In Vitro Studies of Laser Fluorescence for Detection and Quantification of Mineral Loss From Dental Caries

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What is This?
IN VITRO STUDIES OF LASER FLUORESCENCE FOR DETECTION AND QUANTIFICATION OF MINERAL LOSS FROM DENTAL CARIES

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Abstract—Laser fluorescence (LF) is thought to be a quantitative technique for the measurement of mineral loss from dental caries. These studies aimed to demonstrate the quantitative nature of LF, by means of transverse microradiography (TMR), in addition to the possible use of LF on small specimens suitable for in situ studies. Studies also aimed to determine the ability of LF to detect caries adjacent to amalgam restorations. The results demonstrated reasonable correlation between both the histological depth and mineral loss measured by TMR compared with the % change in mean fluorescence radiance measured by LF (r = 0.70 and 0.83, respectively). Studies with small specimens demonstrated that in vitro remineralization could be observed with LF for comparison of the demineralized specimen with an undemineralized reference. ANOVA showed significant mineral gain (p < 0.0001). The detection and quantification of mineral loss adjacent to amalgam restorations may have potential for the management of recurrent caries. Studies have demonstrated that LF can detect such mineral loss, and, with refinement of the image analysis system, LF was capable of detecting remineralization. Remineralization of such lesions is controversial, but it is suggested that LF may have a role in secondary caries management.

Key words: Fluorescence, caries, detection, monitoring.

Studies based on fluorescence observations of laser-induced light scattering have shown the technique to be a quantitative measurement of mineral loss compared with longitudinal microradiography (Hafstrom-Bjorkman et al., 1992). Further studies have demonstrated an in vivo application of the technique to measure remineralization of white-spot lesions on accessible surfaces of teeth (de Josselin de Jong et al., 1995). A detailed description of observed fluorescence from the interaction between Argon laser light and sound and carious tooth tissue has been advanced recently (de Josselin de Jong et al., 1996; ten Bosch, 1996). These papers suggest that fluorescence can be explained fully by light-scattering phenomena within the sound and demineralized tissue. Although the exact nature of the fluorescing chromophores within the sound or demineralized tooth tissue is not known, laser fluorescence detection and quantification of mineral loss may offer advantages over conventional white-light-scattering measurements as a result of increased contrast between sound and demineralized tissue and reduced surface reflections.

This paper aims to describe the context in which laser fluorescence has been investigated as a complement to existing research at the Oral Health Research Institute (OHRI), Indiana University Dental School. In addition, it will review briefly some pilot work regarding the application of the technique to the study of secondary caries and, finally, will discuss some of the current limitations of the technique.

Research work over the past several years at OHRI has demonstrated the utility of an in situ model for the study of the behavior of early white-spot lesions (Stookey et al., 1985; Dunipace et al., in press). Early white-spot remineralization has been quantified by means of serial Knoop hardness and transverse microradiography (TMR) measurements. For the purposes of these studies, early white spots were defined as artificially created caries-like lesions in ground and polished specimens of either human or bovine enamel. These lesions were up to 150 μm deep.

Our first aim was to confirm the quantitative nature of laser fluorescence measurements in relation to an accepted "gold standard", histology, and also in relation to another generally accepted quantitative technique, transverse microradiography.

The second aim was to determine the application of laser fluorescence to the study of mineral changes in specimens used in our in situ model. The specimens used in this model were very small, approximately 3 mm in diameter, retained in a removable partial denture and positioned in a contact-point relationship with a natural abutment tooth. Recent

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developments in microradiographic analysis of these specimens required the creation of “test” and “control” lesions on each specimen. The need for multiple lesions to be created in such a small space left little room for the inclusion of an area of sound enamel as a reference point for laser fluorescence measurements. Therefore, a study was undertaken to determine the suitability of an external sound enamel reference with which changes in demineralized enamel could be compared by means of laser fluorescence.

The final aim was to determine the feasibility of the technique for the study of secondary caries. Current methods of secondary caries diagnosis rely to a large extent on visual assessment of the mineralized tissue adjacent to a restoration, the contour of the interface between the tooth and the restoration, and the radiographic appearance of the tooth. Laser fluorescence may have the ability to detect and possibly monitor longitudinally mineral loss adjacent to restorations. Pilot studies were undertaken to determine the ability of laser fluorescence to detect and monitor repeatedly mineral changes adjacent to amalgam restorations.

**DETERMINATION OF THE ABILITY OF LASER FLUORESCENCE TO QUANTIFY MINERAL LOSS**

**Method**

Eighty-four 3-mm-diameter cores were cut from the labial surfaces of extracted bovine incisor teeth. We mounted these specimens individually in polymethylmethacrylate resin prior to grinding them to remove the outer 50-100 μm of the enamel surface. Specimens were then polished by means of a slurry of 0.05-μm alumina polishing compound and water. We washed, ground, and polished specimens thoroughly in an ultrasonic bath containing de-ionized water and inspected them using a dissecting microscope to ensure that the specimens had no obvious defects at the enamel surface.

We created an exposed window of ground and polished enamel on each specimen by placing a piece of 2 mm x 0.8 mm adhesive tape in the center of the enamel surface. The remainder of the specimen was covered with two applications of acid-resistant nail varnish. When the varnish had set, the adhesive tape was removed and the specimens numbered.

Specimens were divided randomly into 7 equal-sized groups and exposed to a demineralizing solution which contained 0.1 mol/L lactic acid and 0.2% Carbopol 907™ (B.F.Goodrich Co. Chemical Group, Avon Lake, OH), 50% saturated with hydroxyapatite, and adjusted to pH 5 by NaOH (White, 1987) for 0, 2, 4, 8, 12, 16, and 24 hrs, respectively. Specimens in demineralizing solution were stored at 37°C.

After demineralization, specimens were analyzed randomly by laser fluorescence (Fig. 1). A mixed 488- and 514-nm Argon laser source (PCA+, HGM Inc., Salt Lake City, UT) was used to deliver approximately 0.08 Wcm⁻² at the specimen surface via a 300-μm-diameter quartz fiber. We captured images of specimens exposed to the Argon laser using a CCD camera (Panasonic GP-KS152, Panasonic Com-

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![Fig. 1—Schematic diagram to show apparatus for laser fluorescence measurements.](image-url)

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**Fig. 2—Scatter plots to show the change in laser fluorescence, histological and transverse microradiographic measurements with increased exposure to demineralizing fluid. (Note that ‘r’ values represent the correlation coefficients based on a linear correlation between x and y ordinates).**

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DETERMINATION OF THE ABILITY OF LASER FLUORESCENCE TO QUANTIFY MINERAL LOSS

Fig. 3—Graphs to demonstrate the relationship between laser fluorescence, histology and transverse microradiography for the measurement of mineral loss.

Fig. 4—Schematic diagram to demonstrate the preparation of specimens for laser fluorescence detection of remineralization with verification by transverse microradiography.

munications systems Co., Secaucus, NJ) in conjunction with a 540-nm high-pass filter and stored on a PC prior to analysis with customized software (Inspektor Research Systems BV, Amsterdam, The Netherlands). Analysis of images has been described previously (de Josselin de Jong et al., 1995). Five images were captured for each specimen. We calculated a mean % change in fluorescence radiance for each specimen, using individual % change in fluorescence radiance data from each of the 5 images.

Specimens were sectioned by means of a Silverstone-Taylor hard-tissue microtome (Scientific Fabrications Co., Lafayette, CO). Three sections, approximately 100 μm thick, were cut from each specimen, and 1 section, without significant cracking or artefacts, was chosen for analysis by TMR. Specimens were prepared as shown in Fig. 4. This involved partial coverage of each specimen with two pieces of adhesive tape. The remaining exposed enamel was then etched for 15 sec with 37% orthophosphoric acid gel prior to transmitted light using an Olympus microscope (Model BH-2) at 40x magnification so that the lesion depth in microns could be determined. Lesion depth was defined as the deepest extent of the demineralized tissue.

Results

Eighty-four specimens were analyzed by laser fluorescence. However, due to problems in the creation of thin sections, only 63 specimens were suitable for analysis by TMR and histology.

Scatter plots of data for individual specimens are shown in Fig. 2. Comparisons of specific data sets along with correlation coefficients are shown in Fig. 3. These data show a linear correlation between laser fluorescence measurements and both histological lesion depth and transverse microradiographic measurements (r = 0.70, 0.74, and 0.83, respectively) for lesions created by up to 24 hrs of exposure to demineralizing fluid. The negative Δz values recorded by the TMR technique for exposure times of < 8 hrs illustrate some of the difficulties of using this method to determine mineral loss for very early lesions.

DETERMINATION OF MINERAL CHANGES IN SMALL ENAMEL SPECIMENS USED IN AN IN SITU APPLIANCE WITH LASER FLUORESCENCE USED IN CONJUNCTION WITH AN EXTERNAL ENAMEL REFERENCE

Method

Sixteen 3-mm-diameter bovine enamel specimens were created for this experiment. We mounted all specimens in polymethylmethacrylate and ground and polished them to remove the outer 50-100 μm of enamel, as outlined previously.

Specimens were prepared as shown in Fig. 4. This involved partial coverage of each specimen with two pieces of adhesive tape. The remaining exposed enamel was then etched for 15 sec with 37% orthophosphoric acid gel prior to
coverage with a thin layer of opaque fissure sealant, which was light-cured for 60 sec. The adhesive tape was removed, and the specimens were divided randomly into two equal-sized groups which were placed in the demineralizing solution described previously for either 24 or 72 hrs. Once demineralization was complete, each section had a wide demineralized "test" lesion and a narrower demineralized "control" lesion. The area designated as the "control" lesion (Fig. 4) was covered with a further application of fissure sealant. A thin section was cut from one end of each specimen across the two lesions. We analyzed this by TMR to verify that the two lesions on each specimen were approximately the same size. All specimens were then analyzed by laser fluorescence in conjunction with an external sound enamel reference.

We made the external sound enamel reference using the labial surface of a bovine incisor which had been ground and polished in the central part to produce a flat, smooth surface. A small hole, of sufficient width to admit the 3-mm-diameter demineralized enamel specimens, was cut through the bovine incisor, to one side of the central flattened area (Fig. 5).

We made baseline laser fluorescence measurements using the argon laser described previously. However, on this occasion, the 300-μm-diameter quartz fiber was replaced with a 600-μm-diameter fiber to permit a larger area of the specimen and enamel reference to be illuminated at the same time. Images of specimens within the hole cut in the enamel reference were captured by the same high-pass filter and CCD camera system described previously. Captured images required manipulation before they could be analyzed with the customized software. A small piece of the "test" lesion image was "cut" and "pasted" onto the area of sound enamel. Analysis of this "cut" piece of "test" lesion could then be performed in the usual manner (de Josselin de Jong et al., 1995). Five images of each specimen were captured. A mean % change in fluorescence radiance for each specimen was determined from information derived from these images.

Once baseline laser fluorescence measurements had been made, specimens were exposed to an 18-day remineralization protocol (Schemehorn et al., 1994) and repeat laser fluorescence measurements made in the same manner on days 5, 8, and 18. Laser power, source-to-specimen distance, angle of incidence of laser light, and CCD camera aperture were constant for each measurement.

When the 18-day remineralization period had finished, we sectioned specimens across both the test and control lesions using the Silverstone-Taylor hard-tissue microtome to produce thin sections (approx. 100 μm) suitable for analysis by TMR. Between 1 and 3 sections were cut for each specimen. A mean value for mineral loss measurements (Δz) was calculated for both the "test" and "control" lesions for each specimen based on the number of sections suitable for analysis.

Results
Only 10 out of 16 specimens were sectioned successfully to facilitate microradiographic analysis. However, these specimens were divided equally between the 24- and 72-hour demineralization groups. The results of laser fluorescence measurements over the 18-day remineralization period are shown in Fig. 6. With regard to TMR measurements, the mean "control"
lesion $\Delta z$ measurements (vol% mineral x $\mu$m) at the end of the 18-day remineralization period were 1557 (SD 231) and 2506 (SD 367), whereas the mean “test” lesion measurements were 1229 (SD 131) and 1987 (SD 243) for the 24-hour and 72-hour lesions, respectively.

Analysis of variance (ANOVA) of the laser fluorescence results demonstrated significant remineralization over the 18-day remineralization period ($p < 0.0001$ for the 24- and 72-hour lesions, respectively).

Paired $t$ tests demonstrated significant differences between “test” and “control” lesions on the same specimen for TMR $\Delta z$ measurements of 24- and 72-hour lesions at the end of the remineralization period ($p = 0.03$ and 0.01, respectively).

**THE ABILITY OF LASER FLUORESCENCE TO DETECT AND MONITOR LONGITUDINALLY THE MINERAL LOSS ADJACENT TO AMALGAM RESTORATIONS**

Three pilot studies undertaken at OHRI to investigate the application of laser fluorescence to detect and quantify the mineral loss adjacent to dental restorations have used amalgam as a restorative material, since it has a profoundly different appearance compared with enamel or dentin. Furthermore, amalgam does not transmit light, which may have some bearing upon the observation of fluorescence due to laser-induced light scattering. The first pilot study aimed to determine if laser fluorescence could detect demineralization adjacent to an amalgam restoration. The aim of the second pilot study was to determine the relative ability of laser fluorescence and visual inspection to detect early (< 24-hour exposure to demineralizing fluid) demineralization adjacent to an amalgam restoration. The third pilot study aimed to determine the ability of laser fluorescence to observe changes over time in the mineral content of caries lesions adjacent to amalgam restorations. The nature of these changes was verified by TMR.

**Pilot study 1—detection of demineralization**

This study involved the natural buccal surfaces of 20 human molar teeth with small amalgam restorations. Artificial caries lesions were created around the amalgam restorations in 10 teeth. Laser fluorescence observations using the Argon laser and CCD camera in conjunction with the 540-nm high-pass filter enabled two examiners to determine the presence or absence of demineralization. Sensitivity and specificity values for caries detection of between 0.85 and 1.00 were obtained by the examiners (Benedict et al., 1996). However, the criterion for successful lesion creation in this study was a densely white opaque lesion at the periphery of the amalgam restoration created by up to 200-hour exposure to the demineralization solution. Such a lesion could be seen easily by unaided visual inspection and, therefore, was an equivocal test of the laser fluorescence system for caries detection.

**Pilot study 2—relative detection of early demineralization**

This study, and pilot study 3, were made possible by advances in the lesion analysis software. An initial requirement for quantification of mineral loss by laser fluorescence was the presence of sound tooth tissue all around the caries lesion. Clearly, this presented a problem for analysis of caries adjacent to an amalgam restoration. The study described earlier in this paper demonstrated that a sound tissue reference did not need to be an integral part of the specimen. However, recent modifications to the analysis software permitted the comparisons of carious and sound tissue from different parts of the same image without the requirement of sound enamel all around the lesion.

To determine the ability of laser fluorescence to detect demineralization adjacent to amalgam restorations, we divided 50 small (approx. 0.5 x 1.0 mm) exposed windows of ground and polished bovine enamel, each of which was adjacent to a recently placed amalgam restoration, randomly into 5 equal-sized groups and exposed them to demineralizing fluid (White, 1987) for 0, 4, 8, 12, and 24 hrs, respectively. Two dentists assessed laser fluorescence images on a PC monitor to determine the presence or absence of demineralization. The same dentists also examined the speci-
mens using a 60-W light bulb in a desk lamp for illumination to determine, again, the presence or absence of demineralization.

Lesion presence was defined by exposure to demineralizing fluid, and the examiners achieved sensitivities and specificities of between 0.80 and 0.90 for the laser fluorescence technique and 0.40 and 1.0 for the examination using desk lamp illumination (DeSchepper et al., 1996). This wide variation in sensitivity and specificity values for detection of demineralization by means of the desk lamp may be a reflection of insufficient examiner calibration prior to the study. However, the results did show some potential for the laser fluorescence technique to detect early mineral loss.

**Pilot study 3—longitudinal changes in mineral content**

In this study, seven 6-mm-diameter, ground and polished bovine enamel specimens, each with a recently placed amalgam restoration and an exposed 0.5-mm-wide window of enamel around the restoration, were demineralized for 96 hrs. Two 150-µm-thick sections across the amalgam and the adjacent lesions were cut from each specimen. Both of the cut surfaces of each section were varnished and a baseline microradiograph taken. The sections and the remainder of the bulk specimen were subject to a remineralizing protocol (Schemehorn et al., 1994) for 17 days. Laser fluorescence and TMR measurements of mineral loss were made at regular intervals over the 17-day remineralization period.

Results demonstrated significant remineralization of the bulk specimens measured by laser fluorescence (ANOVA p < 0.001) and significant remineralization of the lesions adjacent to the amalgam restorations in the thin sections (ANOVA p < 0.001) measured by TMR (Hall et al., 1996). A scatter plot of the laser fluorescence and TMR measurements from each specimen over the 17-day remineralization period is shown in Fig. 7. The linear correlation coefficient between these two sets of data was 0.66.

**DISCUSSION**

Laser fluorescence with a low-power Argon laser is a repeatable, non-destructive technique which can quantify early mineral loss due to dental caries. Although definitions of early mineral loss may vary, we have observed a linear correlation between laser fluorescence measurements and TMR for lesions up to 200 µm deep (unpublished results).

Current difficulties with the technique include access to lesions on occlusal and interproximal surfaces, the difficulty in distinguishing between hypomineralization due to conditions such as fluorosis and hypomineralization due to dental caries, the effect of incorporation of extrinsic staining into a caries lesion, and the current ease of use of laboratory and clinical equipment.

The occlusal surface has a complex anatomical morphology with complex light-scattering patterns from enamel and underlying dentin. This results in poor contrast between demineralized and undemineralized tissue, which may hinder the early detection of mineral loss (ten Bosch, 1996). Occlusal caries detection remains a difficult problem. A possible application of laser fluorescence may be to determine fluorescence radiance readings for caries-free occlusal surfaces as a reference against which to compare suspicious occlusal surfaces. However, the presence of staining in the occlusal fissure could result in a high number of false positives.

With regard to interproximal caries, the bulk of sound tooth tissue, which has its own inherent light-scattering properties, is superimposed on demineralized tissue when viewed from either the embrasure between teeth or the marginal ridge at the occlusal surface. Additionally, scattering patterns may be further confused by extraneous light from the opposing mesial or distal surface of an adjacent tooth. The possibility exists of preventing light transmission from tooth to tooth by the placement of a suitable medium between the teeth. However, the best arrangement for the sites of the incident light source and observed fluorescence is not known. Suggestions have been made that quantitative laser fluorescence measurements for this type of lesion may develop along lines similar to those of FOTI (ten Bosch, 1996, quoting Vaarkamp et al.).

Hypomineralization of enamel due to fluorosis or other developmental defects which may present in a similar manner may be extremely difficult to distinguish from hypomineralization due to dental caries. It is suggested that the only discriminating factors between these two causes of hypomineralization will be the distribution of the lesion and its ability to change over a period of time when subject to either remineralizing or demineralizing conditions. Typically, dental caries occurs only in areas of plaque stagnation, whereas the distribution of other forms of hypomineralization is not constrained by such conditions. In addition, dental caries is a dynamic process, and mineral loss may progress or regres. To the authors’ knowledge, this is not the case for other forms of hypomineralization once the tooth has erupted.

Staining at the surface of a tooth may be a result of adsorption of stain onto the undemineralized surface of the tooth or the absorption of stain into a subsurface porosity such as dental caries. Stained caries lesions may result in particular difficulties for diagnosis and monitoring by laser fluorescence. Adsorbed stain may often be removed by conventional professional prophylaxis procedures, unlike staining incorporated into a subsurface lesion. The use of different thresholds for caries detection by laser fluorescence may provide some quantitative data for the changes in the degree of mineral loss, and the nature of the response to de- or remineralizing protocols could potentially represent a diagnostic test for stained lesions. However, there is clinical anecdotal evidence that a stained, non-cavitated lesion on a smooth surface is often an indication that the caries process is not progressing and may have arrested (Thylstrup and Fejerskov, 1994). The mineral content of such lesions is not likely to change rapidly with the onset of de- or remineralizing conditions.

Secondary caries diagnosis and the rationale for replacement of restorations have recently been reviewed by Kidd (1996), who considered secondary caries in two parts. These were “an ‘outer lesion’ formed on the surface of the tooth as a result of primary attack and a ‘wall lesion’ which will only be seen if there is leakage of bacteria, fluids,
molecules or hydrogen ions between the restoration and the cavity wall.” Kidd (1996) concluded, from a review of the literature in addition to her own clinical and laboratory studies, that marginal ditching was not a good indicator of secondary caries, although wider ditches resulted in significantly more bacteria at the underlying enamel-dentin junction (EDJ) than narrower ditches or intact restorations. With regard to color change adjacent to restorations, there was no relationship between the color of enamel adjacent to amalgam restorations and the degree of infection at the underlying EDJ, although for tooth-colored restorations, stained margins did show the presence of significantly more outer and wall lesions compared with unstained margins. Furthermore, it was possible for residual caries left behind at the EDJ during cavity preparation to take up extrinsic stain, thereby mimicking secondary caries. According to Kidd, only a cavitated outer lesion was a reliable indicator of active secondary caries around both tooth-colored and amalgam restorations.

In relation to laser fluorescence measurements, secondary caries measurements are problematic. One potential advantage for laser fluorescence is its ability to monitor the changes in the appearance of secondary caries over time. Obviously, access to the lesion is of overriding importance, since secondary caries occurs most frequently at the cervical aspects of amalgam and tooth-colored restorations (Mjör, 1985). However, a combination of threshold changes, to reduce the influence of extrinsic staining on laser fluorescence quantification of mineral loss, and longitudinal observation may determine the activity of an outer lesion in relation to a clinically suspicious (ditched) restoration. The diagnosis, by laser fluorescence, of an active secondary caries lesion prior to cavitation may result in earlier intervention by the dentist and less tissue destruction if it is necessary to replace the restoration.

Current laboratory and clinical apparatus for making measurements by this technique are sensitive to changes in illumination intensity and the angle of incident light as well as changes in camera aperture or focal distance. With laboratory studies, these factors must remain constant. This is somewhat easier with the portable clinical system described by Angmar-Mansson (these proceedings), since the relationship between camera and incident light source may be fixed. However, this device requires training and experience for it to be used competently, and, at the present time, only caries on buccal and lingual surfaces of teeth can be reliably detected and quantified. The incorporation of many of the components of this system into an intra-oral camera format will significantly improve the clinical handling of the system.

Advances in laser fluorescence methodology to quantify and monitor longitudinally mineral loss have been made through laboratory studies, such as those described above, as well as by clinical studies (Angmar-Mansson, these proceedings). The advent of a portable device to capture images and illuminate teeth by the use of homogenous blue light instead of an argon laser has resulted in improved clinical application and the technique being renamed light-induced fluorescence. The findings of laboratory studies, such as those outlined in this paper should be now reproduced by means of either intra-oral models or in vivo clinical studies. Further work is also required to determine the application of the technique to occlusal and interproximal caries detection and quantification, and to detect and monitor secondary caries in vivo.

**CONCLUSIONS**

From the results presented in this paper, it was concluded that laser fluorescence was a suitable technique for the quantification of early mineral loss. The size of the lesion may be quite small, and the sound enamel surrounding such a lesion, which was formerly a requirement for laser fluorescence, may be substituted by the use of a piece of reference enamel and a degree of image manipulation prior to analysis. Additionally, pilot data have demonstrated that early artificial lesions adjacent to amalgam restoration can be detected by laser fluorescence, and changes in the mineral content of such lesions can be observed longitudinally. The nature of these changes has been verified by TMR.

**REFERENCES**


