UvA-DARE (Digital Academic Repository)

Quantified light-induced fluorescence, review of a diagnostic tool in prevention of oral disease

de Josselin de Jong, E.; Higham, S.M.; Smith, P.W.; van Daelen, C.J.; van der Veen, M.H.

Link to publication

Quantified light-induced fluorescence, review of a diagnostic tool in prevention of oral disease

Elbert de Josselin de Jong,[1,2] Susan M. Higham,[1] Philip W. Smith,[1]
Catherina J. van Daelen,[1] and Monique H. van der Veen[1,2]
[1]*School of Dental Sciences, University of Liverpool, The Edwards Building, Daulby Street, Liverpool, L69
3GN, United Kingdom*
[2]*Academic Centre for Dentistry Amsterdam, Vrije Universiteit en Universiteit van Amsterdam, Louwesweg 1,
1066 EA, Amsterdam, The Netherlands*

Diagnostic methods for the use in preventive dentistry are being developed continuously. Few of these find their way into general practice. Although the general trend in medicine is to focus on disease prevention and early diagnostics, in dentistry this is still not the case. Nevertheless, in dental research some of these methods seem to be promising for near future use by the general dental professional. In this paper an overview is given of a method called quantitative light-induced fluorescence or (QLF) in which visible and harmless light excites the teeth in the patient's mouth to produce fluorescent images, which can be stored on disk and computer analyzed. White spots (early dental caries) are detected and quantified as well as bacterial metabolites on and in the teeth. An overview of research to validate the technique and modeling to further the understanding of the technique by Monte Carlo simulation is given and it is shown that the fluorescence phenomena can be described by the simulation model in a qualitative way. A model describing the visibility of red fluorescence from within the dental tissue is added, as this was still lacking in current literature. An overview is given of the clinical images made with the system and of the extensive research which has been done. The QLF™ technology has been shown to be of importance when used in clinical trials with respect to the testing of toothpastes and preventive treatments. It is expected that the QLF™ technology will soon find its way into the general dental practice. © *2009 American Institute of Physics*. [DOI: 10.1063/1.3116138]

I. INTRODUCTION

It is a tradition in dentistry that the dentist looks into the patient's mouth using a dental mirror while holding the dental explorer in the other hand under a powerful dental light that sits on top of the dental chair.[1] The dentist uses these two devices together with occasional x-ray images of the teeth to diagnose the cariogenic status of teeth in the oral environment and has been using this type of diagnostics for over a hundred years. To keep track of changes in the mouth mainly due to invasive treatment by the dentist, a dental status chart is used. In modern practices some digitization has taken place in the form of sophisticated software storing images from intraoral cameras, digital cameras, and x rays in a patient database in the dental practice. However no evident progress has been made in the field of detecting and tracking changes in oral disease phenomena in a quantitative way. It might seem surprising to the reader that detecting techniques have been introduced in the dental research world but have made no real impact on the daily dental practice. One exception might be the DIAGNOdent Pen (KaVo, Biberach, Germany), which is a penlike device that can provide quantitative information at specific positions on the carious state of teeth. This device has found its way to the dental office but is mainly used to indicate invasive treatment to be done by the dental professional and is rarely used in prevention.

This paper presents a review of a dental diagnostic technique called quantitative light-induced fluorescence (QLF) with which oral anomalies such as early and advanced dental caries and bacterial activity on and in the teeth can be detected and quantitatively followed in time. Harmless, visible blue light is used to illuminate the oral environment. The concept was first introduced by Bjelkhagen *et al.*[2] In 1995 (Ref. 3) a prototype clinical device, based on the original concept, called QLF™ was introduced. Since then the method has been widely tested, improved, and used in the dental research world.

II. QLF™ BASIC BACKGROUND

The principle[4] of QLF™ is based on fluorescence where blue light (peak intensity of 405 nm) illuminates and excites tooth tissue. A low cutoff filter ($\lambda > 520$ nm) is used in front of an intraoral charge coupled device (CCD) camera lens to exclude the excitation beam from the image made by the camera (see Fig. 1). By means of a frame grabber digitized live images are stored on a personal computer and displayed real time on a screen.

Basically two types of fluorescence can be observed: green fluorescence (GF) which is generated by tooth tissue and is used to detect very early caries, i.e., incipient caries or so-called white spots in teeth which become visible due to an

---

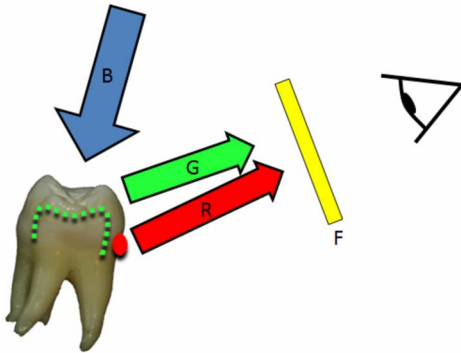a)Electronic mail: e.dejosselindejong@inspektor.nl.

FIG. 1. (Color online) QLF™ concept: a blue excitation light beam (B) with peak intensity at 405 nm illuminates a tooth; a yellow filter (F) transmits green (G) and red (R) fluoresced photons to the eye or camera.

observed GF loss, and red fluorescence (RF) which indicates bacterial activity in and on the teeth, in between the teeth, and on and under the gums (gingiva).

A. Green fluorescence and white spots

GF is an intrinsic property of hard dental tissues. Healthy teeth fluoresce green. At carious sites a loss of GF is observed. The observed fluorescence loss from carious tooth tissue is not caused by a reduction in fluorophores but by a change in light scattering properties. With QLF we observe a white spot lesion as a loss in GF radiance with respect to the fluorescence radiance of the surrounding sound enamel, see circled area in Fig. 2. The fluorescence radiance drop can be explained by the increased scattering coefficient of the lesion compared to that of sound enamel.[5,6] An increase in scattering coefficient implies a decrease in mean free photon path length, and hence the chance of a photon being absorbed by a fluorophore and a fluorescent photon being emitted is lowered. The distribution of the green-emitting fluorophores is higher for dentine than for enamel and greatest at the dentine enamel junction (DEJ). Where a lesion exists, the light travels shorter distances into the tooth, and the view on the DEJ is blocked. As a result we see a dark spot of reduced fluorescence surrounded by bright GF from the sound tooth areas (Fig. 3). The measure of GF is an indirect measure of enamel porosity or lesion severity.[7] Table I shows an overview of sensitivity and specificity experiments done by multiple groups in the last years. There seems to be no significant difference between QLF and visual examination. However
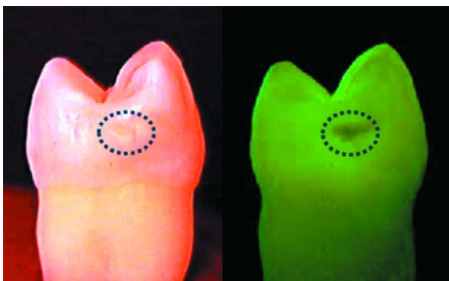

FIG. 2. (Color online) Left: white light image of a premolar visualizing an initial caries lesion visual as a white spot. See encircled area Right: fluorescence image shows GF: the white spot is now shown as a dark spot.
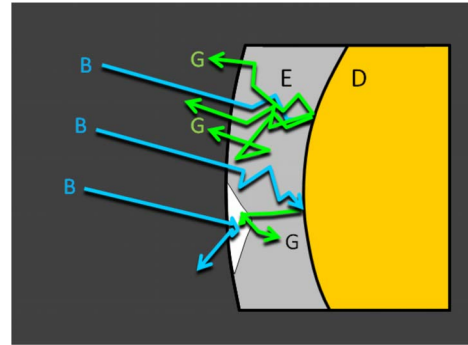

FIG. 3. (Color online) Green Fluorescence (GF) principle shown schematically: blue photons (B) enter the tooth and scatter in enamel and fluoresce green (G) at the dentine (D) and enamel (E) junction DEJ. Where there is a white spot below in the enamel part, blue rays scatter and will not reach the DEJ; fluoresced photons will scatter back at the white spot, both effects resulting in a visual dark spot when viewed with QLF™.

QLF allows following longitudinal changes in white spots quantitatively in time. It can be argued that sensitivity and specificity values for QLF are not valid parameters to evaluate QLF as it is not meant to be a caries detection method.

B. Red fluorescence and presence of bacteria

Excitation of red extrinsic fluorophores from bacterial metabolites with blue light causes red or orange fluorescence.[8,9] Shown in Fig. 4 is a clinical image of a tooth in the mouth of a patient where the tooth surface and gingiva are partly covered by calculus. These bacterial metabolites, porphyrins and also possibly extrinsic and intrinsic polysaccharides, are present in old (anaerobic) plaque as well as in calculus. The RF is also found in more advanced lesions (dentinal lesions) and progressive white spots associated with orthodontic treatment (porous surface structures where large molecules can penetrate) (see Fig. 5). So far the bacteria identified as producing red fluorescing metabolites are mainly related to periodontitis, as can be seen in Table II, and these bacteria are mostly haemin dependent. To date the presence of RF is considered a property of matured biofilm or plaque, which is associated with poor oral hygiene and as such with both caries[10–12] and periodontitis.[13]

C. Monte Carlo simulation model

Both GF and RF can be explained by using the Monte Carlo technique to simulate the photon path in tooth tissue. de Josselin de Jong *et al.*[5] presented a Monte Carlo computer program simulating the light paths in sound and carious enamel placed on a white or a black background. The computer simulations showed that the GF as assessed by QLF can be explained using the scattering properties of enamel only. The same Monte Carlo simulation program can also be used to predict the influence of differences in scattering coefficient or thickness of the sound enamel on the appearance of a lesion. Similarly the appearance of RF can be simulated for lesions of different sizes and depths underneath the tooth surface.

The simulation model was based on a computer-simulated block of sound enamel ($g=0.68$, $s=10$ mm^{-1},

TABLE I. Sensitivity and specificity for the detection of carious lesions with QLF or visual. Both methods are used subjectively.

Authors	Model	Surfaces	Lesion definition	Method	Sensitivity	Specificity
ten Cate		Buccal/lingual[b]	Caries/not caries	QLF	0.79	0.75
et al. 2000[a]	*In vivo*	Occlusal[b]		QLF	0.77	0.71
Ando *et al.* 2000[c]	*In vitro*	Occlusal	depth $<$ 0.2 mm	QLF	0.93	0.7
				Visual Ekstrand	0.87	0.66
			Into dentine	QLF	0.92	0.56
				Visual	0.9	0.55
Ferreira Zandoná				QLF	0.51	0.77
et al. 1998[d]	*In vitro*	Occlusal	Caries/not caries	Visual (color)	0.49	0.69

[a]Reference 48.
[b]Assessments were performed on deciduous teeth (cuspids, first and second molars). The clinical assessment was done *in vivo*. After exfoliation actual caries state was determined.
[c]Reference 49.
[d]Reference 34.

$a = 0.1$ mm^{-1}). Within this block, a carious lesion was simulated ($g = 0.68$, $s = 250$ mm^{-1}, $a = 0.1$ mm^{-1}), where g was defined as the average cosine of the scattering angle of light, s was defined as the linear scattering coefficient, and a as the linear absorption coefficient. The values for g, s, and a at $\lambda = 550$ nm were all computed from the data presented by ten Bosch[6] and Zijp *et al.*[14] The linear scattering coefficient and linear absorption coefficient of white spot enamel were estimated from these data.

The photons were generated randomly at the surface over an extended area around the computer-simulated enamel block and were incident perpendicularly to the tooth surface. The area extension prevented artifacts from developing in the computed images. In "fluorescence mode" the photon started as a blue one. After absorption it fluoresced and then became green or red depending on the type of absorber. In "white light mode" the photon was white and did not change color.

The photon continues its path until it is scattered. The azimuth angle (φ) and the scattering angle (θ) with respect to the previous direction were calculated according to

$$\phi = 2\pi * \text{RN},$$

$$\theta = \arccos\left(\frac{1}{2g} * \left[(1 + g^2) - \frac{(1 - g^2)^2}{(1 + g - 2g * \text{RN})^2} \right] \right), \quad (1)$$

which was derived from the Henyey–Greenstein phase function.[15–17] RN was a random number evenly distributed in the range [0,1].

The free path to the next collision, the scattering free path (τ_s), was determined as follows:

$$\tau_s = - \frac{\ln(\text{RN})}{s}. \quad (2)$$

In the computer simulation model the DEJ is simulated by a white background. A photon, which should exit from the bottom of the simulated enamel block, will not proceed further out of the block. Instead, it will scatter isotropically back into the material. In fluorescence mode the photon will also always fluoresce when it hits the bottom of the simulated enamel block. The simulations have shown that this highly fluorescent white background simulating the DEJ provides a simple yet effective model describing the fluorescence pattern seen for early white spot lesions. The model is further supported by experiments on optical path lengths in incipient caries lesions of various degrees.[18,19]
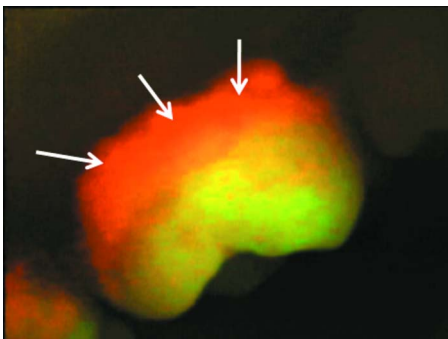

FIG. 4. (Color online) RF. Bacterial metabolites as in cavities and plaque or calculus on the tooth fluoresce bright red. See the areas indicated by the arrows.
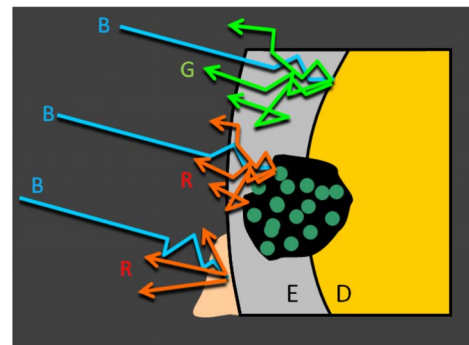

FIG. 5. (Color online) Red (R) and Green (G) Fluorescence schematically: the red fluorescence (RF) is caused by excitation of red extrinsic fluorophores from bacterial metabolites.

TABLE II. Bacteria involved in RF.

Species		Color	Fluorophore	Disease related
Actinomyces gerencseriae	Anaerobe	Red[a]	?	Caries[b]
Actinomyces naeslundii	Anaerobe	Red[c]	Porphyrin[c]	Gingivitis
Actinomyces odontoliticus	Anaerobe	Red[c]	Porphyrin[c]	Gingivitis
Candida albicans	Yeast	Orange[c]	Porphyrin[c]	Candidosis, caries, gingivitis
Fusobacterium nucleatum	Anaerobe	Red[c]	Porphyrin[c]	Gingivitis
Lactobacillus acidophilus	Facultative	Red[a]	?	Caries
Porphyromonas gingivalis	Anaerobe	Red[c]	Porphyrin[c]	Gingivitis
Parvimonas micra	Anaerobe	Red[a]	?	
Veillonella dispar	Anaerobe	Red	Porphyrin	Gingivitis, caries[b]

[a]Reference 10.
[b]Reference 12.
[c]Reference 9.

D. Permanent teeth versus deciduous teeth

The usefulness of QLF for the detection and quantification of white spot lesions in deciduous teeth in comparison to permanent teeth was tested in an *in vitro* experiment[20,21] supported by Monte Carlo modeling.[21] A Monte Carlo simulation of a 4×4 mm^2 illuminated an enamel slab on a highly fluorescent background with a 0.7×0.7 mm^2, 100 μm deep white spot in the center. In that model scattering coefficient of sound enamel s_{SE} was varied between 5 and 160 mm^{-1} (5, 10, 15, 20, 40, 80, and 160 mm^{-1}) and the slab thickness d for each of these scattering coefficients was varied between 500 and 2500 μm in steps of 500 μm. Again a scattering coefficient of the white spot s_{WS} was set to 250 mm^{-1}.[5] Results from the Monte Carlo simulation corresponded well with those of the *in vitro* study comparing lesions in deciduous and permanent teeth. Both studies showed a higher contrast between fluorescence signal from the lesion and fluorescence signal from sound tissue for deciduous teeth than permanent teeth.

E. Red fluorescence in subsurface lesions

RF is observed not only in plaque and calculus found on the surface of teeth but also in advanced dentinal lesions. Since the introduction of fluoride toothpaste in the 1950s, many of these advanced lesions remain unnoticed underneath seemingly intact enamel. To predict if and up to what depth underneath the surface a red fluorescing bacterially infected lesion can be detected a simulation was done on a 2×2 mm^2 block of dry sound human tooth enamel with a thickness of 1 mm. Within this block a cavity with plaque was constructed with a size of 0.7×0.7 mm^2 at a variable depth with respect to the enamel surface extending to the bottom of the enamel. The Henyey–Greenstein phase function (Eq. (1)) was used to derive the path of each photon traveling through the enamel. As previously it was assumed all photons had an average cosine scattering angle $g = 0.68$, a linear scattering coefficient $s = 10$ mm^{-1}, and a linear absorption coefficient $a = 0.1$ mm^{-1}. In this test blue excitation photons entered the tooth tissue perpendicularly at a random position on the outer enamel surface and were scattered around until they left the enamel block or were absorbed, emitted isotropically as a green photon, or hit the cavity block, and were consequently emitted isotropically back into the enamel as a red photon. The number of red (Nr) and green (Ng) fluoresced photons eventually emitting out of the tooth surface was registered and parameter $\Delta R = Nr/Ng$ was calculated. At cavity depths 0, 0.25, 0.50, 0.75, and 0.95 mm ΔR was found to be respectively 570%, 160%, 55%, 15%, and 2%, indicating an exponential-like decrease with cavity
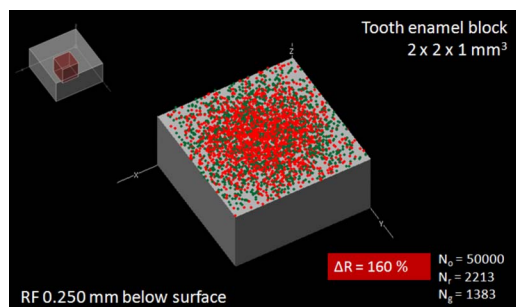

FIG. 6. (Color online) Monte Carlo result of photons which scattered and fluoresced inside a tooth enamel block with inside a block of bacterial metabolites at a depth of 0.250 mm below the surface, as shown at the left top of the figure. The block in the middle shows green and red fluoresced photons that emerged out of the surface. N_o is the number of photons used, N_r the number of fluoresced red photons, N_g the number of fluoresced green photons.
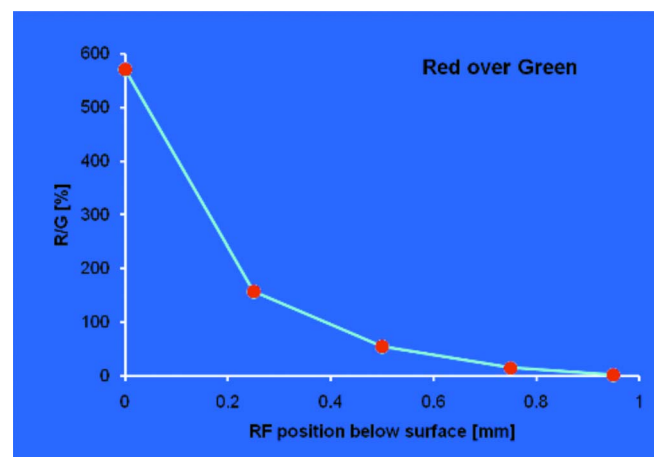

FIG. 7. (Color online) Monte Carlo graph of the red over green number of photons (R/G) that emerged out of the surface in the enamel and bacterial metabolite model of Fig. 6.
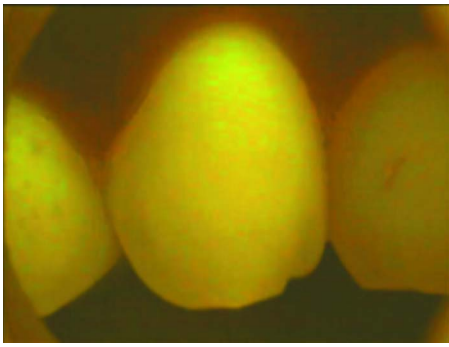
FIG. 8. (Color online) Example of sound and clean teeth as viewed with QLF™.

depth (see Figs. 6 and 7). Hence, it is postulated that a subsurface cavity filled with plaque in human dry enamel up to a depth of 1 mm into the enamel is detectable by the QLF technique.

III. QLF™ INTRAORAL INSTRUMENTATION

Commercially the first QLF system to come on the dental market is called Inspektor Pro™ (Inspektor Research Systems BV, Amsterdam, The Netherlands) and was introduced in The Netherlands in 2004. Figures 8–12 show typical examples of the images which are made with the system. Sound teeth as viewed with QLF™ show an absence of RF and of dark spots (Fig. 11). It is possible to detect removable plaque and calculus which are removable by the dental professional, see Figs. 8 and 10. Figure 11 shows images revealing porphyrines located under the tooth surface. Also the quality of sealants can be checked: Fig. 12 shows an image of a sound sealant and one with a sealant which has been leaking over time.

A. Inspektor Pro™: Quantitative and qualitative assessment

With the Inspektor Pro™ system measurements—the original images—as well as the analyzed images can be shown on a computer screen and can be stored and archived. While the technique was validated both quantitatively by comparison with mineral loss and lesion depth from trans-
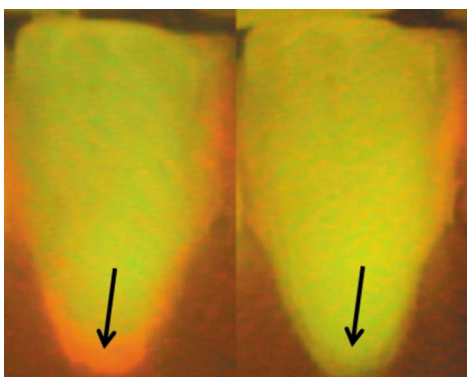

FIG. 9. (Color online) Example of removable signs of bacterial activity. The same element before (left) and after (right) brushing. The arrows point at the areas of interest,
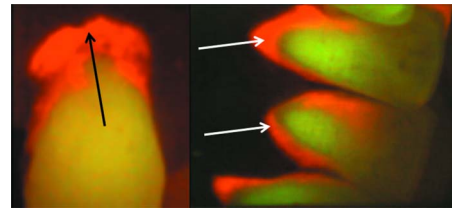

FIG. 10. (Color online) Locations with high concentration of porphyrines are clearly visible as brightly red areas. Plaque is shown in the left image and calculus in the right one.

verse or longitudinal microradiography[22–28] and qualitatively by comparison with visual detection and histology[29–34] the true strength of the technique is not its capability to detect lesions but to follow the oral health over time. The images or series of longitudinal images of a tooth over an extended period in time, can be analyzed for GF loss and presence of RF. This provides valuable information in private practice to the individual but can also be used in clinical trials for testing the efficacy of products.

While the focus in dentistry is slowly shifting from prevention of newly developing caries lesions toward remineralization or stabilization of existing caries lesions, the longitudinal assessment of the remineralization process must be as accurate as for the demineralization process. The remineralization of white spot lesions does not necessarily result in fluorescence gain equal to that lost from the enamel during the initial formation of the white spot lesion. Minerals deposited during remineralization may not follow the crystalline prismatic structure of sound enamel. A recent *in vitro* study comparing fluorescence loss in white spot lesions after initial demineralization and after various remineralization protocols has shown that fluorescence loss for the remineralized lesions and the original lesions have the same linear relation with mineral loss.[35] This, together with the fact that natural caries lesions also show the same relation between fluorescence loss and mineral loss,[27] gives us a good indication that QLF is suited for the assessment of the remineralization process as well.

B. Inspektor TC™: Qualitative assessment

Recently a qualitative device based on the QLF™ technology was introduced. In the Inspektor Pro the yellow filter is built in. With the Inspektor TC™ the yellow filter is applied in the form of adapted glasses that are worn by the observer. Tweaking the filter for this purpose, the contrast
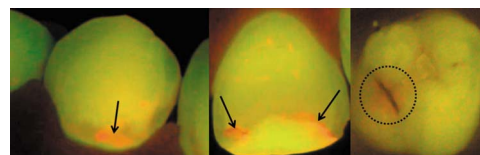

FIG. 11. (Color online) Example of a clear white spot along the gingival margin together with (nonremovable) signs of bacterial activity inside the tooth (left, see arrow). Example of a leaking margin of a composite filling infiltrated with nonremovable plaque (middle, see arrows). Right image: area within the dotted ellipse: occlusal surface showing discoloration at the dark fissure line and signs of bacterial activity underneath the surface. After opening the fissure a dental carious lesion was found underneath.
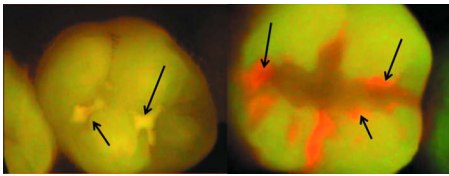
FIG. 12. (Color online) Left: example of a sound sealant: there is no RF visible at the margins of the sealants (see arrows). Right: a compromised sealant where plaque infiltrated the space between sealant and tooth (see regions indicated by the arrows).

between red and GF was enhanced and, as a consequence, the RF (red-over-green) value was increased. Testing of this device in general dental practices has shown that making red fluorescing plaque and calculus visible provides very valuable information not only to the dentist but also to the patient on his or her dental health status. This device is very useful for assessing sealants and restorations with respect to, e.g., leakage and secondary caries.

IV. CLINICAL RESULTS

While the QLF™ technology has been used widely in laboratory studies, the clinical use in trials and general dental practice has spread more slowly. While the initial studies focused on validating the technique by looking at caries progression as well as validation against crude measures of visual inspection, currently the capabilities of QLF as assessment tool of progression or regression of caries have been accepted and we see more studies evolving in which the technique is used. These focus on lesion progression or regression, testing brushing regimes,[36] fluoride varnishes,[37] or, e.g., the removal of fixed appliances at the conclusion of orthodontic treatment.[38] The use of RF during clinical trials has been used for the assessment of sealant integrity,[39] progression of gingivitis,[13] and plaque regrowth studies.[40,41]

V. DISCUSSION

QLF appears to be a technique that has many uses in dentistry. It has been validated both qualitatively and quantitatively and for research purposes the assessment of caries with QLF is to date a well accepted method. Research on the RF seen in QLF images of the oral cavity is taking a flight. Although initially considered a confounding factor when studying incipient caries lesions[29,37,39] the presence or absence of RF seems to be a good indicator of general oral health and an aid in patient understanding.[42] RF can be seen everywhere in the mouth but is found more often in caries sites than healthy sites in the same person.[11] The presence of RF is often noted in the gap between restorative materials and the tooth, which may be an indicator of failure. The absence of RF is also used as indicator when a cavity preparation is clean.[43,44] Current research on RF focuses mainly on bacteria that could be a source for this RF and bacteria interactions and growth conditions.[10,43,45,46] Recently a more clinical approach is found in research of RF in relation to gingivitis, where not the red or GF of the teeth is the object of study but the RF seen from the subgingival plaque growing under the gums.

The use of harmless visual radiance technology makes the inspection of the oral environment on a regular basis ethically a nonissue in contrast to making of x-ray images that do have an impact on the health of patients. With this technology it is possible for the dental professional to focus on the prevention measures such as white spot treatment, the professional cleaning of the oral environment (images before and after), detecting leakage of sealants by just looking at the penetration of bacteria in between sealant and occlusal surface, detecting leakage of fillings before x rays show it, etc.

The technology is also deemed to be instructive and motivational toward the patient as it can be made clear how the health of his or her teeth is improving with time visually as well as in quantitative numbers. This will surely help patients improve on their own health and quality of life.

VI. CONCLUSION

Current systems based on the QLF™ technology are adapted for clinical use, ease of cleaning, and aesthetics within the office. Current software applications are evolving based on clinical application needs and the development of a scientific diagnostic nomenclature for caries, e.g., International Caries Detection and Assessment System (ICDAS).[47] Questions remain about the usability of the method for early caries detection without surgical interventions unless progress is made in reimbursement for this preventive clinical service. Additional research is needed in this area, even though the disclosure and quantification of de- and remineralization are very well documented and validated scientifically. RF of bacterial activity has much promise for the future, both for caries detection and patient education. In conclusion, given its versatility the QLF™ technology is a very promising addition to the dental researcher and the dental practitioner. It has the potential to become a standard of dental care and may well continue to set the trend toward preventive dentistry.

[1] *Oral Health Surveys—Basic Methods*, fourth edition, (WHO Press, Geneva, 1997), p. 39.
[2] H. Bjelkhagen, F. Sundström, B. Angmar-Månsson, and H. Rydén, Swed Dent. J. **6**, 1 (1982).
[3] E. de Josselin de Jong, F. Sundström, H. Westerling, S. Traneaus, J. J. ten Bosch, and B. Angmar-Månsson, Caries Res. **29**, 2 (1995).
[4] B. Angmar-Månsson and J. J. ten Bosch, Dentomaxillofac Radiol. **30**, 298 (2001).
[5] E. de Josselin de Jong, A. F. Hall, and M. H. van der Veen, in *Early Detection of Dental Caries: Proceedings of the 1st Annual Indiana Conference*, Indianapolis, USA, edited by G. K. Stookey (Indiana University, Bloomington, 1996), pp. 91–104.
[6] J. J. ten Bosch, in *Early Detection of Dental Caries: Proceedings of the 1st Annual Indiana Conference*, Indianapolis, USA, edited by G. K. Stookey (Indiana University, Bloomington, 1996), pp. 81–90.
[7] G. K. Stookey, J. Dent. Res. **83**, 84 (2004).
[8] K. König, G. Flemming, and R. Hibst, Cell. Mol. Biol. (Paris) **44**, 1293 (1998).

[9]K. König, R. Hibst, H. Meyer, G. Flemming, and H. Schneckenburger, Adv. Protein Chem. **2080**, 170 (1993).

[10]M. H. van der Veen, R. Z. Thomas, M. C. D. N. J. M. Huysmans, and J. J. de Soet, Caries Res. **40**, 542 (2006).

[11]D. B. Claman, E. J. Leys, and A. L. Griffen, J. Dent. Res. **84**, 3461 (2005).

[12]M. R. Becker, B. J. Paster, E. J. Leys, M. L. Moeschberger, S. G. Kenyon, J. L. Galvin, S. K. Boches, F. E. Dewhirst, and A. L. Griffen, J. Clin. Microbiol. **40**, 1001 (2002).

[13]M. Izu, T. Miyake, M. Uene, H. Tanaka, R. Takashima, N. Nishijima, and M. Kambara, J. Dent. Res. **84**, 1069 (2005).

[14]J. R. Zijp, J. J. ten Bosch, and R. A. Groenhuis, J. Dent. Res. **74**, 1891 (1995).

[15]R. A. Groenhuis, H. A. Ferwerda, and J. J. Ten Bosch, Appl. Opt. **22**, 2456 (1983).

[16]R. A. Groenhuis, J. J. Ten Bosch, and H. A. Ferwerda, Appl. Opt. **22**, 2463 (1983).

[17]L. G. Henyey and J. L. Greenstein, Astrophys. J. **93**, 70 (1941).

[18]C. Mujat, M. H. van der Veen, J. L. Ruben, A. Dogariu, and J. J. Ten Bosch, Caries Res. **38**, 484 (2004).

[19]C. Mujat, M. H. van der Veen, J. L. Ruben, J. J. ten Bosch, and A. Dogariu, Appl. Opt. **42**, 2979 (2003).

[20]M. Ando, M. H. van der Veen, B. R. Schemehorn, and G. K. Stookey, Caries Res. **35**, 464 (2001).

[21]M. H. van der Veen, M. Ando, G. K. Stookey, and E. de Josselin de Jong, Caries Res. **36**, 10 (2002).

[22]S. Al-Khateeb, R. A. Exterkate, B. Angmar-Månsson, and J. M. ten Cate, Acta Odontol. Scand. **58**, 31 (2000).

[23]S. Al-Khateeb, A. Oliveby, E. de Josselin de Jong, and B. Angmar-Månsson, Caries Res. **31**, 132 (1997).

[24]S. Al-Khateeb, J. M. ten Cate, B. Angmar-Månsson, E. de Josselin de Jong, G. Sundström, R. A. Exterkate, and A. Oliveby, Adv. Dent. Res. **11**, 502 (1997).

[25]M. Ando, A. F. Hall, G. J. Eckert, B. R. Schemehorn, M. Analoui, and G. K. Stookey, Caries Res. **31**, 125 (1997).

[26]A. F. Hall, E. DeSchepper, M. Ando, and G. K. Stookey, Adv. Dent. Res. **11**, 507 (1997).

[27]Z. Emami, S. Al-Khateeb, E. de Josselin de Jong, F. Sundström, K. Trollsås, and B. Angmar-Månsson, Acta Odontol. Scand. **54**, 8 (1996).

[28]U. Hafström-Björkman, F. Sundström, E. de Josselin de Jong, A. Oliveby, and B. Angmar-Månsson, Caries Res. **26**, 241 (1992).

[29]J. Kühnisch, S. Ifland, S. Tranaeus, R. Hickel, L. Stösser, and R. Heinrich-Weltzien, Acta Odontol. Scand. **65**, 183 (2007).

[30]A. M. Lennon, W. Buchalla, B. Rassner, K. Becker, and T. Attin, Oper. Dent. **31**, 551 (2006).

[31]J. G. Boersma, M. H. van der Veen, M. D. Lagerweij, B. Bokhout, and B. Prahl-Andersen, Caries Res. **39**, 41 (2005).

[32]M. Ando, C. Gonzalez-Cabezas, R. L. Isaacs, G. J. Eckert, and G. K. Stookey, Caries Res. **38**, 350 (2004).

[33]A. R. Biesbrock, R. K. Chesters, R. P. Ellwood, and S. R. Smith, J. Dent. Res. **83**, 53 (2004).

[34]A. G. Ferreira Zandona, M. Analoui, B. B. Beiswanger, R. L. Isaacs, A. H. Kafrawy, G. J. Eckert, and G. K. Stookey, Caries Res. **32**, 210 (1998).

[35]H. Fujikawa, K. Matsuyama, A. Uchiyama, S. Nakashima, and T. Ujiie, Caries Res. **42**, 37 (2008).

[36]I. A. Pretty and R. P. Ellwood, Caries Res. **41**, 115 (2007).

[37]S. Tranaeus, S. Al-Khateeb, S. Björkman, S. Twetman, and B. Angmar-Månsson, Eur. J. Oral Sci. **109**, 71 (2001).

[38]T. Mattousch, M. H. van der Veen, and A. Zentner, Eur. J. Orthod. **29**, 294 (2007).

[39]R. Heinrich-Weltzien, J. Kühnisch, M. H. van der Veen, E. de Josselin de Jong, and L. Stösser, Caries Res. **37**, 278 (2003).

[40]I. A. Pretty, W. M. Edgar, and S. M. Higham, Br. Dent. J. **197**, 561 (2004).

[41]I. A. Pretty, W. M. Edgar, P. W. Smith, and S. M. Higham, J. Dent. **33**, 193 (2005).

[42]E. Waller, E. de Josselin de Jong, and M. van der Veen, Dent. J. **1**, 8 (2003).

[43]A. M. Lennon, Oper. Dent. **28**, 341 (2003).

[44]A. M. Lennon, W. Buchalla, L. Brune, O. Zimmermann, U. Gross, and T. Attin, Caries Res. **40**, 2 (2006).

[45]R. Z. Thomas, H. C. van der Mei, M. H. van der Veen, J. J. de Soet, and M. C. Huysmans, Oral Microbiol. Immunol. **23**, 7 (2008).

[46]L. Coulthwaite, I. A. Pretty, P. W. Smith, S. M. Higham, and J. Verran, Caries Res. **40**, 112 (2006).

[47]A. I. Ismail, D. Banting, H. Eggertsson, K. Ekstrand, A. Ferreira-Zandona, C. Longbottom, N. B. Pitts, E. Reich, D. Ricketts, R. Selwitz, S. Sohn, G. V. A. Topping, and D. Zero, in *Clinical Models Workshop: Remin-Demin, Precavitation, Caries. Proceedings of the 7th Indiana Conference*, Indianapolis, USA, edited by G. K. Stookey (Indiana University, Bloomington, 2005), p. 161.

[48]J. M. ten Cate, M. D. Lagerweij, and J. S. Wefel, in *Early Detection of Dental Caries II. Proceedings of the 4th Annual Indiana Conference*, Indiana, USA, edited by G. K. Stookey (Indiana University, Bloomington, 2000), p. 231.

[49]M. Ando, H. Eggertson, R. Isaacs, M. Analoui, and G. K. Stookey, in *Early Detection of Dental Caries II. Proceedings of the 4th Annual Indiana Conference*, Indiana, USA, edited by G. K. Stookey (Indiana University, Bloomington, 2000), p. 279.