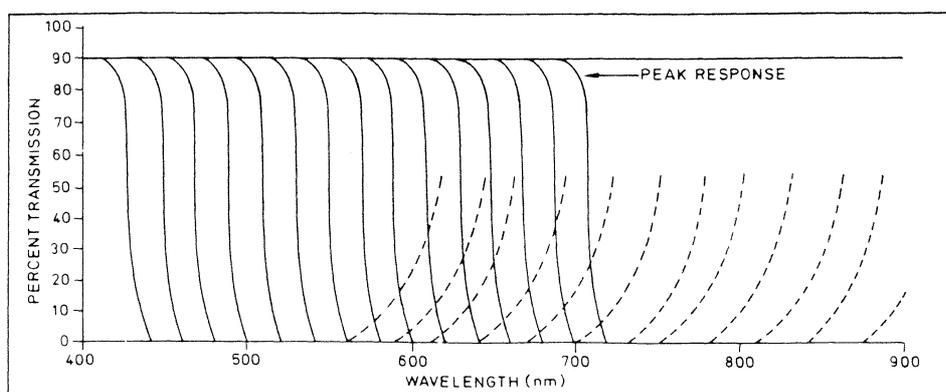


Fig. 11. Transmission of some Ditrac short wavelength pass filters. The filter with a cut-on at about 480 nm was used for additional filtering of halogen light sources.

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Address:
Sundström
Department of Cariology
Karolinska Institute
Box 3207
S-103 64 Stockholm
Sweden