

Cancer Detection Using Biophotonics

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Introduction

Tissue visualization of patients with early malignant disease is still performed by the naked eye and illumination with white light sources. Compared to the sophisticated diagnostic methods available prior to surgical intervention, this appears almost medieval. Fluorescence imaging and spectroscopy, however, allows us access to entirely different optical information on tissue constituents. Interpretation of relevant optical data will greatly enhance tissue diagnostics.

Interest in the use of optical technologies to detect premalignant lesions has grown in the recent years. Optical technologies offer the ability to non-invasively diagnose and monitor precancers in-vivo with the use of optical technology and fluorescent drugs that localize in cancers. For many precancers and early cancers the current standard of care relies on histopathological assessment of directed biopsies in order to obtain a final diagnosis. Fluorescence diagnosis can be achieved in real time, using automated techniques, without removing tissue for biopsies.. Fluorescence imaging and spectroscopy is an emerging optical technology that offers particular promise for the diagnosis of disease, in part because of a large number of naturally occurring (endogenous) biological fluorescent substances (fluorophores) such as aromatic amino acids, reduced cofactors enzymes as well as extra-cellular matrix such as collagen and elastin. Fluorescence imaging and spectroscopy offer a means of assessing both the structural and the biochemical progression using these novel technologies, clinical studies have achieved promising results in multiple organs sites including bladder, lung, oral cavity, gastrointestinal tract and the cervix.

Principles of Fluorescence Diagnostics

What is fluorescence? Once a molecule has been electronically excited (for example by absorption of a photon with appropriate wavelength), it has several options to return to its ground state. One of these options involves emission of a photon, which carries the energy difference of the transition from the upper to the lower electronic state. The higher this difference, the higher the frequency or the shorter the wavelength of the photon. High energy photons are able to excite the 'blue' cones in our retina; low energy photons only the 'red' ones. By the law of energy conservation, the emitted photon can only carry less or the same energy compared to the excited one. So, fluorescence light is usually red shifted with respect to the light used for excitation.

This wavelength shift can be exploited for very sensitive detection of fluorescent substances, as the excitation light can be completely blocked by appropriate filtering, and the absence of background light (back scattered light) gives excellent contrast such as in light scattering spectroscopy and imaging. The fluorescence intensity observed is proportional to the concentration of the fluorescent substance.

The time delay between the absorption and the emission process can also be measured. This fluorescence lifetime is usually measured in nanoseconds, requiring fast electronics to observe small differences in fluorescence lifetimes. The signals can be generated in the time domain by using short-pulsed lasers or in the frequency domain using high-frequency modulated lasers. Time-gated imaging can be used to identify the fluorescence of a certain fluorophore thus reducing background fluorescence. Real lifetime images code the local lifetime in pseudo-colours and are no longer dependent on the fluorophore concentration and can provide information on the local binding site or pH in the cell milieu.

Today's Possibilities

***In-vivo* Autofluorescence**

After extensive studies of spectral properties of natural fluorescence or autofluorescence of tissue constituents, we now have some knowledge of the fluorescence fingerprints of normal and diseased tissue.

With different fluorochrome concentrations within the tissue of interest, the signals generated are often dependent on the layering of the tissue and tissue optical parameters. Many image analysis methods are currently employed to improve this technique, making localisation of small lesions within larger areas possible in real time. Among the commercial systems that apply some of these techniques are those produced by Xillix and Pentax systems (LIFE system for lung and gastric cancer) and also Storz systems (D-Light AF for lung and bladder cancer).

Drug or Dye-induced Fluorescence

A substantial increase in contrast can be obtained if a fluorophore selectively labels the target tissue. Most fluorophores or photosensitisers as they are usually referred to be used for photodynamic therapy (PDT). Many of these drugs also show fluorescence emission when activated by light and have been tested for their diagnostic potential. However, as any direct therapeutic benefit from diagnosis can only in some cases be assumed, potential side-effects of drug application cannot be tolerated to the same

extent as for a therapeutic purpose. Therefore, Photofrin®, a potent 1st generation photosensitizer approved in several countries, is considered unsuitable for routine diagnosis. Many of the first and second generation drug-photosensitizers suffer from the same restriction of prolonged general skin photosensitivity.

Today, many second and third generation photosensitizers based on old and new chemistries of their earlier counterparts but with better characteristics of rapid uptake and fast clearance are being designed for fluorescence diagnosis for early detection of cancers.

Bladder cancer detection using such photosensitizers is very promising (Fig. 1). Both topical and systemic use of drugs such as 5-ALA and analogous, and also hypericin for the detection of bladder cancer, head and neck cancers, gynaecological cancers and malignant neurological tumours are widely used all over the world.

However, how is fluorescence imaging of selectively accumulated photosensitizers best performed? As we know from fluorescence microscopy, proper filtering in the excitation and fluorescence emission light paths is necessary.

This is illustrated in Fig. 2. The technical implementation is very simple, but it still provides very impressive and reliable real time fluorescence images visible to the naked eye.



Fig.1. PDD system by Karl Storz in a urological operating theatre, comprising an excitation light source (D-Light), a light-efficient endoscope with observation filter and a CCD camera system.

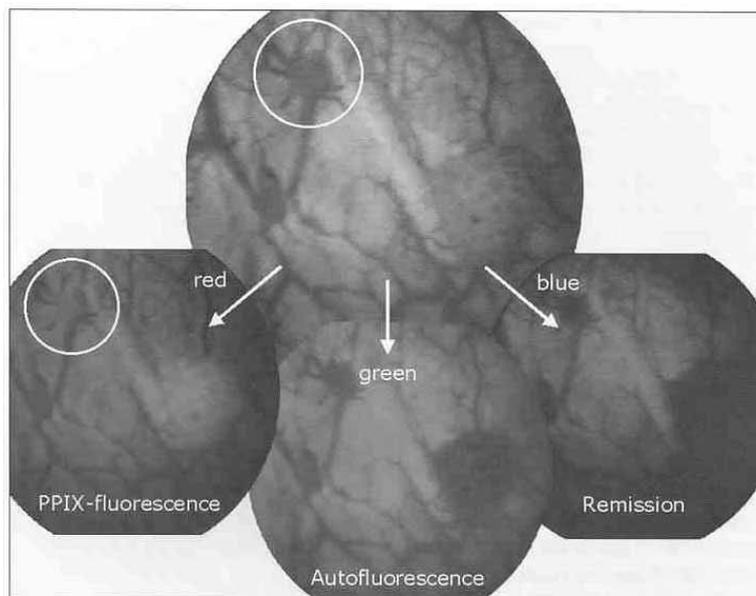
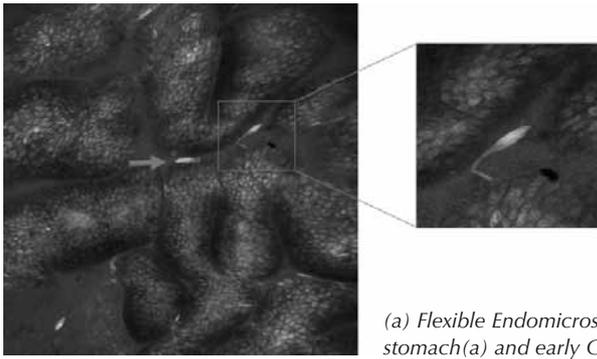


Fig. 2. Bladder cancer detection by Drug induced fluorescence and autofluorescence.. Monochrome fluorescence observation in a wavelength range covering the drug emission peak (red) does not give satisfactory results (lower left). Only the combination with the remission image (blue) and the autofluorescence (green) provides reliable information on the existence and localization of the tumours.

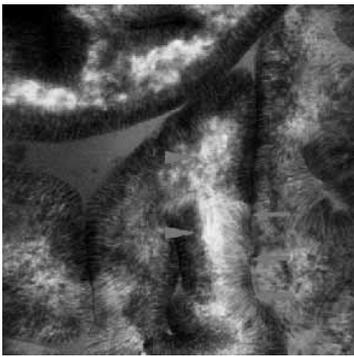
Advances in State-of-the-Art Optical Imaging

Confocal endomicroscopy

The function of the in vivo confocal endomicroscope is to non-invasively produce confocal 3-dimensional digital images of cells lining various hollow organs in real time. Fluorescence confocal endomicroscopy enables the visible detection of abnormal cells both on the surface and sub-surface of the mucosal lining of any hollow organ. The development of such a method for both macroscopically and microscopically assessing the lining of hollow organs was only possible with the recent invention of the Optiscan fibre confocal endomicroscope. Real-time image acquisition and digitisation of optical biopsies would allow for further image processing enabling derivation of meaningful real time information of the inspected site. This technique would be immensely useful in the determination of tumour margins and tumour staging during surgery. Fibre optic fluorescence endoscopic confocal imaging technology could potentially be applied to a number of organ systems including the oral cavity, aerodigestive tract, genito-urinary systems and tracheo-bronchial systems to detect early disease or predisposing conditions causing premalignant changes.



(a) Flexible Endomicroscope used In imaging *Helicobacter pylori* (arrow) in the stomach (a) and early Gastric cancer (b).



Light Scattering Spectroscopy

Biomedical imaging with light scattering spectroscopy (LSS) is a novel optical technology developed to probe the structure of living epithelial cells in situ without the need for tissue removal. LSS makes it possible to distinguish between single back scattered and multiple back scattered light from epithelial cell nuclei. The spectrum of the single backscattering component is further analysed to provide quantitative information about the epithelial cell. LSS imaging allows mapping these histological properties over wide areas of epithelial lining. Principal features of nuclear atypia associated with precancerous and cancerous changes in epithelium of organs could potentially be used to detect precancerous lesions in optically accessible organs.

Conclusion

Although the development of fluorescence detection has come a long way, there is still a long way to go. Firstly, more information on both endogenous tissue and exogenous fluorophores is required. Spectroscopy provides the possibility of detecting very subtle changes in relative concentrations of different fluorophores. Computing algorithms, ranging from multivariate linear regression over neural networks and principle components analysis, have been proposed to extract the most relevant features from spectral measurements. Fluorescence lifetime imaging can be used to eliminate background fluorescence or to provide information on the microenvironment of selected fluorophores.

Finally, fluorescence detection cannot be regarded as a stand-alone diagnostic tool. Properly analysed remission signals, optical coherence tomography, confocal techniques, polarised light scattered spectroscopy, and endoscopic ultrasound, navigational techniques based on computed tomography (CT), magnetic resonance imaging (MRI) or optical tomography will have to be rationally combined. This will certainly present a great step forward, not only for interventional diagnostics but also for other medical applications of clinical photonics in therapeutics. It is conceivable in the near future that highly sensitive endoscopic and non-endoscopic tumour detection methods can be successfully complemented by online staging and grading, thus finally fully justifying the term 'optical biopsy'. 🌐

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