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Fluorescence Kinematics: An Innovative & Promising Tool for the Diagnosis & Treatment Monitoring of Malignancy in Early Stage

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Abstract: ***For many cancers, early detection is the key for improving survival and reducing the morbidity associated with radical resections due to late presentation. Detection requests specific recognition, the advent of fluorescence imaging (FI) for cancer cell detection in the field of oncology is promising for both cancer screening and surgical resection. early detection is the cornerstone for improving outcomes and reducing cancer - related morbidity and mortality. Tumor markers have to be ideally present on the surface of cancer cells. Their targeting with ligands coupled to imaging agents make them visible/detectable. While the establishment brags that more cancer patients survive than ever before, the horrific side effects inflicted by conventional therapy often leave patients partially or severely debilitated, and set the stage for deadly secondary diseases. For those patient suffering from cancer the dangerous mix of chemotherapy and surgery not only failed to cure the cancer, but destroyed their remaining quality of life. The laser treatment has the potential to destroy the malignant cells in primary stage. This unique treatment can also seek and destroy cancer cells that have spread (metastasized) from the original tumor to other parts of the body. This is especially critical as metastasized cells are the primary cause of cancer death.*** *A laser beam* ***operating in the "near - infrared frequency of light. This beam heats tissue to a depth of several centimeters, allowing the beam to penetrate directly into a solid tumor with minimal damage to normal tissue because it can be focused directly through intact skin, no surgical incision is required. Fluorescence lifetime imaging (FLIM) is a novel imaging technique that generates image contrast between different states of tissue due to differences in fluorescence decay rates. presented fluorescence diagnostic system in combination with new fluorescent probes has the potential to distinguish between cancerous tissue samples with high enzymatic activity and non - cancerous tissue samples with lower enzymatic activity.***

Keywords: health, surgery, chemotherapy, laser

Abbreviation: Fluorescence lifetime imaging (FLIM)

Principle:
Fluorescence lifetime (FLT) of fluorophores is sensitive to the changes in their surrounding microenvironment, and hence it can quantitatively reveal the physiological characterization of the tissue under investigation. Fluorescence lifetime imaging microscopy (FLIM) provides not only morphological but also functional information of the tissue by producing spatially resolved image of fluorophore lifetime, which can be used as a signature of disorder and/or malignancy in diseased tissues.

1. Introduction

In the field of oncology, many tools have been developed for early cancer detection. Despite advances in modern imaging, cancer screening and surveillance remain imperfect. Fluorescence imaging (FI) for cancer cell targeting utilizes a variety of optical imaging technologies in order to improve detection of early neoplasia based on molecular signatures specific to cancer.

In recent years there has been much interest in the use of optical diagnostics in cancer detection. Early diagnosis of cancer affords early intervention and greatest chance of cure. Laser induced Fluorescence spectroscopy (LIF); Synchronous Luminescence Spectroscopy (SL) and Raman spectroscopy are some of the techniques used to detect the cancer in early stage. New screening tools are needed for women for whom x ray mammography is not suitable. We present a new and novel modality called "fluorescence lifetime imaging" as a potential tool for breast cancer detection and diagnosis. In this technique, the contrast for the diseased tissue is provided by a change in lifetime of fluorescent contrast agents such as porphyrins or some of the newly synthesized lifetime sensitive dyes.

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2. Results and Discussion

Fluorescence molecular imaging, using tumor targeted fluorescent dyes, is being widely explored for tumor detection during surgeries and for cancer staging (12 - 13)

Why the NIR (near infra - red) light?

In tissue fluorescence imaging, it is necessary to take into account five important parameters: reflection, absorption, refraction, background autofluorescence and distribution of photons emitted by the fluorochrome targeted to tissues. Skin is an obstacle because the emitted light is reflected by this barrier and this reflection brings a loss in the penetration of the excitation light.

Near - infrared (NIR) fluorescence cancer imaging is a growing field for both preclinical and clinical application to the clinical management for cancer patients due to its advantageous features, including a high spatial resolution, portability, real - time display and detailed molecular profiling with the multiplexed use of fluorescent probes. In this review, we present a basic concept of NIR fluorescence imaging and overview its potential clinical applications for *in vivo* cancer imaging, including cancer detection/ characterization, lymphatic imaging.

NIR fluorescence imaging can compensate some limitations of conventional imaging modalities, and thus it could play an important role for cancer imaging combined with other modalities in clinical practice.

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Basal cell carcinoma (BCC) of skin is the commonest form of cancer worldwide and its incidence is increasing. Approximately 800 000 - 900000 new cases are diagnosed each year in the U. S. A. It predominantly affects Caucasians and although typically a disease of older patients, it is also becoming more common in the population aged under 40 years. Most BCCs occur in areas of sun - exposed skin, with 80% being found on the head or neck. Although BCCs rarely metastasize, they have considerable potential for morbidity and disfiguration. Approximately $500 million is spent annually by Medicare for the diagnosis and management of non - melanoma skin cancer.

Recently, there has been considerable attention given to time - resolved studies of NADH fluorescence ([1 - 4]). A key advantage of the time resolved method is that the excited - state lifetime of a fluorophore is independent of its concentration. Furthermore, because the lifetime of the fluorophore directly relates to its microenvironment includes factors such as local pH, temperature, oxygen concentration and protein binding ([5 - 7]). Flim, which relies on the temporally resolved fluorescence signal, is advantageous over fluorescence intensity or spectral measurements for small volume tissue imaging because it is generally independent of fluorophore concentration.

This method can obtain information about the interaction of the fluorophore with the surrounding system, because the fluorescence lifetime of intracellular NADH molecules that coexist in both bound (localized mainly within the mitochondria) and free (localized mainly within the cytoplasm) forms are inherently different ([8]), this technique can be used to quantify the ratio of the free and bound forms of NADH despite the fact that both have similar spectral characteristics ([9]).

Most of these studies have discriminated between normal and neo - plastic tissue by detecting differences in the measured steady - state fluorescence intensities or spectra. Unfortunately, both techniques have limitations. Intensity measurements are sensitive to fluctuations in excitation intensity, and are difficult to quantify and compare between samples. ([10]) Spectrally resolved measurements are hampered by the fact that the broad fluorescence spectra of many tissue fluorophores overlap, limiting the degree of discrimination that can be obtained. ([11])

Time - resolved techniques, such as fluorescence lifetime imaging (FLIM), add a further dimension to fluorescence data by analyzing the temporal properties of the fluorescence. FLIM is an imaging technique that measures the rate of decay ('lifetime') of fluorescence at each point in the image after pulsed laser excitation ([12 - 14]) and plots the distribution of fluorescence 'lifetime' values. FLIM provides more reliable quantitative data than steady - state intensity imaging. Fluorescence typically decays over a period of picoseconds to nanoseconds after an excitation pulse ([15]) and the rate of this decay (or lifetime) depends not only on the fluorophores present but also on their surrounding microenvironment. [16] Fluorescence decay profiles are therefore sensitive to both the composition and the function of tissue. ([17]) For simple fluorescence decay profiles, the data can be accurately fitted to a mono - exponential decay model. For more complex decay profiles, such as those often observed from tissue AF, it is possible to fit such data to a mono - exponential decay model and obtain an apparent (effective) fluorescence lifetime value. Alternatively, the data can be fitted to a more complex model such as a stretched exponential decay profile, which corresponds to a continuous distribution of fluorophore lifetimes at each pixel that may be represented by a mean lifetime.

The object of the present study was the ultra - fast photodynamic processes of hematoporphyrin derivative (HPD) for diagnosis and therapy of cancer. Time - resolved fluorescence spectra of cancerous and normal cells were measured using an ultra - short pulse laser spectral technique and pico - second time - correlated single - photon counting system. The fast part of cancerous and normal cells fluorescence decay was approximately 150 and 300 ps, the fluorescence peak intensity of cancerous and normal cells decayed about 10% and 55% in 12 hour, the lifetime of cancerous and normal cells was about 824 and 1798 ps by calculating data of fluorescence decay, and HPD stay time was about 17 and 6 days in the cancerous and normal cells sample respectively. The data shows that cancerous cells were greatly intimate with HPD. The results obtained can be

used as an important basis for the diagnosis of cancer based on ultra - short pulse laser spectral technique. The results will contribute to feebleness ultra - fast fluorescence of biology sample for real time measurement.

This study uses multi - photon fluorescence lifetime imaging (FLIM) to characterize the fluorescence lifetime of normal and neo - plastic epithelial tissues in vivo. Multi - photon FLIM is a promising tool for the study and diagnosis of cancer in vivo because it non - invasively provides chemically specific information about tissue fluorophores and fluorophore microenvironment, and it is independent of fluorescence intensity. This technique exploits the intrinsic fluorescence of molecules already present in tissue, such as the metabolic coenzyme reduced nicotinamide adenine dinucleotide (NADH), thus obviating the need for exogenous contrast agents. Multiphoton microscopy can generate high resolution, three - dimensional fluorescence images deep within tissue while maintaining tissue viability, thus allowing for the visualization of cellular and sub - cellular structures. Our group has previously shown that multi - photon microscopy of epithelial tissues reveals statistically significant differences in tissue morphology and endogenous fluorescence intensity between normal, pre - cancerous and cancerous tissues (Skala et al., Cancer Res, 2004 in review). The current study provides an additional layer of functional information by characterizing changes in the fluorescence lifetimes and relative abundance of fluorophores in normal and pre - cancerous epithelial tissues.

The development of non - invasive, biomedical optical imaging from frequency - domain photon migration (FDPM) measurements of near - infrared (NIR) light propagation depends upon (i) the measurements of optical signal on the boundary of tissues and (ii) the numerical techniques enabling the reconstruction of interior optical properties from such measurements. From the mapping of interior optical properties, it is envisioned that diseased tissues can be identified and diagnosed based upon the differences in absorption and scattering properties. Briefly, FDPM consists of launching intensity - modulated light at the air - tissue interface and detecting the phase - delay and amplitude attenuation at another point distant from the incident point source. In the Purdue Photon Migration Laboratory (PPML), we have developed rapid multi - pixel methods for acquiring large data sets of phase - delay and amplitude attenuation across a tissue surface for use in an inversion algorithm in order to perform image reconstruction. In addition, since we have found that the endogenous contrast offered by absorption and scattering may be insufficient for biomedical imaging, we have invented a method for inducing contrast using fluorescent contrast agents. Algorithm development for biomedical fluorescence lifetime imaging was conducted under USAMRMC support. Using fluorescent agents, we have shown that the inverse problem may be better posed and that bio - diagnostic information can be obtained from assessing the fluorescent decay kinetics within the tissue.

This article describes changes in the fluorescence lifetimes and contributions of fluorescence emitting species in normal human breast epithelial cells at a two - photon excitation wavelength of 740 nm that correlate well with imposed changes in the intracellular reduction - oxidation ratio. Based on previous studies, we infer that these fluorescence signals likely arise from the protein - bound and free forms of NADH. Our studies suggest that the fluorescence lifetime of both the free and the protein - bound components of NADH and the ratio of free to protein - bound NADH is related to changes in the NADH/NAD^+ ratio.

In the presence of oxygen, eukaryotic cells depend on the mitochondrial electron - transport chain to produce useful chemical energy in the form of ATP. The first complex of this system, NAD^+ (an active coenzyme of niacin), functions as an important intermediary in the transfer of two electrons for cellular energy metabolism. Although it exists in an oxidized (NAD^+) and a reduced (dihydro - NAD^+, NADH) form, the photo - physics is such that NADH is an intrinsically fluorescent molecule, whereas its oxidized product (NAD^+) is not. In 1962, Chance et al. exploited this phenomenon to show that micro - fluorometry of NADH in intact cells and tissues give a continuous measurement of intracellular oxidation - reduction states in vivo ([18]). This pioneering work served as a precursor for a variety of studies that used direct monitoring of NADH fluorescence to dynamically interpret the metabolic activity within the cell ([19 - 20]). An intuitive application of this technique is to study carcinogenesis in a variety of organ sites such as the breast, which are known to have increased metabolic rates [21]. In the past decade, several groups have shown using quantitative NADH fluorescence measurements from tissues in a variety of different organs (including the breast and oral cavity) that significant differences exist between malignant and normal tissue types ([22 - 25]).

The key advantage of using the lifetime components rather than the intensity of NADH is that the lifetime is independent of NADH concentration and does not need to be calibrated for variations in the throughput of the instrument. We expect the outcome of this study to be a precursor for future work directed at functional mapping of normal and malignant breast epithelial cells, which may provide a novel way to track carcinogenesis in breast cells and ultimately, in breast tissues.

FLIM of NADH can detect changes in tissue metabolism with pre - cancer development in vivo using multi - photon microscopy techniques (Figure 1) in the same hamster cheek pouch model of oral carcinogenesis. The most important finding from this study is a statistically significant decrease in the relative abundance and lifetime of protein - bound NADH fluorescence lifetime with the development of pre - cancer in vivo ([26]). In the near - term, the findings from multi - photon FLIM studies could guide the design and development of practical time - gated fluorescence detection schemes for clinical applications. In long - term, portable technology could be engineered to enable multi - photon FLIM in a clinical setting. This technology could be used for epithelial pre - cancer detection and metabolic monitoring for tumor therapy.

Our study involves experimental and computational approaches to explore the technical feasibility of this technique. Experimental studies are planned which include frequency domain measurements of the fluorescent light in a multiple detector - multiple source arrangement.

Measurements will be performed for analyze sensitive dyes in heterogeneous tissue phantoms. These measurements will be used as inputs to a developed inverse imaging algorithm to obtain an image of lifetime. Since probe or dye lifetimes are sensitive to biochemical environment, diagnostic information is also obtained in addition to detection of disease. Preliminary computational and experimental results are promising and suggest a new imaging modality with specificity for breast disease.

The first fluorescence lifetime imaging (FLIM) studies of the free and bound forms of NADH was reported by Lakowicz et al. in 1992, where lifetime values of unbound NADH (free in solution) and NADH bound to malate dehydrogenase were obtained to reveal distinct differences in the excited - state lifetimes of the two species ([27]). This critical investigation suggested that FLIM - based techniques have the potential to provide researchers with functional maps of free and protein - bound forms of NADH and their corresponding lifetimes. FLIM of free and protein - bound NADH in cultivated endothelial cells from calf aorta ([28]) revealed lifetime values of ~0.4 to 0.5 and 2.0 to 2.5 nanoseconds for free and bound molecules, respectively. A few studies have been reported that exploit the intrinsic fluorescence lifetime of NADH for discriminating between malignant and nonmalignant cells/tissues. Time - resolved fluorescence studies of metastatic and non - metastatic murine melanoma cell lines, as well as human tumorigenic lung cancer and bronchiolar epithelial cells, showed that the average lifetime of NADH was lower in metastatic cells than in non - metastatic cells ([29]). Specifically, nonmalignant cells exhibited mean lifetimes in the range of 1.4 to 1.9 nanoseconds, whereas malignant cell lifetimes were in the range of 0.5 to 0.85 nanosecond. A single study has been conducted that uses the unique properties of FLIM to study breast cancer, primarily to show the feasibility of this approach for histopathologic assessment ([30]). This study, which used fixed but unstained tissue sections revealed statistically significant differences in the fluorescence lifetimes of benign and malignant tissues. Whereas this was the first FLIM study to be conducted that directly compared benign versus malignant breast tissue, the effects of sample fixation are likely to significantly alter fluorescence lifetimes compared with that of unfixed tissue. When recording the fluorescence lifetimes of metabolites related to cellular metabolism, it is clearly preferable to use viable cells and tissues.

3. Conclusion

Despite advances in diagnostic techniques and adjuvant therapies, the global burden of cancer - related disease remains exceedingly high.

Cancer treatment, diagnosis, and follow - up pose immense costs to both patient and healthcare industry. The use of FI as both a diagnostic tool and surgical guide enhancement has the potential to ameliorate the increasing cost of healthcare while simultaneously improving survival and QOL outcomes [34]. As new epidemiologic patterns evolve in response to improved life expectancies and changing lifestyle habits of the developing world, there is evidence that the incidence of cancer is growing While imperfections in cancer screening and surveillance are multifactorial, there is a pressing need for both improved cancer detection and innovative, cost - effective imaging modalities. Thus, the shift to develop low - cost, tumor specific fluorescent screening devices and contrast agents represent a major advantage in minimizing healthcare disparities among industrialized and developing nations while making a formidable impact on the global burden of several cancer types.

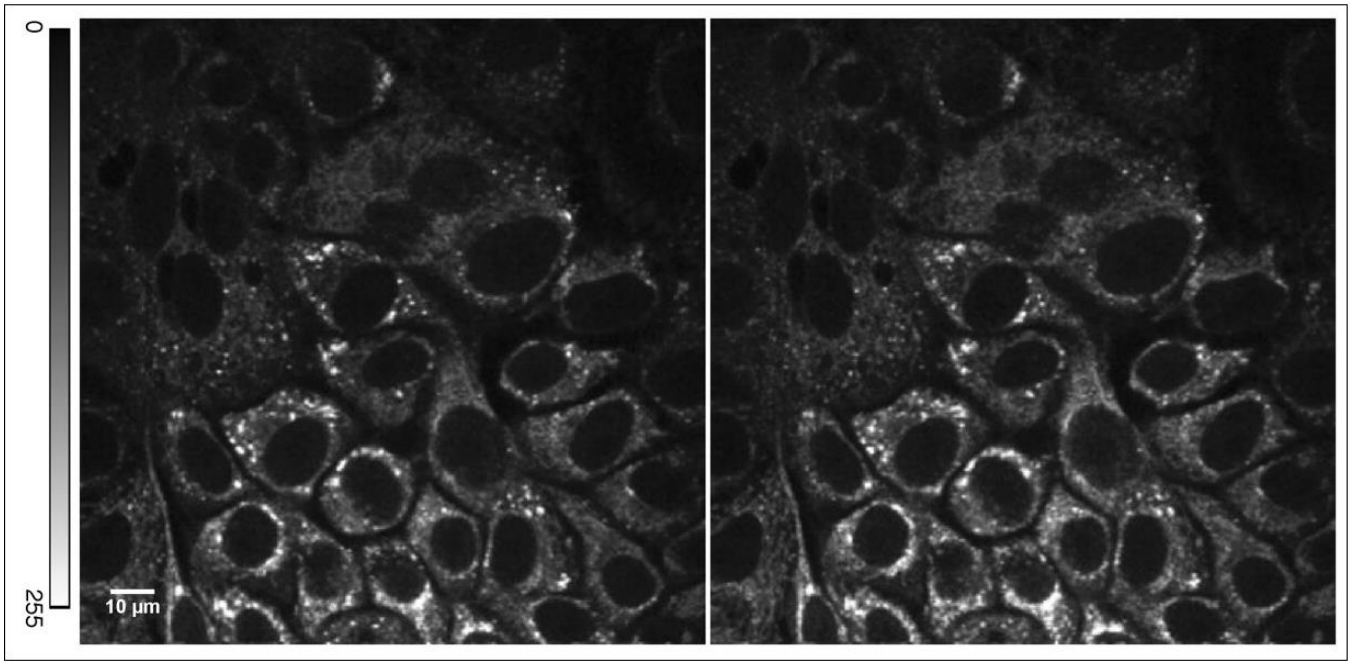

Figure 1: Two consecutively recorded multiphoton fluorescence intensity images of MCF 10A Pzip cells at a 1, 000, 000 confluence.

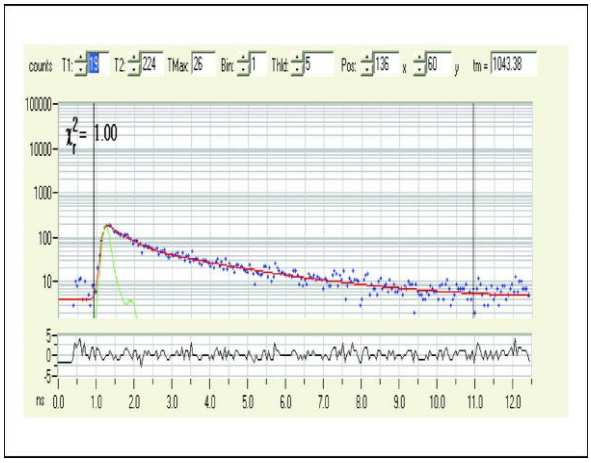

Figure 2: Fluorescence lifetime decay curve (blue), the instrument response function (green), best fit (red) and residuals (black) of a single pixel selected from within a multiphoton fluorescence intensity image of an MCF 10 A pZIP cell in a monolayer of cells at a 1, 000, 000 confluence.

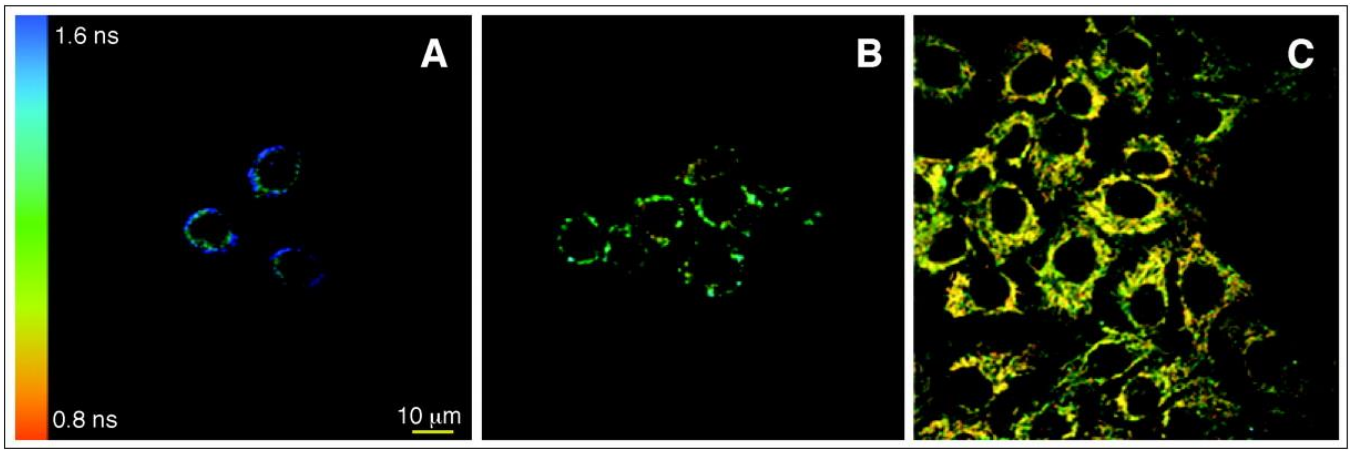

Figure 3: Represntative fluorescence lifetime images of MCF 10 A pZIP cells at (A) an early (25, 000 cells), (B) mid (100, 000cells, logarithmic), and (C) late (1, 000, 000 cells, confluent) point on the cellular growth curve

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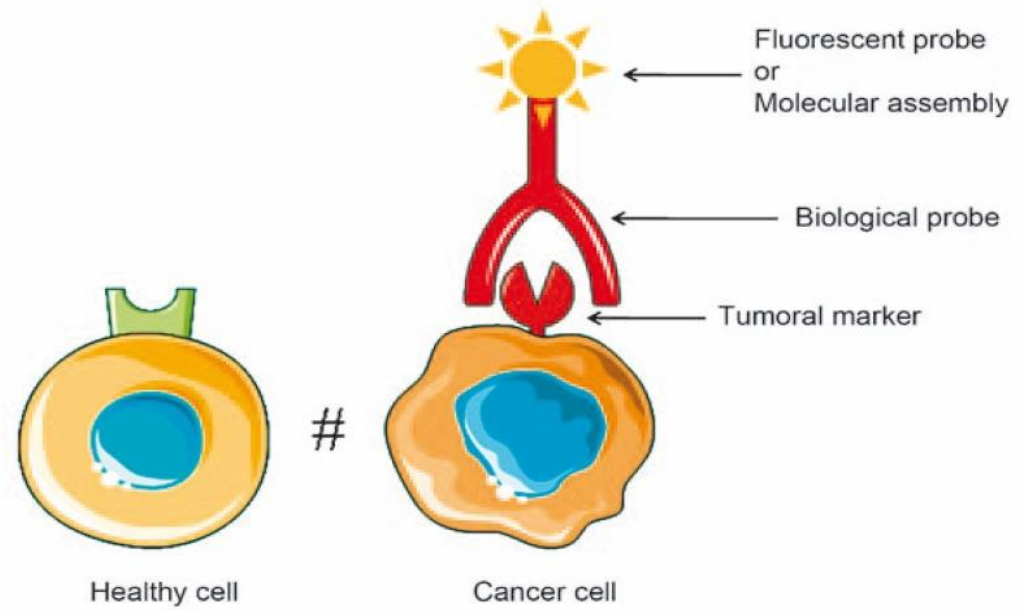

Figure 2: Principle of targeting tumor cells by fluorescence imaging.