T.2: Photonics based point-of-care devices for improved healthcare – the activities at RRCAT

Shovan K. Majumder, Rashmi Shrivastava, Hemant Krishna, Khan Mohammad Khan and Khageswar Sahu
Laser Biomedical Applications Division
Email: shkm@rrcat.gov.in

Abstract

Translation of research from 'Lab-to-Land' is an essential requirement for efficient healthcare management in developing countries like India. Necessity of affordable, rapid and reliable technologies is fast increasing in the field of medical diagnosis. Recent research has demonstrated significant potential of photonics-based technology for rapid and reliable diagnosis of diseases. We, at RRCAT, are dedicated towards research and development of point-of-care devices based on optical imaging and spectroscopy for healthcare. Towards this objective, we have developed three point-of-care devices: TuBerculoScope, OncoVision (a vision enhancement module) and OncoDiagnoScope. These devices are targeted for early diagnosis of two ubiquitous diseases in India: tuberculosis and cancer (oral and cervical). TuBerculoScope is a portable and affordable fluorescence imaging device that is intended for rapid detection of tuberculosis causing bacteria in dye-stained sputum smears. OncoVision helps identifying oral tissue transforming towards malignancy based on the differences in the fluorescence emission characteristics from an abnormal and a normal tissue type. OncoDiagnoScope is a point-of-care optical device for non-invasive screening of oral cavity for abnormalities leading to cancer. It performs fluorescence and diffuse reflectance spectroscopy based characterization of oral tissue into normal and abnormal types (tissues transforming towards cancer). The technology of TuBerculoScope and OncoDiagnoscope has been transferred to the industry and the systems are also deployed in hospitals.

1. Introduction

Recent times are witnessing increasing developments in the healthcare technologies, which are specially driven by emerging needs for affordable, non-invasive or minimally invasive, rapid and sensitive diagnosis for efficient disease management. Optical techniques are inherently advantageous for being rapid and sensitive and also for their capability in providing non-invasive or minimally invasive disease diagnosis [1-3]. Optical approaches may be divided into two broad areas, viz., imaging and spectroscopy. Broadly speaking, conventional techniques for clinical diagnosis, especially imaging, are primarily subjective and require expertise for image analysis and interpretation to arrive at a proper diagnosis. Optical imaging may be defined as the use of light for an investigational imaging intended for medical applications. Optical imaging offers a very specific benefit over conventional radiological imaging techniques. Unlike radiological imaging techniques, it uses a non-ionizing, and relatively harmless visible light for either *in-vivo* or *in-vitro* imaging of biological samples. Optical spectroscopy, on the other hand, is the study of the spectral properties of the light emerging upon interaction with matter. It can further be classified into absorption, reflectance, fluorescence and Raman spectroscopy, based on the type of light-matter interaction being investigated. Optical spectroscopy provides biochemical as well as morphological information of the tissue being investigated [1-3].

Towards these endeavors, biophotonics was initially explored at Raja Ramanna Centre for Advanced Technology (RRCAT) for understanding laser/ light tissue interaction. The transformation of a normal tissue towards malignancy results in several biochemical changes which are reflected in the optical signatures obtained from the tissue. Subsequently laser-induced fluorescence (LIF) was explored to identify characteristic differences in the fluorescence emitted from normal and malignant tissue sites [4-7]. Further, intensive studies to identify the excitation wavelength(s) which can lead to large differences in the fluorescence characteristics of normal and diseased tissue sites and the development of algorithms which can exploit these differences for diagnosis were carried out [8]. These studies resulted in development of a nitrogen laser based prototype system for optical spectroscopy based non-invasive distinction of normal and abnormal oral tissue types [9].

On the basis of the previous studies carried out at RRCAT, biophotonics based point-of-care diagnostic devices are developed for applications in actual clinical settings. These portable devices, TuBerculoScope, OncoVision (a vision enhancement module) and OncoDiagnoScope are intended to provide rapid, cost effective and easy approach for diagnosis of two major challenging diseases affecting Indian population by large, viz., tuberculosis and oral cancer, respectively. The current article provides in-depth information about the development and principle of operation behind these devices.

2. Point-of-care devices developed at RRCAT

2.1 TuBerculoScope

Tuberculosis (TB) is one of the top ten causes of death world-wide. India is the country with the highest burden of TB. According to the World Health Organisation (WHO) TB statistics of the year 2016, an estimated incidence figure of 2.79 million cases of TB were present in India [10]. TB is caused by the bacteria *Mycobacterium tuberculosis* (Mtb) that most often affect the lungs. Tuberculosis is curable and preventable and thus early, rapid and accurate diagnosis is crucial to reduce the global burden of the disease.

Sputum smear microscopy remains the standard method of clinical diagnosis of pulmonary tuberculosis. Conventionally Ziehl–Neelsen (ZN) stain based light microscopy was used for visualizing Mtb in sputum smears. Recently, based on its increased sensitivity, the WHO has recommended the application of fluorescence microscopy as an alternative to ZN microscopy. For fluorescence microscopy, the sputum smears are stained with fluorescent dye, auramine O, which binds specifically to the mycolic acid present on the cell wall of Mtb [11]. When such a stained slide is observed in an illumination with blue light under a fluorescence microscope, green fluorescent bacteria (Mtb) are clearly visible.

Conventional fluorescence microscopes are bulky and costly and their availability is limited only to a few hospitals. An easy-to-use, compact and portable fluorescence imaging device intended for point-of-care microscopy based detection of Mtb bacteria in auramine O stained sputum smears was developed by us. Further, a graphical user interface (GUI) software enables automated counting of the Mtb bacteria in the 'field of view' of the microscope objective. This device, named TuBerculoScope, can be operated in a limited resource setting in remote areas allowing rapid diagnosis of pulmonary tuberculosis.

TuBerculoScope is a light-emitting diode (LED) based fluorescence microscope (Figure T.2.1). The illumination light from a blue LED is focused onto the dye-stained sputum smear on the glass slide through a microscope objective and the backscattered fluorescence signal is collected by the same objective and detected by a CCD camera.

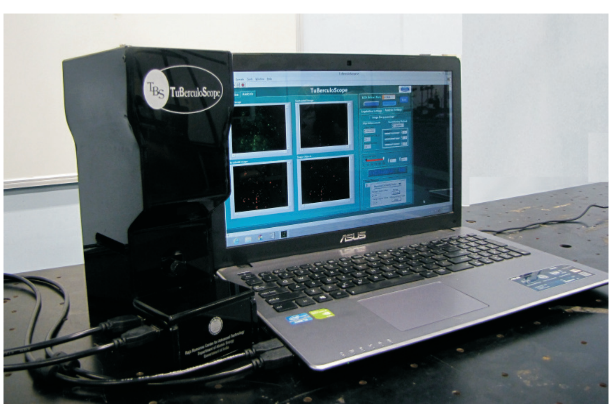


Fig. T.2.1: TuBerculoScope.

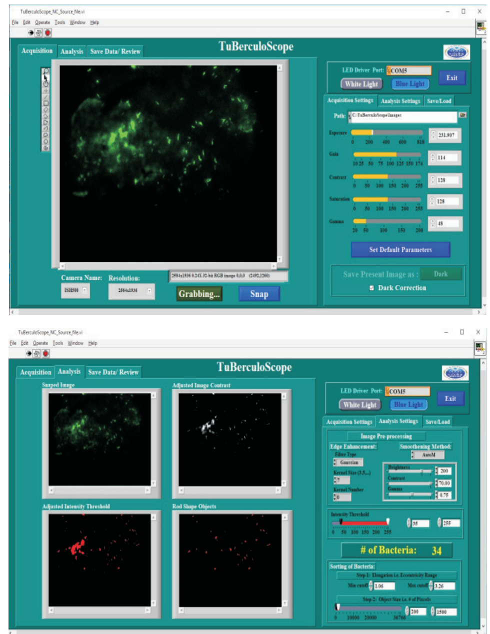


Fig. T.2.2: Graphical user interfaces for TuBerculoScope.

A dichroic and blue cut-off filter combination is used to block the elastically scattered excitation light reaching the camera. The device possesses a slide insertion slot where auramine O stained sputum smears are placed for observation. The magnification provided by TuBerculoScope is 400x. The Mtb present in sputum smears binds with auramine O dye to give green fluorescence upon excitation with blue light.

The main module of the TuBerculoScope is integrated with a GUI software. This enables hardware control of the device and automated counting of the rod shaped Mtb bacteria in a particular field of view of the microscope objective. The front panel of the GUI has three tabs, (i) Acquisition, (ii) Analysis, (iii) Save Data/Review, for grabbing of fluorescence image in the field of view, subsequent analysis of the grabbed image, and saving the image for further recall and offline analysis, respectively (Figure T.2.2).

TuBerculoScope was validated by comparing its results with that obtained from manual observations in a commercial fluorescence microscope and also with the clinical findings for 35 sputum smear slides. The slides included negative control samples as well as varying load of Mtb, graded as 1+, 2+ and 3+ depending upon the number of bacteria observed (according to Table T.2.1) in each length of the smear. TuBerculoScope was found to have 100% diagnostic accuracy and was found equivalent to the commercially available system.

Table T.2.1: Grading criteria for auramine O stained sputum smear slides

Result	**Fluorescence** (400x magnification: 1 length = 30 fields)
Negative	Zero AFB / 1 length
Scanty	1–19 AFB / 1 length
1+	20–199 AFB / 1 length
2+	5–50 AFB / 1 field on average
3+	>50 AFB / 1 field on average

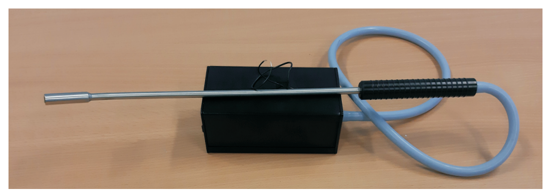
The technology of the device has been transferred to M/s RKT Meditech, Indore. One unit of the device has been deployed in Homi Bhabha Cancer Hospital, Varanasi and the other in the Department of Biochemistry, Banaras Hindu University, Varanasi where they are undergoing clinical validation.

2.2 OncoVision (a vision enhancement module)

OncoVision is a simple, hand-held device for real-time, non-contact and in-situ imaging of fluorescence from superficial tissues like human oral cavity, cervix, skin etc. intended for improved visual assessment. The device utilizes the fact established by previous studies that the fluorescence emission properties of a tissue transforming to a cancerous stage is different from that of a healthy tissue. Using this instrument, regions of lesions in oral cavity, cervix or skin can be better identified against the healthy tissues based on their natural characteristics in response to light. The method involves shining light from the multiple UV LEDs, onto the tissues for inducing fluorescence in the native tissues. The fluorescence emitted from the tissues is detected by a CCD camera attached to a high-pass optical filter ($\lambda > 380$ nm) to generate two-dimensional fluorescence spectral images. All the components of the system, including the LEDs, the CCD and the optical high-pass filter are accommodated in an in-house fabricated cylindrical case made of Perspex (Figure T.2.3(a)). The device is powered through a USB port. The system facilitates acquisition of fluorescence emission information from larger areas of the tissue surface for the purpose of rapid screening of the oral cavity and identifying the abnormal regions.

A GUI software (Figure T.2.3(b)) developed and integrated with this imaging tool enables automated acquisition and processing of the tissue fluorescence images for highlighting the difference in the fluorescence characteristics of the different tissue types. The software allows online display of fluorescence images of tissues in the video mode as well as has provision to take a snapshot of the displayed image at any instant in the still mode on the computer screen. It also has the ability to process the grabbed images offline using different color composites (RGB component) for highlighting the differences in the fluorescence characteristics of the different tissue types. In general, healthy oral tissues showed uniform green fluorescence and an increase in red fluorescence intensity was frequently observed in the images recorded from oral squamous cell carcinoma (OSCC) lesions.

The system was found to identify the lesions in the oral cavity and is validated at hospitals and cancer screening camps on over 500 subjects who enrolled for routine examination. OncoVision has potential to be a first-hand examination tool, aiding a physician to identify abnormalities leading to cancer in superficial tissues of oral cavity, cervix or skin.



(a)

(b)

Fig. T.2.3: (a) OncoVision, and, (b) GUI software for OncoVision.

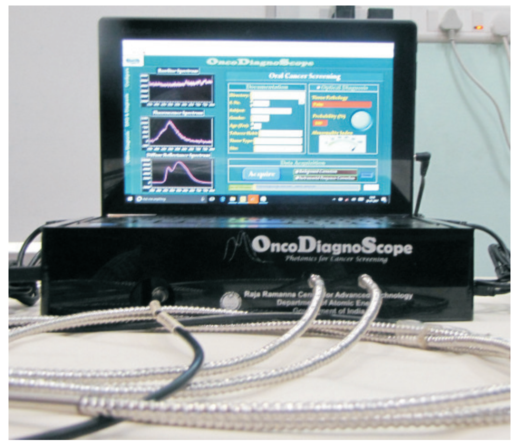
OncoVision can easily be augmented with the point-spectroscopy based system, the OncoDiagnoScope for simultaneous use. The synergistic use of the two devices may lead to better management of cancer in a real-time diagnostic setting. This is because while the former allows better visual identification of regions of lesions (often missed by visual examination under white light illumination) against the healthy tissues, the latter can be used to quantitatively ascertain the pathology of these lesions.

2.3. OncoDiagnoScope

Oral cavity cancer is amongst the most prevalent cancers world-wide. Currently, India too bears a huge burden with more than 1 million incidences of oral cancer and accounting for around 7.6% cancer related deaths in India. This is largely associated with the extensive use of tobacco, including smokeless tobacco and excessive consumption of alcohol, which are the prominent risk factors [12]. Early detection through screening of high risk as well as asymptomatic individuals is the best approach to identify susceptible and primary stage cancer patients and thereby reducing the mortality rate related to oral cancer. At present, the only available means of obtaining the true status of a suspicious lesion in the oral cavity is invasive biopsy followed by histopathology, which is considered as the gold standard for definitive diagnosis of tissue pathology [13]. As biopsy is invasive and causes patient trauma, it is unrealistic and unethical to conduct a biopsy-screening of asymptomatic individuals for the purpose of early identification of oral lesions. Therefore, non-invasive devices are the need of the hour to conduct screening of oral cavity for early detection of oral cancer. Optical spectroscopic techniques are well suited for this kind of application. Its major attraction comes from two important facts, first, the biochemical and morphological changes, that the tissue undergoes as it changes from normal to neoplastic, can be sensitively probed by optical spectroscopic techniques even before the appearance of disease symptoms, and second, the usability of the techniques for making point-of-care optical devices for *in-situ* screening and detection.

For achieving the final goal of routinely using optical spectroscopy for the management of oral cancer in a clinical setting, it is imperative to deliver diagnostic results in real time. This requires development of a stand-alone optical spectroscopic device capable of providing non-invasive and accurate diagnostic feedback in real-time. Thus, it is essential to equip the device with an appropriate software that not only provides the necessary interface for the hardware control of the device and automation of data acquisition, but also have the ability to deliver online diagnosis of the interrogated tissue sites based on an instant analysis of the spectra being acquired thereof. For making reliable diagnostic predictions, the software needs to be integrated with a robust diagnostic algorithm appropriately trained on a statistically significant number of previously measured (using the device) optical spectra from various pathologically certified oral lesions as well as healthy oral tissues.

(a)



(b)

Fig. T.2.4: (a) OncoDiagnoScope, and, (b) GUI for OncoDiagnoScope.

Towards non-invasive early diagnosis of malformities in the oral tissue, we developed a LED based compact and portable diagnostic system intended for use in a clinical environment (Figure T.2.4(a)). A touch-screen enabled GUI software developed and installed in a tablet computer integrated with the device provides the necessary interface for the hardware control of the device and automation of data acquisition and analyses (Figure T.2.4(b)). The changes in the optical properties of oral mucosa taking place during carcinogenesis can be measured with this USB powered device using a pencil-sized stainless steel fiber optic probe. The fiber optic probe is brought in contact with the suspected tissue of the oral cavity of a patient and light is shone upon it. The light coming out of the tissue is captured and fed to the tablet computer where it is analyzed by a smart diagnostic algorithm which can instantly determine whether the tissue is normal or progressing towards cancer. Two types of spectroscopic measurements (fluorescence and diffuse reflectance) are performed following illumination of the oral tissue by light from two LEDs of two types- broad band white LED for diffuse reflectance and UV-LED (365 nm). Light delivery to and collection from the target tissue is achieved with the trifurcated fiber-optic probe. The three legs of the probe, each comprising a fused silica fiber (0.22 NA) of core diameter of

600 µm, merge to form a common fiber bundle enclosed in an SS tube of 5 mm outer diameter and 200 mm length. Two of the fibers sequentially deliver (fluorescence and diffuse reflectance) excitation light to the tissue surface and the third fiber collects fluorescence or diffuse reflectance from the surface area directly illuminated by the excitation light. While the white LED light is directly coupled to the SMA connected end of one of the excitation fibers, the light from the 365 nm LED is spectrally cleaned by passing through a narrow band-pass filter before coupling to the SMA connected end of the other excitation fiber. A custom-made miniaturized 385 nm long-pass filter placed at the tip of the SMA connected end of the collection fiber blocks the back-scattered 365 nm light from entering the spectrometer.

The result is used to determine the pathology status without disturbing or destroying the tissue. The entire investigation procedure per subject using this device is less than 15 minutes as compared to several hours required by the conventional procedure of biopsy followed by histopathology. The software developed is user friendly with the probability of a tissue site being normal or abnormal represented by a colourcoded output- red, green or orange flash with red and green implying confirmed (probability > cutoff decided by the physician) abnormal and normal respectively, while the orange indicating borderline case. The borderline cases are the ones for which the posterior probability is less than the cut-off decided by the examining doctor and may, probably, require re-assessment.

The device has been validated in several cancer screening camps as well as in various hospitals including Tata Memorial Hospital, Mumbai on around 1500 individuals with different oral tissue abnormalities. The technology of this device has been transferred to M/s. Applied Optical Technologies Pvt. Ltd., Ambarnath, Thane. This device is also fabricated by Electronics Corporation of India Ltd. (ECIL), Hyderabad through technology absorption and available for supply. Two numbers of OncoDiagnoScope have been handed over for clinical trials to Homi Bhabha Cancer Hospital, Varanasi.

3. Future direction

The results of various clinical studies conducted using the developed point-of-care devices- TuBerculoScope, OncoVision and OncoDiagnoScope are encouraging. The results demonstrated with evidence that techniques based on optical spectroscopy and imaging are a promising tool in the detection of diseases like TB and cancer in a clinical setting. This has inspired the deployment of multiple sets of the devices to different hospitals and multi- centre clinical trials shall be conducted.

Further, an advanced version of TuBerculoScope, named 'Automated TuBerculoScope' has been developed which aims at reducing manual intervention in analysis of sputum smears for TB diagnosis. This device is augmented with a software controlled motorized stage which can be preprogrammed for automatic scanning and counting of TB bacteria per field of view within much shorter time than the TuBerculoScope.

Besides oral cancer, OncoVision as well as OncoDiagnoScope both have potential for applications in diagnosis of other superficial cancers like cervical and skin cancer. Detailed clinical study to generate a large number of patient dataset is required to extend the use of these devices in cases of cervical and skin cancer.

Potential of wider applications of OncoDiagnoScope, like probing deep tissues and internal organs like oesophagus and colon by modification of probe design needs to be explored. A feasibility study on optical spectroscopy-guided tumor tissue demarcation method during surgery is also envisioned. Exploiting complete potential of optical imaging and spectroscopy through development of clinically amicable point-of-care devices shall definitely bring a paradigm shift in disease diagnostics in recent times.

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