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# Clinical assessment of a low-cost, hand-held, smartphone-attached intraoral imaging probe for ALA PDT monitoring and guidance

Shakir Khan PhD[a, b, d], Bofan Song PhD[c], Srivalleesha Mallidi PhD[b], Shaobai Li PhD[c], Hui Liu PhD[a], M. A. Bilal Hussain MSc[d], Mayra S BaptistaLopesTeix[a], Shaista Siddiqui FRCR[e], Amjad P. Khan PhD[b], Kafil Akhtar MD[g], Shahid Ali Siddiqui MD[d], Syed Abrar Hasan MS [h], Colin Hopper FRCS(Ed)[f], Stephen G. Bown FRCP[f], Rongguang Liang PhD[c], Tayyaba Hasan PhD[*b], and Jonathan P. Celli PhD[*a]

[a]University of Massachusetts at Boston, Boston, Massachusetts, United States.
[b]Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, United States.
[c]The University of Arizona, Wyant College of Optical Sciences, Tucson, Arizona, United States.
[d]Aligarh Muslim University, Jawaharlal Nehru Medical College, Department of Radiotherapy, Aligarh, India.
[e]Aligarh Muslim University, Jawaharlal Nehru Medical College, Department of Radiodiagnosis, Aligarh, India.
[f]University College London, London, England, United Kingdom.
[g]Aligarh Muslim University, Jawaharlal Nehru Medical College, Department of Pathology, Aligarh, India.
[h]Aligarh Muslim University, Jawaharlal Nehru Medical College, Department of Otorhinolaryngology (E.N.T.), Aligarh, India.

## ABSTRACT

India has one of the highest rates of oral squamous cell carcinoma (OSCC) in the world, with an incidence of 15 per 100,000 and more than 70,000 deaths per year. The problem is exacerbated by lack of medical infrastructure and routine screening, especially in rural areas. This collaboration recently developed, and clinically validated, a low-cost, portable and easy-to-use platform for intraoral photodynamic therapy (PDT) specifically engineered for use in global health settings. Here, we explore the implementation of our low-cost PDT system in conjunction with a small, handheld smartphone-coupled, multichannel fluorescence and white-light oral cancer imaging probe, which was also developed for global health settings. Our study aimed to use this mobile intraoral imaging device for treatment guidance and monitoring PDT using 5-aminolevulinic acid (ALA)-induced protoporphyrin IX (PS; PpIX) fluorescence. A total of 12 patients with 14 lesions having moderately/well-differentiated micro-invasive OSCC lesions (<2 cm diameter, depth <5 mm) were systemically administered with three doses of 20mg/kg ALA (total 60mg/kg). Lesion site PpIX and auto fluorescence was analyzed before/after ALA administration, and again after light delivery (fractionated, total 100 J/cm$^2$ of 630nm red LED light). Quantification of relative PpIX fluorescence enables lesion area segmentation to improve guidance of light delivery and reports extent of photobleaching. These results indicate the utility of this approach for image-guided PDT and treatment monitoring while also laying groundwork for an integrated approach, combining cancer screening and treatment with the same hardware.

**Keywords:** Oral cancers, Smartphone, Intra-oral probe, Photodynamic therapy (PDT), ALA, PpIX, Fluorescence imaging.

## 1. INTRODUCTION

Superficial oral cancer diagnosis and treatment are the best preventive methods as compared to late-stage treatment modalities. Oral cancer accounts 2% new cases (377,713) and 1.8% mortality rate (177,757 death) worldwide (GLOBOCAN, 2020),[1] where India comprises an alarming rate of new age-adjusted oral cancer cases (36%, 135,929) and mortality rate (42.4%, 75, 290 deaths) as compared to other nations. The widespread and

* Corresponding authors: **Tayyaba Hasan** PhD, Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston MA 02114, E-mail: thasan@mgh.harvard.edu, **Jonathan P Celli** PhD, Physics Department, University of Massachusetts at Boston, Boston MA 02125 -3393, E-mail: Jonathan.celli@umb.edu

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proliferating status of oral cancer in India is attributed to popular tobacco chewing habits such as 'Gutka' and other smokeless tobacco products.[2] Early standard clinical oral evaluation methods such as biopsy and ultrasonography diagnosis and subsequent oral cancer surgery, chemotherapy, and radiotherapy are expensive for rural patients. Hence, remote health care setup requires a portable, low-cost, and early cancer noninvasive theragnostic module, which not only helps in lesion visualization but also monitors and navigates in lesion treatment.

In our clinical study, a low-cost, portable, and smartphone-attached intra-oral optical method was applied on oral cancer PDT trial in India.[3] This was a noninvasive treatment trial where ALA (precursor of PpIX) localized to the lesion site and produce PpIX. The PpIX departs red fluorescence, as well as singlet oxygen ($^1O_2$), mediated phototoxicity against cancer cells.[4] Herein, an intra-oral optical method was applied on 12 patients (14 lesions) and outcome of post-PDT was very successful where 13 lesions showed no evidence of cancer till current date of follow-ups. Intraoral device-mediated relative fluorescence segmentation enhanced lesion margin visualization during PDT as compared to standalone autofluorescence as well as white light imaging. Further, after the PDT treatment, probe helped in lesion site PpIX bleached imaging for confirmation of targeted site PDT. This device has potential not only for monitoring and guidance in PDT but also PpIX fluorescence-based pre-malignant and malignant lesion screening.

## 2. MATERIALS AND METHODS

### 2.1 Subject Selection and Imaging timeline

The intraoral probe was used for imaging on 14 oral lesions (10 patients each with unilateral lesion, + 2 patients each with bilateral lesion, 1 female, 11 males, median age: 39 years, age range: 25 to 53 years). The imaging study was conducted at Departments of Radiotherapy and Clinical Oncology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, India. All oral lesion sizes were less than 2 cm diameter with histologically proven $T_1M_0N_0$ OSCC. White light and autofluorescence images were taken before the first oral dose of ALA, 20mg/kg dissolved in fruit juice. Second and third doses of ALA (20mg/kg each) were given at hourly intervals. Second PpIX fluorescence imaging was taken after the third dose of ALA (Fig.1). The light-emitting diode (LED) light(640 nm peak) with power (maximum irradiance 48.7 mW/cm$^2$) was delivered to the lesion surface (total dose 100 J/cm$^2$) using modular 3D-printed component (mouth prop).[5] The total light treatment is fractionated two times with 2 min. interval and total time was 34 min. Third PpIX fluorescence image was taken after the light treatment to measure lesion surface PpIX and auto-fluorescence bleaching.

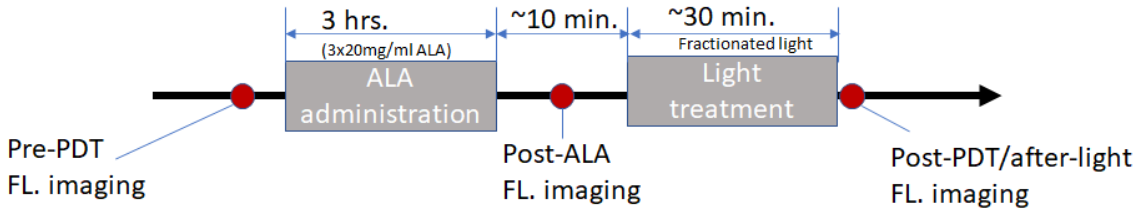


Figure 1. Three time-point lesion site imaging (WL; white light and FL; fluorescence) during the ALA-PDT treatment. The time-points are pre-PDT; just before ALA administration, post-ALA; after total dose of ALA(60 mg/kg) within 10 min., post light treatment; just after 100 J/cm$^2$ light dose.

### 2.2 Intra-oral device

The intra-oral probe was developed for pre-malignant and malignant oral cancer screening and it was successfully implemented on 5000 patients.[6–9] In previous studies, the device exploited only white light and autofluorescence imaging modality for the screening using 405 nm light excitation on the lesion. The Narrow-band filters and a long-pass filter in front of UV LED and camera, respectively enhance the auto-fluorescence contrast. This device is the third generation of hardware having a dual-mode, wide field of view (FOV) for fluorescence imaging (FL) and polarized white light imaging (WL). Device components are commercial smartphone (Moto G5 Android),



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handheld intraoral imaging probe, light-emitting diode driver, smartphone case attached rechargeable lithium battery, and mobile application software. Both back of smartphone case and probe have four 405 nm Luxeon UV U1 LEDs (Lumileds, Amsterdam, Netherlands) for FL imaging and four 4000-K Luxeon Z ES LEDs for WL imaging. The existing mobile imaging system has two imaging mode options, (1) intraoral imaging with the flexible intraoral probe (with front view of the phone case) and (2) whole mouth imaging with the phone camera (back view of the phone case). This semi-flexible intra-oral probe had increased clinical ergonomics, can be bent to access all areas of the oral cavity where the probe is straight, bent for inward imaging and outward imaging (Fig.2).

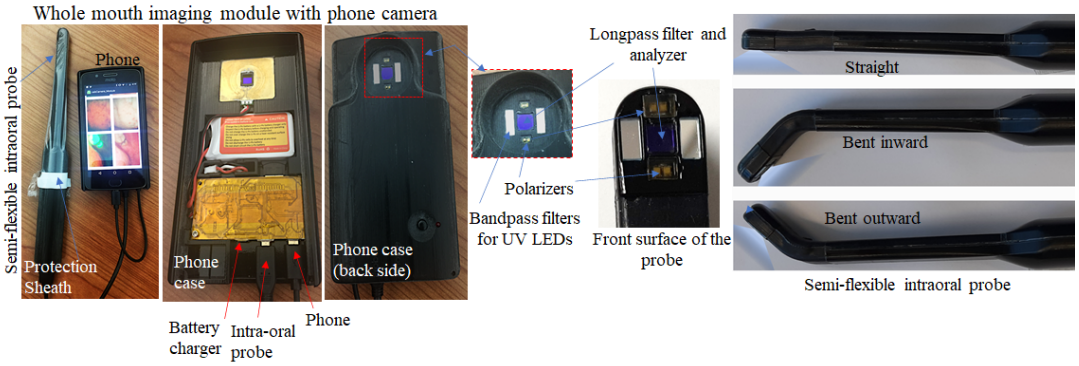


Figure 2. The oral lesion imaging module with the attached smartphone. The module has a semi-flexible intra-oral probe with a camera, and another camera is installed on the backside of the phone case for whole mouth imaging. The flexibility of intra-oral probe due to bending adjustment according to location of lesion.

### 2.3 *In-vitro*, intra-oral device based Protoporphyrin IX fluorescence imaging

The device was calibrated on the head and neck cancer OSCC cell line TR146 (Cat. no. ECACC 10032305, source histology: well-differentiated keratinizing squamous cell carcinoma), where generated PpIX fluorescence images was analyzed. Here, TR146 cells were cultured in two 75 $cm^2$ T-flasks at $37^0$C in Ham's F-12K (Kaighn's) medium containing L-glutamine and sodium bicarbonate buffer system and supplemented with 10% FBS, 100 µg/ml penicillin/streptomycin, 0.5 µg/ml amphotericin-B. The cells were trypsinized (trypsin 25%) and harvested when cell density was achieved around 0.5 x $10^6$ cells/ml in each T-flask. Cells were incubated with 3mM ALA (5-Aminolevulinic acid HCI, Sigma Aldrich, Israel) for 4 hours in a new T-flask. ALA incubated cells were pellet down after 5 min centrifugation at 5000 rpm. TR146 generated PpIX fluorescence images were taken in disposable polymethyl methacrylate (PMMA or "acrylic") cuvettes, where aggregated cells (dissolved in $TiO_2$; 0.58g/l) were pipetted down in 2% (w/v) sodium alginate hydrogel phantom (crosslinked by DPBS supplemented with $Ca^{+2}$ and $Mg^{+2}$ ions).

### 2.4 Lesion image segmentation

Lesion captured 8 bit unsigned integer RGB image was splitted to red and green channel (using Python Numpy module) where dominantly green channel represents the auto-fluorescence and red channel represents PpIX fluorescence.[10,11] Lesion with margins and non-malignant surrounding tissue was segmented on relative red (PpIX fluor.) and green (auto-) fluorescence intensity ($R_{value}$). The red ($I_{redNorm}$) and green ($I_{greenNorm}$) pixel intensity were normalized on total RGB intensity ($I_{total(1-255)}$). The Python OpenCV package was used for fluorescence and $R_{value}$ image segmentation.[12]

### 2.5 Statistical analysis

Central value of lesion site as well *in-vitro* parameters (i.e., lesion site red, green fluorescence parameters, correlation of $TiO_2$ phantom produced PpIX fluorescence intensity-concentration) were analyzed by open-source statistical software R (Comprehensive R Archive Network).[13] The difference between mean values of red, green intensities and their relative values during three-time points; pre-ALA, post-ALA, post-light was assessed Kruskal



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Wallis test, an alternate one-way ANOVA test. The significance values are considered by calculating the $P$-values at: ***, $P< 0.001$; **, $P< 0.01$; *, $P< 0.05$.

## 3. RESULTS AND DISCUSSION

### 3.1 Protoporphyrin IX and autofluorescence during PDT

The intraoral device was initially assessed in the lab to simulate the approach of PpIX fluorescence imaging on various concentrations of PpIX including buccal mucosal TR146 OSCC produced PpIX. At lower concentration of PpIX (5$\mu$g/ml), intraoral probe detect the red PpIX fluorescence in cuvette (Fig.3). TR146 Aggregated and dissolved in $TiO_2$ showed the generated PpIX fluorescence and clearly differentiate with contrast in PpIX red fluorescence signal from control having only $TiO_2$ (0.58g/l) and PpIX.

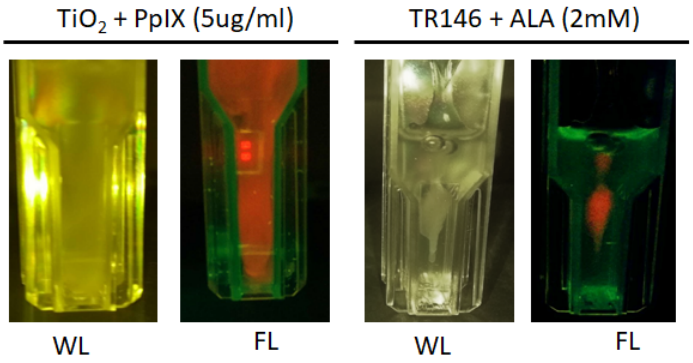


Figure 3. Imaging of PpIX in Tissue phantom $TiO_2$ and imaging of OSSC TR146 produced PpIX in SA hydrogel.

Cancerous lesion with clear margin visualized after fluorescence imaging and successively light treatment was applied using 3D printed intraoral applicator.[3] The fluorescence images splitted into red and green channel where green channel represents the auto-FL and red channel represents PpIX fluorescence. The auto-FL attribute to normal tissue's intrinsic fluorophores such as collagen, flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide (NADH) and elastin. The combination of these fluorophores produce the broad spectrum of fluorescence after the near UV excitation (300nm to 450nm).[14] In several clinical and non-clinical studies demonstrated the decreased auto-FL intensity in the tumor tissues as compared to normal tissues. This auto-FL property has been exploited in demarcation of lesion having malignant, nonmalignant, oral dysplasia ( mild to sever dysplasia).[9,15,16] After ALA administration, laesion site auto-FL was decreased (Fig.4) and post-ALA panel show the ALA produced PpIX fluorescence. The bleached PpIX fluorescence after light treatment confirmed the delivered light dose on the lesion surface including surrounding margins.

Further, segmentation of image, base on relative fluorescence pixel intensity ($R_{value}$) shows the good lesion demarcated fluorescence (Fig.4b). The increased $R_{value}$ corresponds a reduction of auto-F and an increase in PpIX fluorescence intensity. In the earlier studies, the relative fluorescence intensity of split channels (R, G, B) predicts the malignant and normal tissue.[17,18] Even, Sharwani *et.al.*. not only differentiated the cancer tissue but also confirmed oral premalignant lesion stages such as dysplasia, hyperplastic tissue, and inflammation, where dysplastic tissue and carcinoma *in situ* (CIS) showed the higher $R_{value}$ with 83-90% sensitivity and 79-89% specificity.[11] Sharwani *et.al.* study set the threshold value of $R_{value}$ at 1.2 to differentiate the normal to dysplastic/CIS tissue. In our study set threshold value ($R_{value}$) image segmentation guides the PDT light applicator on the lesion surface with a margin for targeted light dosimetery.

## 4. CONCLUSION

The intra-oral probe with smartphone has been shown the pre-malignant and malignant screening potential among 5000 patients with successful validation.[9] In our clinical ALA-PDT study, intraoral probe shows relative fluorescence contrast not only during PDT but also in post-PDT with bleached PpIX and confirms the light



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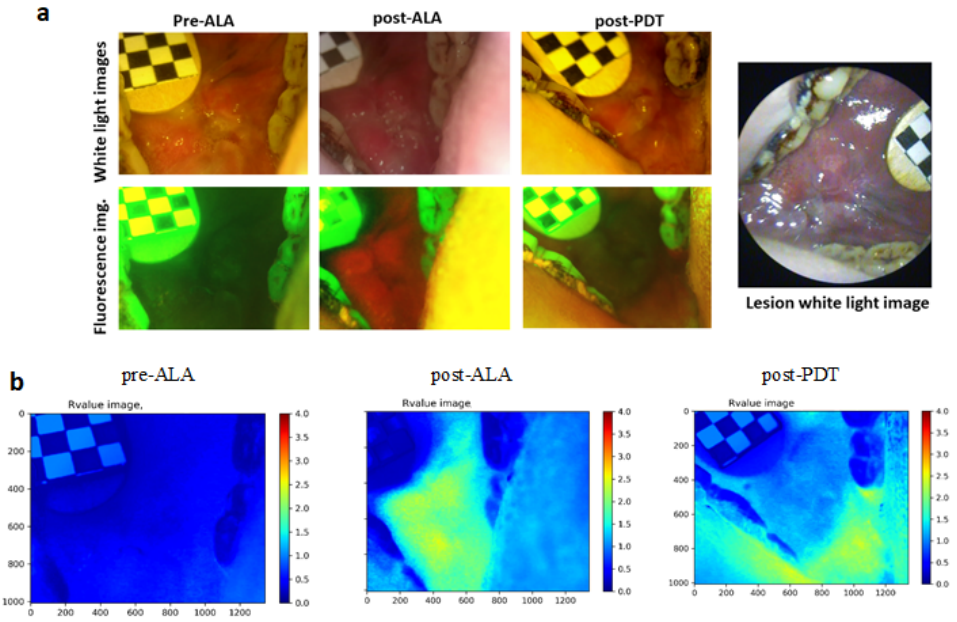


Figure 4. Image segmentation of three time points fluorescence and white light images (pre-ALA, post-ALA, post-PDT).

dosimetery. The wide availability and popularity of smartphones, particularly in the developing countries, make this device promising as a low-cost, portable, and capable theragnostic cancer technology for global health.[19] Further, large-scale case studies will required to validate and calibrate the $R_{value}$ imaging during monitoring and guidance of PDT. In future this potential approach can discriminate between low to severe dysplasia before developing to CIS. Oral lesion could be screened and treated simultaneously at the remote rural clinical settings with integration of PDT and intraoral device in same hardware.

## Disclosures

The authors have no relevant financial interests in this article and no potential conflicts of interest to disclose.

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