Optical imaging of dental plaque pH

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ABSTRACT

Tooth decay is one of the most common chronic infectious diseases worldwide. Bacteria from the oral biofilm create a local acidic environment that demineralizes the enamel in the caries disease process. By optically imaging plaque pH in pits and fissures and contacting surfaces of teeth, then medicinal therapies can be accurately applied to prevent or monitor the reversal of caries. To achieve this goal, the fluorescence emission from an aqueous solution of sodium fluorescein was measured using a multimodal scanning fiber endoscope (mmSFE). The 1.6-millimeter diameter mmSFE scans 424nm laser light and collects wide-field reflectance for navigational purposes in grayscale at 30 Hz. Two fluorescence channels centered at 520 and 549 nm are acquired and ratiometric analysis produces a pseudo-color overlay of pH. In vitro measurements calibrate the pH heat maps in the range 4.7 to 7.2 pH (0.2 standard deviation). In vivo measurements of a single case study provides informative images of interproximal biofilm before and after a sugar rinse. Post processing a time series of images provides a method that calculates the average pH changes of oral biofilm, replicating the Stephan Curve. These spatio-temporal records of oral biofilm pH can provide a new method of assessing the risk of tooth decay, guide the application of preventative therapies, and provide a quantitative monitor of overall oral health. The non-contact in vivo optical imaging of pH may be extended to measurements of wound healing, tumor environment, and other food processing surfaces since it relies on low power laser light and a US FDA approved dye.

Keywords: Optical pH measurement, dental imaging, caries detection, oral biofilm, fluorescence medical imaging, scanning fiber endoscope

1. INTRODUCTION

Sugar rich diets, snacking, and poor dental hygiene promote the formation of a biofilm (plaque) that strongly adheres to the dental enamel surface and fosters the evolution of aciduric bacteria. The acid production contributes to demineralization of the exterior tooth enamel which accelerates after the pH drops from neutral to below a critical value for extended time periods, 5.5 pH is a generally accepted threshold. If the locations of maximum plaque acidification can be found, then specific interventions of medicinal therapies can be applied to these locations to manage the bacterial infection and remineralize the enamel (Featherstone 2018)[2]. The significance of this new approach provides a quantitative tool for assisting the dental field to shift from a surgical model based on restorative procedures (waiting until necessary to drill and fill), to a disease treatment model that helps the enamel remineralize.

There is an unmet need to rapidly evaluate the acid production capability of plaque deposits, especially in the pits and fissures of occlusal (biting surfaces) and interproximal (between teeth) regions. Although real-time measurement of plaque pH has been accomplished over 75 years ago using pH electrodes (Stephan 1944)[8], the method is considered too challenging to perform in dental offices, and the physical probe limits access to the highest risk regions of pits, fissures, and interproximal spaces (Carlen 2010)[1]. Custom semi-quantitative pH strips have been successful in a research environment (Carlen 2010)[1], but low-cost paper strips cannot be used in pits and fissures and are not designed for tight interproximal spaces. A non-contact optical imaging method would be ideal, but pH indicator dyes typically require full spectral analysis or multiple biocompatible dyes which limit clinical translation. A simplified spectroscopic approach using a single FDA approved fluorescence dye as the pH indicator has recently been demonstrated in pilot clinical testing (Sharma 2019)[7]. However, spectral analysis that measures pH is from a single spot of LED illumination limits the capability of guiding therapeutic interventions and adds variability to any quantitative analysis over time due to probe placement errors (Zhou 2017)[11].

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To improve clinical utility, we have created a prototype optical pH imaging wand that uses the multimodal scanning fiber endoscope (mmSFE) technology (Lee 2010)[4]. Dental imaging using early mmSFE prototypes have relied on natural sources of fluorescence and the reflectance from blue laser illumination (Zhang 2013[10]; Timoshchuk 2015[9]). To measure pH in vivo, a biocompatible pH indicator dye (fluorescein) is applied as a spray or mouth wash. Instead of spectral analysis based on full spectra of fluorescence emission changes with pH, selected bands were chosen and a ratiometric approach was used to measure pH within the range of 4.5 to 7.3. In addition to guiding the application of remineralization therapies (Featherstone 2018), quantitative analysis of oral plaque pH mapping over time may provide a new predictive measure of the location of caries and provide an overall measure of enamel health over time.

2. METHODS

2.1 Setup

The mmSFE scans the distal end of a single 80 micron diameter optical fiber in a spiral pattern at 10-12 KHz using a custom tubular piezoelectric actuator and a custom lens assembly (Lee 2010)[4]. The vibrating singlemode fiber emits 424 nm light (Nichia laser diode with Thor Labs Fiberport and clean up Semrock Brightline bandpass filter at 420+/-5nm) that is nearly collimated for a forward view from the mmSFE tip. By collecting backscattered reflectance (B-channel) and emitted fluorescence channels (G channel centered at 520 nm and R channel centered at 549 nm) in a ring of multimode plastic optical fibers, three spectral bands of RGB are created after filtering and photomultiplier detection, see block diagram **Figure 1**.

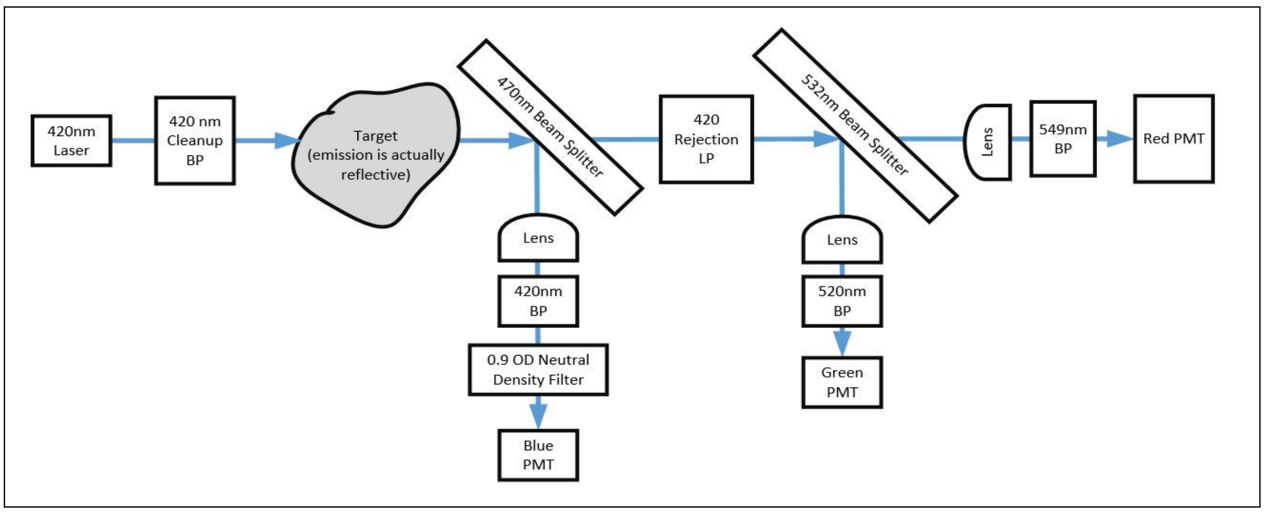


Figure 1: The optical block diagram is displayed in transmission, although the measurements were obtained in the reflectance mode, as shown in Figure 3. The collected fluorescence and reflectance light is collected from plastic optical fibers (250-micron diameter). All bandpass (BP) filters and beamsplitters used were Semrock Brightline, except the 450 long pass (LP) from Edmund Scientific. All photomultiplier tubes (PMTs) were from Hamamatsu Corporation.

After remapping the spiral scan, a full color video display at 30 Hz is generated and multiple individual video frames are saved for post processing (e.g. averaging). The overall mmSFE scope diameter is a continuous 1.6 mm with a rigid tip of 9 mm at the end of a 2-meter long flexible shaft. At a maximum voltage of +/- 15 V, a maximum field of view (FOV) of 100 degrees of full angle is formed. The useful FOV of the mmSFE was reduced due to intensity falloff in the periphery for accurate calculation of pH, as shown in the LabView clinical interface **Figure 2a**.

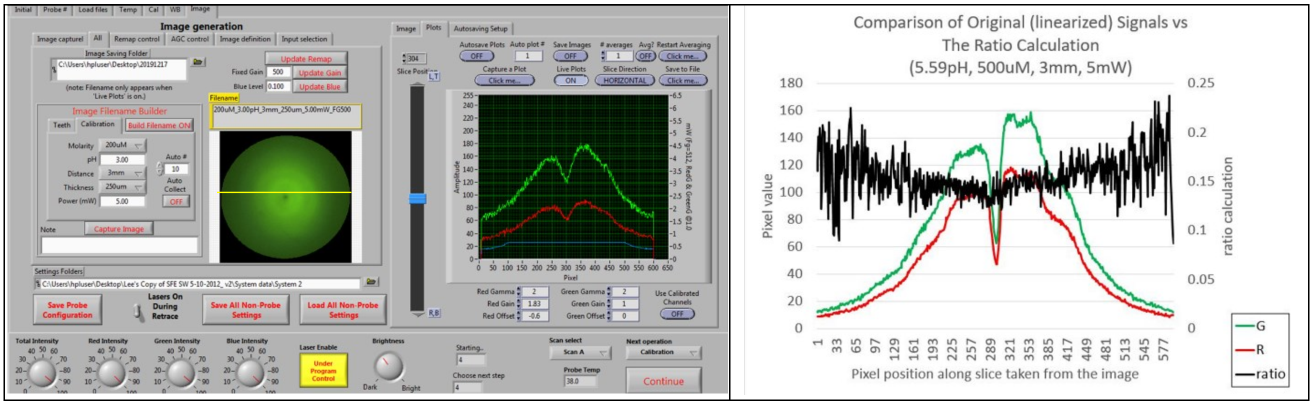


Figure 2. (**a-left**) LabView interface during measurement of pH calibration solutions. On left is single video frame showing 8-pixel horizontal line bisecting mmSFE FOV, and at right is a plot showing intensity values of green (G) and red (R) fluorescence and blue (B) reflectance channel versus horizontal pixel number. (**b-right**). The plot of the ratio calculation of (G-R)/(G+R) over G & R intensity plots across the mmSFE FOV, demonstrating an increase in noise at the periphery of the mmSFE image. Central dip in G and R signals are from photobleaching.

2.2 In vitro Measurement

To optically measure pH in vitro, aqueous solutions of sodium fluorescein dye at 0.5 mM concentrations of known pH using lab meter (Orion Star A211, ThermoScientific) were measured using the mmSFE. Typically pH values range from 4.5 to 7.3 which span the physiologically relevant range for oral biofilms that cover health and carious enamel. Each aqueous pH sample was contained in a rectangular glass cuvette (Fireflysci) with 1-mm solution thickness that was reduced to 0.25 mm by addition of glass coverslips. Ten individual video frames were acquired over a 10 second period to improve spatial signal to noise in the fluorescence channels and integrate any photobleaching effects. To measure spatial resolution, a USAF 1951 test target was imaged with the blue reflectance channel providing the contrast at the operational working distance of roughly 6 mm.

For both in vitro and in vivo measurements, the calculation of pH used the ratiometric fluorescence value of (G - R)/(G + R) which has been shown to be insensitive to photobleaching (**Figure 2b**). To reduce photobleaching and maintain Class IIIR level, the laser emission was limited to ≤5 mW.

2.3 In vivo Measurement

The single human case is the study PI with IRB approval to have laboratory mmSFE measurements taken to develop future clinical testing protocols in oral health. The adult volunteer had no recent history of caries, and was measured 7 months after his last cleaning. At the time of measurement, the volunteer had withheld from tooth brushing and flossing on the selected teeth for 5 days, and fasted for over 3 hours prior to the measurements.

The protocol for the in vivo case study consisted of three rinses as listed below.
Rinse 1: 10 ml of 0.5 mM Sodium Fluorescein for 15 sec.
Rinse 2: 15 ml of 0.3 M sucrose solution (DI water pH 7.4)
Rinse 3: 10 ml of 0.5 mM Sodium Fluorescein for 15 sec.
Images were taken after rinse 1 to obtain resting pH (REST), then a minute after rinse 2 and 3 to measure the drop in pH after the sugar rinse (DROP). In the following 30 minutes, five sets of mmSFE images were taken every 6 minutes to measure the rise in pH (RISE). Each mmSFE measurement consisted of a single video frame acquired from continuous 1 to 2 seconds of 30Hz video. The mmSFE measurement was non-contact, as shown in **Figure 3**. Although the mmSFE was held with fingers to image selected teeth, the same small flexible scope can easily fit onto a pediatric dental wand (Rugg 2016)[6], or be added as an eye that guides the topical application of medicinal therapies, such as fluoride varnish.

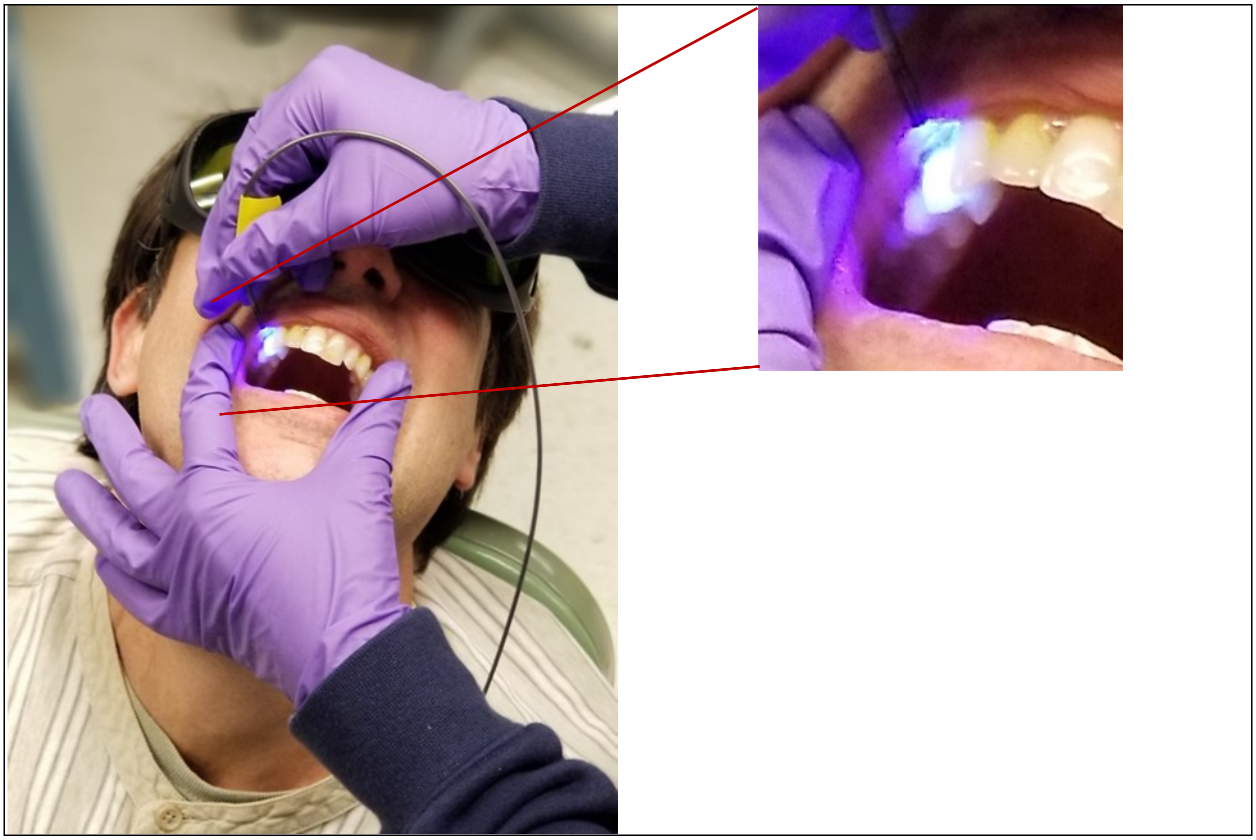


Figure 3. Non-contact optical imaging of oral biofilm pH using the flexible mmSFE device

2.4 Image Processing

The prototype mmSFE optical pH imaging of a single human case was restricted to the lower occlusal (biting) surface of molars and the interproximal spaces of the upper teeth. Following the protocol of 3 rinses, plaque pH was measured first after rinse 1 and labeled as REST. After the rinses 2 & 3 (sugar & fluorescein rinses) a drop in pH on the same surfaces is expected, and labeled as DROP. Even with storing the previous mmSFE image as a reference, it was difficult to acquire matching pairs of images. Thus, post processing of video frames from the same region of occlusal and interproximal regions are analyzed to provide an overall measure of pH change to the enamel surfaces. To illustrate the challenges in imaging processing, we focus on interproximal Teeth 2-3 and 3-4, with an example pair of video frames shown in **Figure 4**.

To maintain the precision of the system, the noise and registration error is minimized in the following process. A 7-by-7 Gaussian filter is applied to reduce the noise during capturing. For the settings of the filter, the window size for convolution is determined by the in-vitro calibration, where a similar size was used. In addition, some pixels will be labeled as risk pixels which means it's possible that the calculation with these pixels involved will have errors than what we can accept. Pixels in some regions might have saturated due to the limited maximum range of fluorescence detection, which results in the loss of information, and it will lead to errors in calculating pH values. These saturated pixels will be labeled in this step. Also, pixels that have too low of intensity are labeled, (the threshold set at <60 pixel value in the R channel), because low intensity will lead to low signal to noise ratio. In the calculation of pH, all labeled pixels are eliminated in the following process and analysis.

For the purpose of determining the pH change of biofilm surrounding teeth, the images having sufficient fluorescence signal should be paired to the same location. This means that the images after processing will have the same geometric location for every pixel, although only overlapping parts of the original images will be displayed. The distance and angle between the mmSFE tip and the surface of teeth has been unconstrained when imaging the plaque. Consequently, the teeth locations in all the images are not the same, making paired-up analysis difficult. For this problem, perspective transformation was applied to calculate the transformation matrix (Haralick 1980)[3].

$$\begin{bmatrix} x'_i \\ y'_i \\ 1 \end{bmatrix} \cong \begin{bmatrix} h_{00} & h_{01} & h_{02} \\ h_{10} & h_{11} & h_{12} \\ h_{20} & h_{21} & h_{22} \end{bmatrix} \begin{bmatrix} x_i \\ y_i \\ 1 \end{bmatrix}$$

Though the transformation of image content is straightforward, the widely used methods for finding interesting points for registration such as SIFT, SURF, and Harris corner detector do not work well due to the lack of features within the mmSFE images of teeth. Hence, four distinct points (the top points of two teeth and the both end of the interproximal groove) are picked manually according to the geometry of the teeth as the **Figure 4** illustrates. Obviously, not the whole image but the edge region of the teeth is the most informative. For the purpose of improving calculation speed, the informative region is extracted. The region is a square shape, and the center of square locates at the center of the groove between two teeth, the length of the square is twice as the distance of the line which links two top points and the center of the groove. The steps involved in pairing images are shown in **Figure 5**. The interesting regions, such as the groove and the edge of the teeth, have been paired precisely; though it is admitted that some parts (always uninteresting parts) after processing may have distortions. Besides, some regions in paired image edge are totally dark because the corresponding area in the reference image doesn't show up in the original pair image, and they are labeled as well, so they will not be taken into consideration when estimating the pH value and the analysis of pH change. Once the image is paired to the reference image and the informative region is extracted, the next step is finding the corresponding location in the paring image for every interesting region pixel in reference image by perspective transformation matrix. In most cases, the corresponding location results are not integers, so we estimate the pixel value by bilinear interpolation.

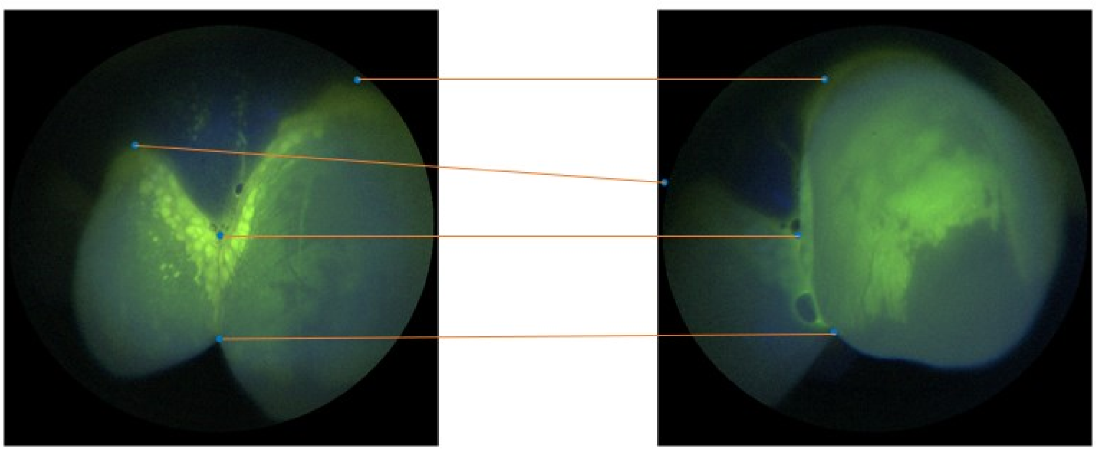
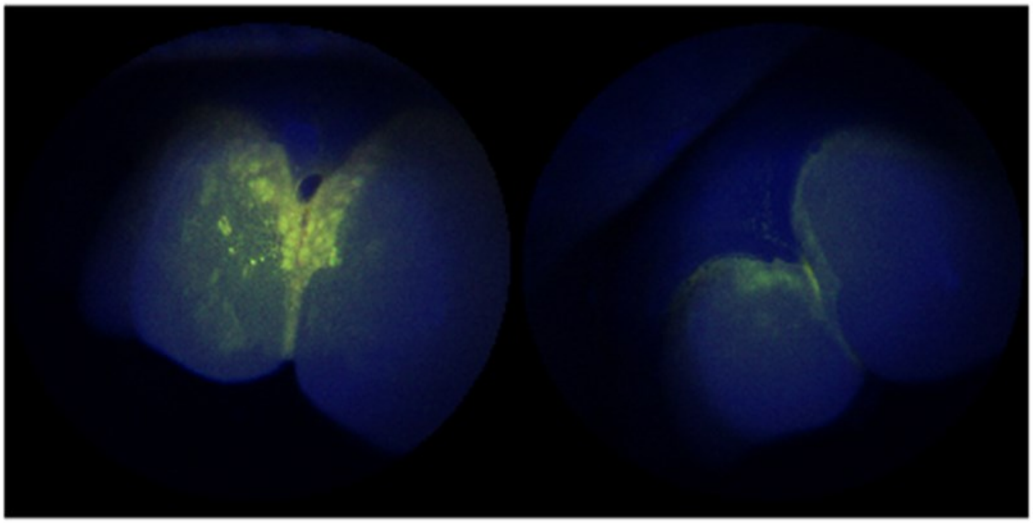
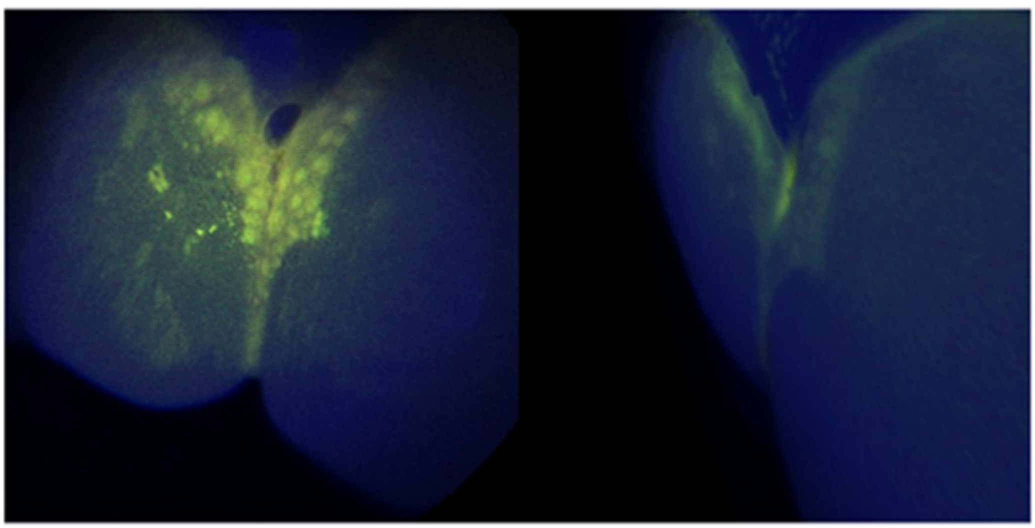


Figure 4. Challenging example of image pairing, teeth 3-4.

After the images from different type (REST, DROP and RISE) are paired, the pH value will be calculated for each pixel in the images except the labeled. A heat map will be created over the background image, which is the reference image in registration for better viewing experience. Besides, the difference between images is calculated, and only in the overlapping area, which means the pixels are not labeled in both images. Though the errors for individual pixels are not eliminated, the average pH value for a region can be considered close to the ground truth pH value. For the comparison between different types, the average pH of the informative region of the image will be calculated.



(a) DROP image (original) (b) REST image (original)



(c) DROP image (region of interest) (d) REST image (paired to DROP)

Figure 5. Choosing example REST image to be paired to DROP image and paired results, teeth 2-3 following procedure of images (a) to (d).

3. RESULTS

3.1 In vitro testing

Using a series of 0.5mM fluorescein buffered solutions with pH between 4.5-7.3 in a glass cuvette (0.25-mm optical-path length), a calibration curve was generated for two ranges of fluorescence signal (High and Medium bands), as shown in **Figure 6**. A non-linear correlation was obtained using the ratio of the two fluorescein channels. Additional measurements were taken to estimate the variability of the measurement, which are shown in the **Table 1** for both High and Medium bands. Highest spatial resolution of mmSFE operation was approximately 30 microns.

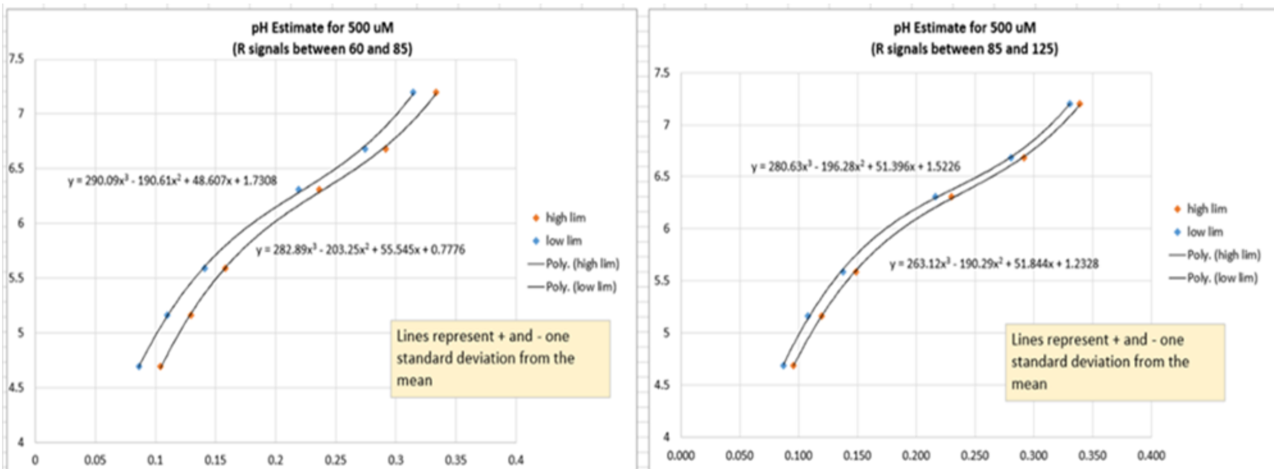


Figure 6. Plots of pH versus (G-R)/(G+R) ratio values from 6 different calibration solutions containing 0.5 mM fluorescein, with the Medium band (left) and High band (right) of fluorescence R signals. Ratio values were not calculated for fluorescence values below R=60.

Table 1: Mean and standard deviation for medium and High Bands.

pH	Medium Band (mean)	Standard Deviation	High Band (mean)	Standard Deviation
7.20	7.23	0.4	7.20	0.2
6.68	6.74	0.2	6.70	0.1
6.31	6.27	0.1	6.29	0.1
5.59	5.58	0.3	5.60	0.2
5.16	5.13	0.5	5.14	0.3
4.69	4.64	0.6	4.69	0.3

3.2 In vivo testing

Using the method described in previous section, we compared images of teeth before and after a sugar rinse. The pH was calculated using the calibration curve shown in **Figure 6**. Ratio values were not calculated at low fluorescence signal levels (R channel <60) which corresponded to a lack of sufficient oral biofilm on the smoother surfaces of the enamel. Heat maps shown in **Figure 7** were obtained from single mmSFE video frames for interproximal regions 2 and 3. An average pH drop of 1.51 was obtained between the resting pH before (REST) and after a sugar rinse (DROP). Similarly, between the RISE (2nd measurement) and DROP images from the same teeth 2-3, we obtained a mean difference of 0.82 pH. The spatial mapping of pH in both DROP and RISE images displayed lower pH at the interproximal space between the teeth as expected.

4. DISCUSSION

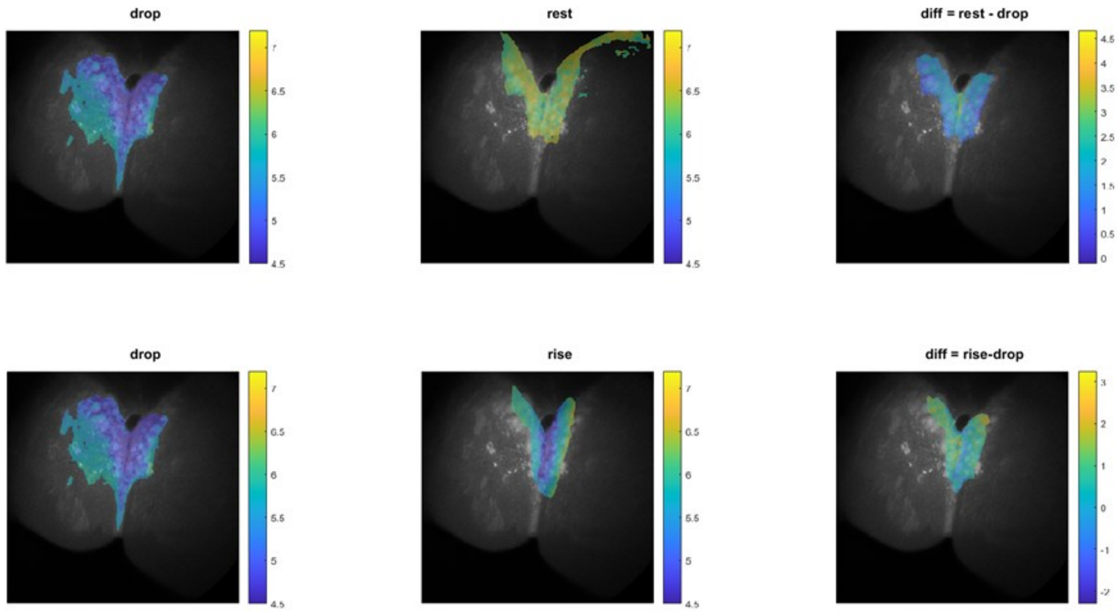


Figure 7. Heat maps of in vivo oral biofilm pH are shown for teeth 2-3 with color scale in pH.

Non-contact temporal changes of spatial pH mapping were created using the mmSFE in a case study of human oral biofilms. The quantitative mapping of pH may open new frontiers in the clinical analysis of oral biofilm for individuals, especially those who are at high risk of caries. The single human case did not have underlying caries when his conventional dental appointment removed the biofilms and the enamel was visually inspected to be ICDAS II ranking of 0. Therefore, the absolute values of pH and also the changes in biofilm pH during DROP and RISE were larger than expected from the standard Stephan Curves for the level of caries progression found in the single subject (Stephan 1944)[8]. One explanation is that the fluorescein is providing an indication of pH across the biofilm thickness, which is unlike previous contact based measurements that were performed with microelectrodes or pH strips. Lingström et al., (1993)[5] compared different methods of measuring pH, including growing oral biofilms on an electrode sensor in vivo. They reported significantly lower pH readings when the microelectrode was reading from the substrate or enamel surface rather than from the external or saliva surface of the oral biofilm. Thus, the values for biofilm pH in the conventional Stephan Curve may need to be adjusted lower when determining the average pH across the thickness of biofilm when using a fast diffusing dye like fluorescein.

This preliminary study has several limitations and challenges for clinical implementation. Firstly, the contribution of autofluorescence was simply ignored if the signal was less than 20% of the weakest fluorescence signal level. (R channel in this study). The rationale for this exclusion of weak signal was made because the measurement of buffers containing fluorescein at known pH did not change when this criteria was applied during measurements on extracted teeth. Furthermore, there is an expectation that many regions of the tooth surface will have insufficient biofilm to create conditions of acidification, so weak fluorescence signals relative to the autofluorescence is a reasonable criteria for excluding regions for pH biofilm measurement. In addition, the optical properties of biofilm and glucose are being ignored in this pilot study. To address these limitations, an initialization image of the region of interest can be made prior to the rinse of fluorescein where the baseline autofluorescence and optical properties of the biofilm can be mapped as the baseline for the optical pH mapping in the first REST images.

Secondly, there are many technical challenges that provide many opportunities for improving the accuracy, precision, and speed of measuring the biofilm pH spatially and temporally in vivo. Currently, the mmSFE console takes around a second to image one interproximal or occlusal region. For a video rate imaging system, this limitation is simply a computer interface issue. Future image acquisition will record video of the dental surfaces instead of imaging regions of interest in snapshots. Without mapping the teeth surfaces to a 3D reconstruction, the 2D image pairing requires several image processing steps that are currently performed manually. Automated image processing is challenged by the fact that there are limited features that can be used with standard computer vision techniques to align the mmSFE images. Finally, all the mmSFE images were taken in a darkened room. For clinical translation, the mmSFE must be engineered with optical and/or temporal filtering so that room lights can remain on in the dental clinic. Additionally, fluorescein dispensation as well as saliva contributes to bubble formation in the mouth which the present algorithm is unable to correct for pH calculation. So, the pixels which correspond to bubbles are removed from analysis. At the time of data collection, bubbles were reduced by blowing it with air from a hand air dispenser. This process helped decrease the bubble formation at interproximal locations, but lower occlusal surfaces retained bubbles, which had more saliva and bubble accumulation. Addition of a pressurized air dispenser at the tip of the SFE would help mitigate the loss of information when bubbles are initially present. These future improvements will likely reduce the current high variability of pH measurement in vivo, which is exacerbated by weak fluorescence signals.

5. CONCLUSIONS

Non-contact, rapid, optical pH mapping at the physiologically relevant range of 4.7-7.2 pH for early caries management was demonstrated in vitro and in vivo for the first time, at least for mmSFE. The small size and flexibility of the mmSFE allows measurements in smaller mouths of children (Rugg 2016)[6]. This new technique of locating and tracking the oral biofilm acidification will allow quantitative oral health monitoring of children. In vivo non-contact optical mapping of oral biofilm pH is important to understanding and managing the caries process in applications of prevention, guiding treatments, and monitoring therapy. In addition, the technique is safe, simple, and will become rapid with automated image processing. The colorful and dynamic visual display provides the basis to build a new visual learning experience for educating children on the effects of sugary drinks on their teeth.

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