Optical Systems of Biopsy: The Invisible Eye

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Abstract
The exploration of new methods and techniques for the diagnosis of malignant tumours has always attracted the attention of scientists. The development of adjunct tools to facilitate the non invasive screening of high risk lesions in real time has the potential to significantly improve our ability to reduce the dismal morbidity and mortality of oral cancer. Despite easy accessibility of the oral cavity to examination, there is no satisfactory method to adequately screen and detect precancers non-invasively. The current method of oral cancer diagnosis clinically relies heavily on visual examination of the oral cavity. However, discerning potentially malignant and early malignant lesions from common benign inflammatory conditions can be difficult at times. There is a need for an objective method that could provide real-time results and be routinely applied to a large population. Though science is yet to present such a perfect technique, Optical Biopsy Systems developed using knowledge of light and tissue interaction, can provide a plausible option.

Key word: Optical Biopsy systems, Fluorescence, Oral Pre Cancer, Oral Cancer diagnosis.

Introduction
Oral cancer is the sixth most common malignancy worldwide. It is a highly lethal and disfiguring disease. The 5-year survival rate for oral cancer patients has remained unchanged at 50% for the past five decades, despite advancements in therapeutics. At the time of diagnosis, majority of the lesions are found to be at Stage III, with more than 50% of these cases exhibiting metastatic lymphadenopathy. However, if diagnosed early, oral cancer is often curable and causes lesser morbidity to the patient.

The common procedure for detecting malignant lesions consists of visual inspection, followed by biopsy of any suspicious lesions found. However, benign lesions - which are very common and diverse (lichen planus, candida infections, inflammation, hyperkeratosis, ulcerations etc) - may appear similar to early malignant or premalignant lesions, which makes it difficult to distinguish them even for experienced clinicians. On the other hand, “Mirror image” biopsies of normal looking mucosa from patients with oral cancer and precancer involving the contralateral side revealed that 58% of these apparently normal-looking mucosa demonstrated abnormal histological findings ranging from reactive changes to frank carcinoma.

Visual screening has been reported to have a sensitivity of only 74% and a specificity of 99%. This is because naked eye examination of human tissue under white light has a penetration depth of approximately 1 mm (eg: nail bed through a transparent nail). Also, the white light should be diffusely incident on the tissue, as reflection is a major variable on moist and shiny human tissue.

The main limitation in the present day scenario is that differential diagnosis of oral malignancy from other similar clinically presenting lesions is challenging even for experienced specialists. Patients may be reluctant to submit to an invasive and sometimes, painful biopsy. Also, the processing of biopsy material and the interpretation of results leads to diagnostic delay. Furthermore, there is increasing awareness that early squamous cell carcinomas (SCC) are clinically occult and not visible under standard white light examination. This escalates the possibility of taking an unrepresentative sample.

To ameliorate the diagnosis of early or secondary malignant transformations of oral mucosa; several different screening optical methods have been alternatively developed and discussed.

Discussion
Luminescence is the general term for emission of radiation by a surface or object. Fluorescence is the stimulation and emission of radiation from a subject by the impact of higher energy radiation upon it. Unlike Phosphorescence, fluorescence ceases almost immediately when the excitation is removed. This inherent fluorescence of many naturally occurring substances as well as human tissue is termed as Primary Fluorescence or Autofluorescence. Alternatively, fluorescent marker dyes, or fluorochromes can be introduced into an object and the resulting phenomenon on excitation is called Secondary Fluorescence.
Various techniques employed under optical biopsy systems are:

1. **Endoscopic examination**
   The Stroz bronchoscope has white light of broad wavelength distribution and a fluence of 60 mW. The Olympus gastroscope has a fluence of 40 mW. The image focus is not distance dependent. Information obtained is related to observer experience and visual contrast of the disease to normal surrounding tissue.

2. **Fluorescence photography**
   Onizawa et al developed a custom made camera with ultra violet flash lamps adjusted to excitation spectrum of 360 nm. They found maximum applicability in diagnosing superficial and ulcerative lesions.

3. **Enhanced/dye fluorescence**
   Individual molecules can be excited to a so-called singlet state, and subsequently relax to the ground state resulting in the emission of a longer wavelength of light than incident/excitation light. Intracellular molecules to which this applies include porphyrins such as Protoporphyrin IX (PpIX). The intracellular concentration of this molecule can be enhanced by the topical or systemic application of 5-amino laevulinic acid (ALA), a precursor of the haem biosynthetic pathway. PpIX fluoresces in the red spectrum of light near 636 nm after excitation with ultra violet/blue light (360-420 nm) and this feature is useful in tumour mapping. Images are obtained using a charge couple device (CCD) camera by an endocoupler to enable the study of hollow organs. Refinements include the subtraction of background fluorescence and use of polarization. This allows individual molecules and dyes to be studied in great detail.

4. **Fluorescence/Autofluorescence (AF) spectroscopy**
   When cells interact with light, they become excited and re-emit light of varying colours (fluorescence) and this can be detected by sensitive spectrometers. All tissues fluoresce due to the presence of fluorescent chromophores within them. Fluorescence spectroscopy can detect these substances and provide characteristic spectra that reflect biochemical changes occurring within the tissue. The resultant spectra not only detect the light that is fluoresced but also are sensitive to structures that absorb light. E.g: haemoglobin. With instrumentation standardization and advent of diagnostic algorithms, fluorescence spectroscopy has great potential for acting as a screening tool for early detection of malignant changes in oral tissues.

5. **Ratio Imaging**
   This technique compares a photochemical agent or its metabolic end product that is known to be increased in diseased state. Amino laevulinic acid converted to Protoporphyrin IX fluoresces red after excitation with blue light. The same excitation results in green fluorescence of molecules such as NAD and FAD, which become depleted in high metabolic states and this red: green ratio may be important in diagnosing degrees of dysplasia and setting thresholds for frank malignancy. This tissue examination takes place at two frames per second and can scan large mucosal and cutaneous areas.

6. **Elastic Scattering Spectroscopy (ESS)**
   ESS is an emerging technique that generates a wavelength dependant spectrum that reflects structural and morphological change within tissues. Elastic scattering implies that the light returns with same kinetic energy as the incident photons. The incident light can undergo single, or more commonly, multiple scattering events before being collected again at the same surface by an optical probe and the data analyzed. The acquired data reflects both the scattering and absorptive properties of that tissue. This scattering process has been shown to occur at the gradients in the optical index of refraction resulting from differences in densities that occur at a cellular and sub-cellular level. The structures that induce scattering are the nucleus, chromatin concentration and sub-cellular organelles. The study of malignancy using ESS is in still in developmental stages.

ESS optical signature of a lesion is greatly dependant on the morphology of the tumour, as it depends on the penetrating depth of the chosen wavelength and the fact that the greatest scatter will be obtained from these molecules that are the same size as photons scattering them. ESS is considered superior to other optical diagnostics in terms of early detection of low-grade dysplasia.

7. **Raman Spectroscopy (Vibrational Spectroscopy)**
   A Raman spectrum is a form of inelastic scattering and is generated by shift in frequency in the incident excitation light. This is caused by discrete changes in emergent light, above and below the wavelength of the incident photons due to the vibrational frequencies of the bio molecules that constitute the tissue. It is extremely sensitive and is most accurate of all the optical techniques so far, but the signal is extremely weak (in order of one trillionth of the incident beam).

Within the biological tissue, there are four principle components that contribute to the spectra: water, lipids (cell membranes), nucleic acids (DNA and RNA) and proteins (hormones, isoenzymes, immunoglobulins and keratins.) the resultant spectra from these structures give a characteristic signature for that tissue. The choice of wavelength also
enables the operator to probe different depths of tissue due to different wavelength penetrations and the technique, therefore represents a true form of optical histochemistry. The disadvantage is that, it is expensive, complex and difficult to adapt for in vivo use due to superimposed optical fiber and autofluorescence complicating the spectra.

Spectral identification of malignancy and earlier abnormal changes were achieved in a number of studies on laryngeal carcinoma (sensitivity 92%, specificity 90%), laryngeal dysplasia (sensitivity 76%, specificity 91%). Pharyngeal carcinoma was found to be easily diagnosed using this technique. Raman spectroscopy was also found to be a potential tool for the objective identification and classification of Barrett's oesophagus. Lau et al suggested that Raman spectroscopy could be a useful tool to distinguish cancer from normal mucosa.

8. Trimodal Spectroscopy
Combining Fluorescence spectroscopy, ESS and Raman spectroscopy can increase the accuracy of optical techniques. This is known as Trimodal spectroscopy. This combination enabled Muller et al to diagnose malignant/ precancerous tissue with a sensitivity and specificity of 96%, and to distinguish cancer from dysplastic tissue with a sensitivity of 64% and specificity of 90%. Again, this may prove to be very expensive and time consuming.

9. Electrical impedance spectroscopy (EIS)
It can be used to monitor the effects of chemotherapy or photodynamic therapy, and hence has applications during treatment and follow up. Tissue physiology can be documented below 10 kHz. This method is invasive and requires the insertion of probes into the tissue studied, and variables such as tissue impedance, especially with muscle and skin, need to be taken into account.

10. Optical Coherence Tomography (OCT)
OCT is analogous to cellular ultrasound imaging, and light waves as opposed to ultrasonic waves are used. The probing depth is limited to 300 m, although longer wavelengths of light allow imaging to 3 mm. It provides a cross section of tissue in contact with the probe. The resolution is 10 times that of any currently available in vivo diagnostic modality. A reference arm is used in parallel to the sample arm of the detector, and a temporal phase delay is incorporated. The number and wavelength of photons is digitally recorded. Parameters distinguishing different disease processes still need to be set.

11. Nuclear Magnetic Resonance Spectroscopy
This technology allows the three-dimensional study of atoms in a molecule, and the larger the magnet, more sensitive the device. It is possible to view the way in which the proteins link up to DNA and document the events surrounding gene transcription and signal transduction. The device is very bulky and costs astronomical amounts. Smaller and cheaper versions are becoming available and hand held devices are under exploration.

Conclusion
The field of head and neck oncology is increasingly using light spectroscopy techniques to not only diagnose dysplasia and malignancy but also to monitor treatment and potential complications. It may be used to monitor surgical margins and when used in conjunction with sentinel node biopsy, may have a role to play detection of cervical node metastasis. At present, large multicentre trials will be necessary to determine the sensitivity and specificity of individual and combined techniques, as well as to assess and improve their ability to favourably influence the early detection, management of head and neck oncology.

It has been suggested that combining optical techniques in the assessment of head and neck cancer with routine procedure will increase the accuracy and could definitely augment standard histopathological assessment and decrease the rate of recurrence. Though histopathology still remains the gold standard for confirmatory diagnosis, optical systems can provide an effective adjunct which is non invasive and aids in identifying representative site for biopsy as well. Further research in this field can open entirely new avenues in the arena of cancer diagnosis.

References


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