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Clinical evaluation of smartphone-based fluorescence imaging for guidance and monitoring of ALA PDT

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ABSTRACT

India has one of the highest rates of oral cancer incidence in the world, with an estimated 80,000 new cases per year, accounting for 30% of reported cancers. In rural areas, a lack of adequate medical infrastructure contributes to unchecked disease progression and dismal mortality rates. PDT emerges as a potential modality which can be implemented in resource limited settings, while photosensitizer fluorescence can be leveraged for treatment guidance. Here, as part of an ongoing clinical study evaluating low-cost technology for ALA PDT treatment, we evaluated the capability of a simple smartphone-based device for imaging ALA-induced PpIX fluorescence for treatment guidance and monitoring. The imaging device itself consists of an annulus of 405nm LEDs for PpIX excitation with emission filter in the center mounted over the phone camera. 21 subjects having <2 cm diameter (mean size; ~ 1.29 cm²) lesions with micro-invasive (≤ 5 mm. depth) moderately/well-differentiated squamous cell carcinoma were administered 60 mg/kg ALA in oral solution and imaged before and after delivery of 100 J/cm² total light dose to the lesion surface. We will present comparative analysis of pre-and post-treatment fluorescence, white light (WL), and ultrasound images. In general, PpIX fluorescence images obtained prior to therapeutic light delivery are able to resolve lesion margins while dramatic photobleaching in post-treatment images confirms the irradiated zone. Overall this approach is able to generate sufficient fluorescence contrast for treatment guidance and monitoring photobleaching while the use of a smartphone-based device provides a low-cost, widely available platform with potential for telemedicine integration.

Keywords: Oral cancers, Smartphone, Photodynamic therapy (PDT), PpIX, Fluorescence imaging

1. INTRODUCTION

The increasing incidence of head and neck cancers in South Asia has been described as a global health crisis.¹ Particularly in India, the highest incidence of oral cancers is ascribed to the popular addiction of chewing “gutka” (a compound mixture of tobacco, acacia and betel nut extracts). Moreover, in rural areas, there is limited accessibility of early-stage medical screening and imaging. Furthermore, the economic burden of late state treatments such as complex surgical procedures and/or radiation therapies exacerbate oral cancer management.² Recently, photodynamic therapy (PDT) has emerged as an alternative and a non-evasive early stage anticancer

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treatment modality.³ The PDT is a light-based treatment in which a precursor or photosensitizer localized to the lesion sites and depart the singlet oxygen ($^1\text{O}_2$)-mediated photocytotoxicity against cancer cells. Here, we used the ALA (5-aminolevulinic acid as Levulan[®], DUSA, SUN Pharmaceuticals, Inc.) precursor against buccal mucosa lesion, where ALA localized to neoplastic tissue and enhanced PpIX (5-ALA-induced Protoporphyrin IX) production.⁴ This PpIX photosensitizer not only acts as a fluorescent probe but also departs the antitumor phototoxicity. This dual property has been made the successful treatment modality for superficial cancers such as head and neck lesions in the oral cavity.^{5,6} In the clinical settings, PpIX fluorescence and bleaching imaging are a diagnostic as well as a treatment monitoring tool.⁷ Recently, a smartphone with fluorescence imaging capability has been used as a low-cost device for pre-malignant oral screening.⁸ Since widely availability and popularity of smartphone, particularly in the developing countries, this combination suggested as a low-cost, portable and capable theragnostic cancer technology for global health⁹⁻¹¹

Here, we initiated the low-cost based fluorescence imaging in the clinical settings for guidance and monitoring of PDT treatment and simulate the rural settings where afford the advanced cost-effective monitoring are an economic burden.

2. METHODS

2.1 Subject selection

Twenty-one subjects (2 female, 19 males, median age; ~ 42 years, age range; 24-64 years) with T₁N₀M₀ stage oral buccal mucosa lesion (<2 cm. lesion diameter) were enrolled in for the study. The subject was excluded if they had a history of photosensitivity or photosensitive diseases, were taking any photosensitive medications, have any history of malignant disease treatment and had any allergies of ALA formulation. Subjects were given written, audio and video informed consent to participate in the clinical trial. This study protocol was approved by the India Council of Medical Research (ICMR), India.

2.2 PDT treatment

The total 60 mg/kg dose of 5-ALA (20 mg/kg each) was administrated orally to the patients at 0, 1, and 2 hours. After the third dose of ALA and 15 minutes break, 100 J/cm² fractionated light dose was given to the patients (each fractionated light dose for 10 minutes with 2 minutes inter-fraction intervals). 635 nm light was delivered to the buccal mucosa lesion site using flexible optical fibre attached to a portable and battery-operated LED source as previously described.¹² Light delivery to target lesions was achieved using custom light delivery applicators to control spot size and position depending upon the size of the lesion and mouth opening. The LED light spot covered the lesion as well as margins of normal tissue. Each patient was treated with a total light dose of 100 J/cm² delivered at an irradiance of approximately 50 mW/cm².

2.3 Smartphone-based PpIX imaging

The PpIX fluorescence imaging was performed on 24 lesion sites at the buccal mucosa (i.e. among 21 patients, three patients had two lesion sites). The hand-held smartphone device was used for the imaging. It has the attachment with a 405 nm LED array (modified FluoroVu device, by Eigen Imaging) fitted with a 610-710 nm emission filter as previously described¹³ (Figure 1a). The smartphone device was positioned in various possible orientations to get the maximum pre-PDT PpIX fluorescence and post-PDT bleaching (Figure 1b). The smartphone tried to hold in same angle and distance from the oral lesion for the fluorescence and bleaching imaging. The basic principle of smartphone-based fluorescence depends upon specific localization of PpIX in the neoplastic tissue/oral cancer lesion, which excitation with blue light (405 nm.) emits the red light fluorescence. The surrounding non-fluorescence tissue emits lower intensity red light due to backscattering light phenomena (Figure 1c).

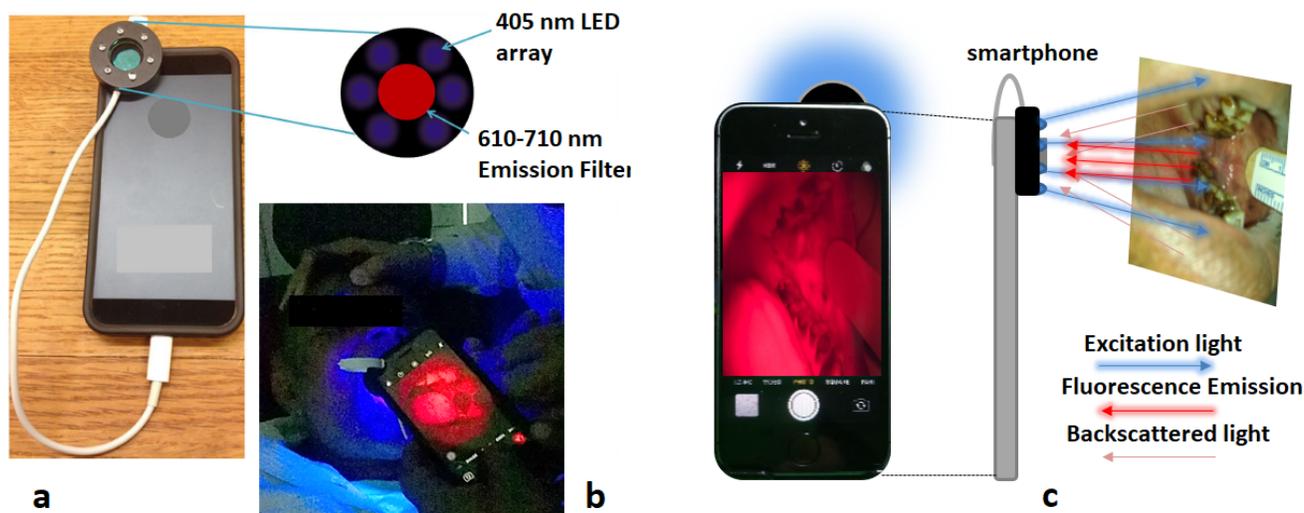


Figure 1. A Prototype smartphone for PpIX fluorescence imaging. (a) A smartphone attached with a 405 nm LED array (modified FluoroVu device, by Eigen Imaging) fitted with a 610-710 nm emission filter.¹³ (b) Hand-held smartphone based lesion site PpIX fluorescence imaging during the buccal mucosa PDT treatment. (c) Illustrative presentation of smartphone-based basic principle of PpIX fluorescence detection.

2.4 Lesion-site imaging (pre-, post and during PDT treatment)

Initially, before the ALA administration, the white light (WL) and autofluorescence image of the lesion were taken. The PpIX fluorescence image was immediately taken after the third dose of ALA. For the targeted PDT monitoring, the post-PDT PpIX bleaching image was captured after the last fractionated light dose. The ultrasonography scanning (USG) was used as an auxiliary method to get the maximum dimensionality of the lesion to co-relate the fluorescence findings. The lesion site USG imaging was taken before ALA administration as well as after the PDT treatment (i.e. on 7-10th day after the PDT). The WL and fluorescence image analysis was performed by ImageJ NIH software.¹⁴ The python OpenCV package was used for fluorescence as well as WL image segmentation (<https://docs.opencv.org/3.0-beta/index.html>).

2.5 Statistical analysis

The significant and central values of PpIX fluorescence, bleaching, WL lesion, USG lesion size were analysed by open source statistical software R (Comprehensive R Archive Network; CRAN).¹⁵ The difference between mean/central values was assessed by student's t-test. The values of $P < \alpha = 0.05$ and $P < \alpha = 0.01$ were considered to be significant (following the confidence level of 95%). Graph analysis was done by package ("ggplot2") downloaded in R console.

3. RESULTS AND DISCUSSION

3.1 Imaging-based guidance of PDT light delivery

The appropriate determination of the maximum lateral extent of the lesion is critical to insure that beam spot fully covers the lesion and healthy tissue margins (Figure 2a). In our study, we get the maximum width of the lesion by WL, USG, post-ALA lesion site PpIX fluorescence (Figure 2b, c, d). To enhance display contrast and aid in visualization of lesion boundaries, the red channel from RGB images can be displayed using an alternate look up table (LUT) such as the 16 color LUT (16 LUT) shown in Figure 2c'. The maximum width decides the appropriate diameter of a light applicator. The box-plot distribution of lesion width measured by USG and fluorescence imaging showed the almost equal central values (i.e. mean width ≈ 14 mm.). Although, USG observation showed the

maximum inter-quartile range (i.e. q_3-q_1 ; 8.6 mm.) but the uniformity of PpIX fluorescence width distribution corresponding to the actual extent of the malignant lesion (Figure 2e).

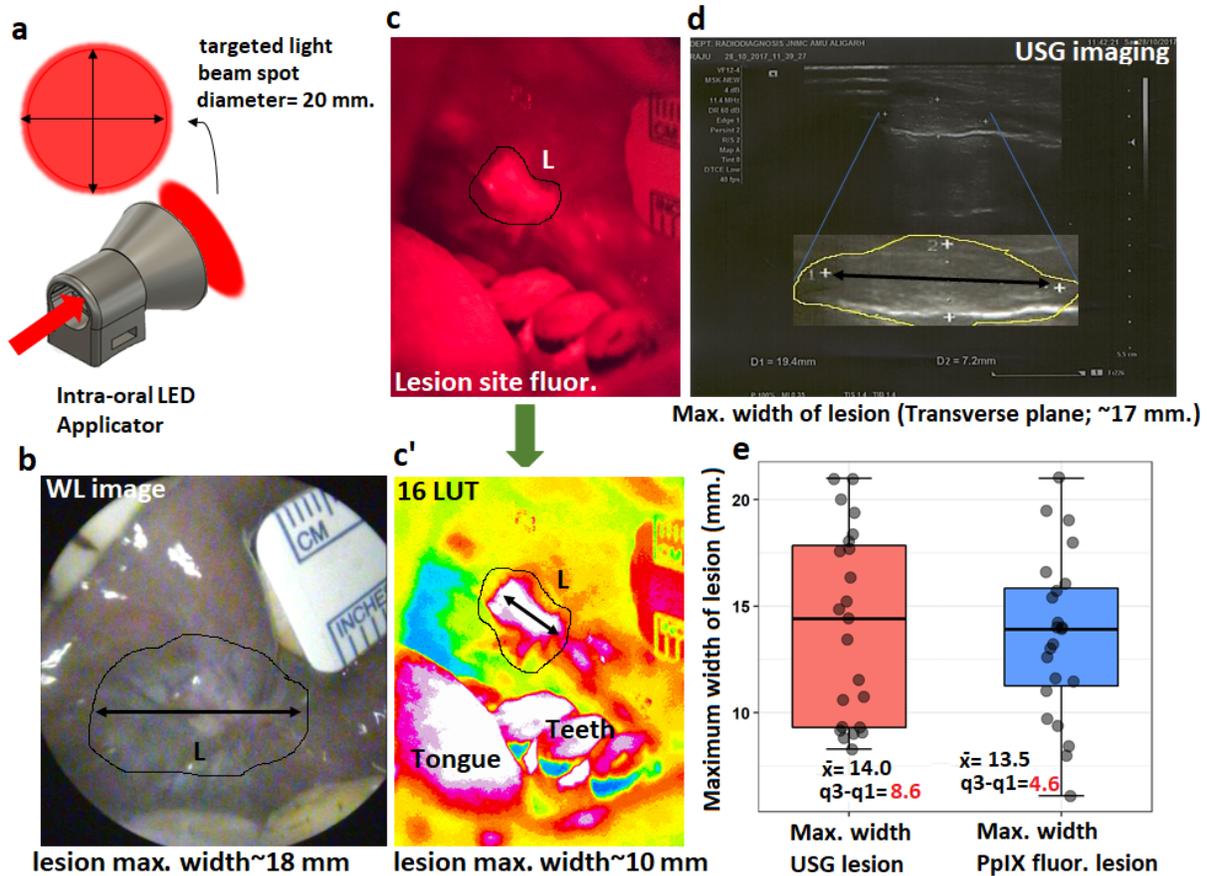


Figure 2. The PpIX fluorescence based guidance of LED applicator to cover the margins of lesions. (a) The dimension of the Intraoral applicator with light beam spot. (b, c) The measurement of max. dimension lesion with help of smartphone white light and fluorescence imaging. (c') The fluorescence image applied 16 colour LUT for the measurement of maximum lesion width. (d) USG for the maximum width of the lesion in transverse plane. (e) The boxplot of the maximum lesion width measured from the USG and PpIX fluorescence imaging on 24 lesion sites.

3.2 Comparison of lesion identification by white light and fluorescence imaging

The oral lesion having the invasive *in situ* carcinoma is inconspicuous by the naked eye or white light imaging to demarcate the extent of buccal mucosa surfaced lesion spread. Although post-ALA induced PpIX fluorescence and 16 LUT imaging is useful to locate and demarcate the surfaced lesion (Figure 3a), we also sought to corroborate conclusions from fluorescence image by an independent analysis of lesion area based on the abnormal color and texture of the diseased tissue visible even in a white light image. Here, we use WL HSV (hue, saturation and brightness) colour spacing segmentation, where we masked the WL-original and WL-gray image with the help of tunable and threshold ranges of HSV values trackbar (Figure 3b). This WL HSV segmentation resulted in same visible lesion dimensions as in processed fluorescence image.

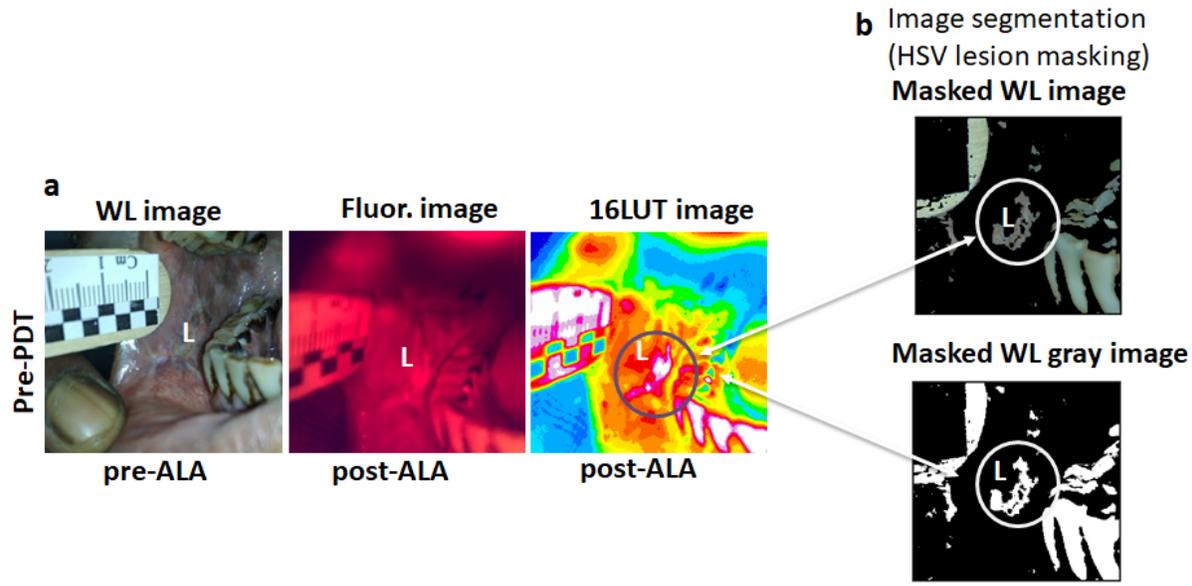


Figure 3. The identification of lesion by WL and fluorescence imaging. (a) The post-ALA fluorescence imaging and identification of lesion by 16 colour LUT. (b) The pre-ALA WL image's HSV segmentation is showing the identification of the lesion.

3.3 Lesion site fluorescence assessment (pre-, post- and during PDT treatment)

Pre-ALA WL images showed the leukoplakia type white surface (Figure 4a). When visualized with PpIX fluorescence contrast, the surface is shiny and semi-smooth (Figure 4b). However, the interpretation of the raw fluorescence signal is confounded by non-specific fluorescence from other structures in the oral cavity. For example, the bright red fluorescence of teeth is supposed to be due to the reflection of blue light (i.e. excitation light, which has such high relative intensity that it makes a significant contribution to total signal even after attenuation through the red emission filter) and autofluorescence of microbial biofilm on teeth surface (Figure 4c). After the last dose of ALA, the lesion is visible due to generated PpIX fluorescence. The fluorescence of tongue is supposed to be due to the presence of microbes with strong endogenous fluorescence (Figure 4d). It was reported that tumour tissue shows 12.5 times brighter fluorescence than surrounding tissue after oral administration of ALA (200 mg) at 1 to 2.5 hours.¹⁶ In our studies, after the first dose of ALA (at 1 hour), the PpIX fluorescence becomes conspicuous to identify the tumour tissue and after the last dose of ALA tumour tissue show brighter fluorescence. Although increased fluorescence intensity is useful to the lesion identification, here we didn't correlate it to the quantification of PpIX production. The colour segmentation of red fluorescence using 16 pseudo-colour LUT help to visualize the extent of the lesion (Figure 4e, f). After the last dose of light, bleached area due to PpIX bleaching is easily visible (Figure 4g).

3.4 Comparative study of lesion site PpIX fluorescence, bleaching and WL area imaging

In our study, WL lesions (area parameter; cm^2) are quite small (mean = 1.29 cm^2) and distribution of observations are in within the range ($q_3 - q_1 = 0.68 \text{ cm}^2$) (Figure 5). The fluorescence area of the lesions showed the uniformly distributed observations within the maximum and minimum limits (mean = 1.87 cm^2 and $q_3 - q_1 = 1.54 \text{ cm}^2$). Interestingly, photobleaching of the lesion sites is the maximum extent of area parameter covering the lesion sites. The photobleaching caused by the PDT generated singlet oxygen, which reacts to the ground state of PpIX photosensitizer leading to the irreversible destruction.¹⁷ The greater area of photobleaching is likely hood of the covering of the photobleaching of the PpIX as well as surrounding tissue autofluorescence.¹⁸ Hence, extent of photobleaching

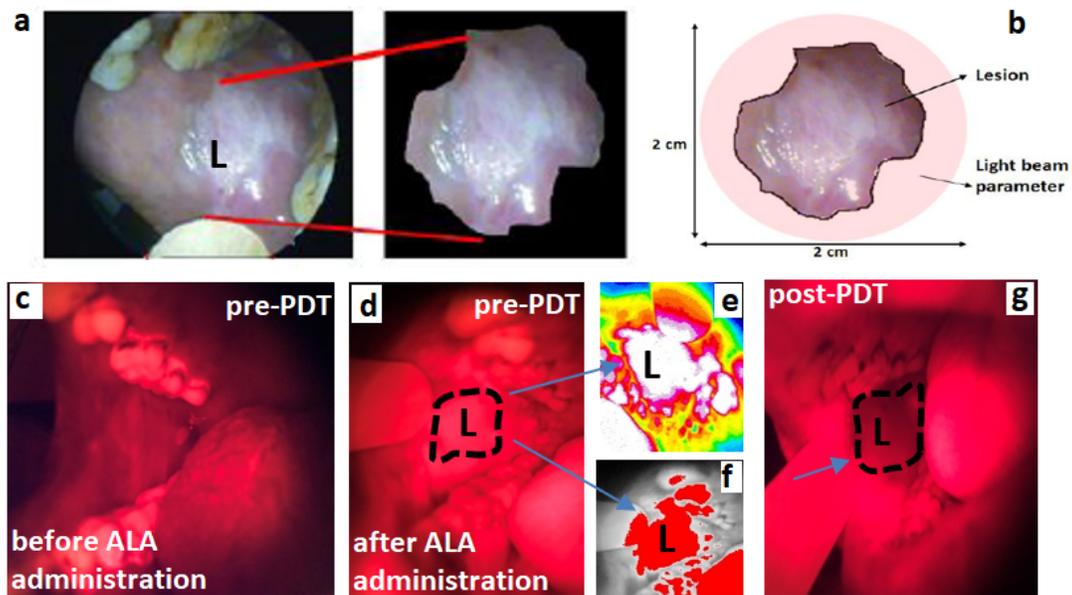


Figure 4. The lesion site smartphone based PpIX fluorescence assessment. (a, b) Measurement of two dimension parameter of buccal mucosa lesion by WL imaging. (c, d) Pre- and post-ALA administrated PpIX imaging and corresponding image processing with ImageJ (e, f). (g) Post-PDT PpIX bleaching.

is a useful measurement to confirm that light was indeed delivered to the target site. If combined with spatial co-registration of the pre-PDT imaging, the overlaid photobleaching map could also be used to confirm that light delivery achieved desired margins around the lesion.

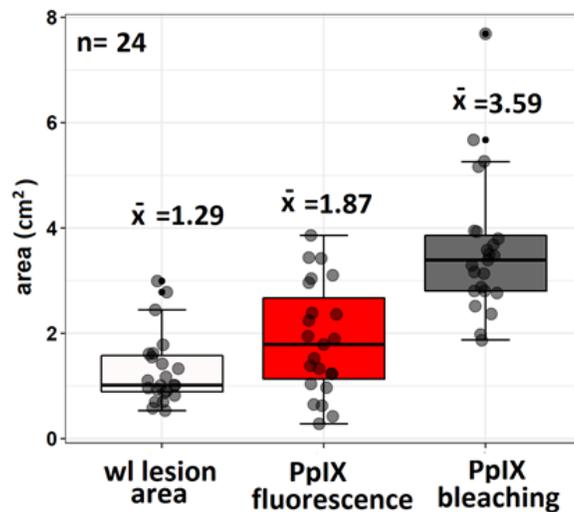


Figure 5. The comparative boxplot analysis of PpIX fluorescence, post-PDT bleaching with pre-PDT WL lesion area. Larger area of photobleached region following PDT is consistent with expectations based on the treatment design, using a light delivery applicator which treats the full lesion area plus margins.

4. CONCLUSIONS

Here we show that a simple smartphone-based attachment for PpIX fluorescence imaging can be used to reliably demarcate boundaries of ALA-photosensitized oral lesions. Lesion areas obtained by fluorescence image data were validated by independent analysis of ultrasound images from the same sites as well as a custom analysis based on hue, saturation and value of white light images of the same lesions. The use of smartphone-based imaging is sufficiently streamlined as to be conducive to implementation for treatment guidance (determination of target area for therapeutic light delivery) during the clinical PDT procedure. In addition to treatment guidance, the analysis of fluorescence contrast also proves useful post-treatment to confirm the location and diameter of the photobleached area, which has excellent contrast in the smartphone display. Here we show in particular that the size of the photobleached region confirms margins around the lesion were achieved and size is consistent with the light applicator (hence beam spot size) used for treatment. The photobleaching analysis could be further leveraged for treatment monitoring, for example by obtaining photobleaching image data during light delivery fraction breaks and interpreting feedback to inform modulation of light delivery parameters during subsequent fractions. While the simple device here which mounts directly over the smartphone camera is useful, a potential future design improvement would be to couple the phone to a handheld imaging probe with form factor similar to a commercial dental camera.

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