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Laser-Induced Fluorescence for Imaging Cancer Cells

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Abstract

Cancer is one of the deadly diseases that threaten human life, so many researchers have studied diagnostic techniques as a step before surgery. These techniques are considered one of the effective methods in early detection of cancer cells. One of these modern methods is the use of laser fluorescence spectrum to image tumors, as this method has several advantages, including high sensitivity and locating the tumor in the body. In this research, the possibility of a laser operating in the visual region (532 nm) and silver nanoparticles is studied for vivo imaging of various cancerous tumors. The used detector, CCD (Genetic) type, with high sensitivity, in addition to an optical analyzer of the ocean 2000 type. A type of optical microscope was used to study the surface behavior of the tissue. The ability to diagnose the affected area using the laser induced fluorescence spectrum was observed at a very high rate, in addition to changes in the intensity of the fluorescence spectra, and the use of nanoparticles helped to identify affected cells within a single tissue.

Keywords: Fluorescence, Cancer Imaging, Laser, Fluorescent Imaging; Image-Guided Surgery

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طيف التألق المحتث بالليزر لتصوير الخلايا السرطانية

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الخلاصة

يعد السرطان واحد من الامراض الفتاكة التي تهدد حياة الانسان ، لذا درس العديد من الباحثين تقنيات التشخيص كخطوة تسبق الجراحة . وتعد هذه التقنيات من الطرق الفعالة في الكشف المبكر عن الخلايا السرطانية . ومن هذه الطرق الحديثة هي استخدام طيف التالق (الفلورة) الليزر لتصوير الاورام ، اذ تمتلك هذه الطريقة عدة محاسن منها الحساسية العالية وتحديد مكان الورم في الجسم. في هذا البحث يتم دراسة امكانية ليزر يعمل في المنطقة االمرئية (532 نانومتر) ودقائق الفضة النانوية لتصوير الاورام السرطانية المختلفة خارج الجسم الحي . الكاشف المستخدم CCD نوع (Genetic) ذو حساسية عالية بالإضافة الى محلل بصري نوع ocean 2000 كما تم استخدام نوع من المجاهر البصرية النافذة لدراسة سلوك سطح النسيج. تمت ملاحظة القدرة على التشخيص للمنطقة المصابة باستخدام طيف الفلورة المحتثة بالليزر بنسبة عالية جدا بالاضافة الى حدوث تغييرات في شدة أطياف الفلورة، كما ان استخدام الفضة النانوية ساعد على تحديد الخلايا المصابة ضمن النسيج الواحد.

الكلمات المفتاحية: الفلورة، تصوير السرطان، الليزر، التصوير الفلوري؛ الجراحة الموجهة بالصور.

Introduction:

Cancer may be a group of diseases with the power to invade or spread to other parts of the body involving irregular cell growth. Some sorts of cancer cause rapid climb of cells, while others cause cells to grow at a slower pace and divide. Some types of cancer lead to tumor-like visible growths, while others such as leukemia, do not [1]. The fluorescent imaging system benefits

from its protection, high-spatial resolution, and real-time capability, unlike traditional anatomical and molecular imaging technologies, and has therefore become a highly adaptable imaging method for clinical tumor detection and image-guided surgery[2].

Fluorescence spectroscopy is a class of techniques that by observing their interactions with fluorescent probe molecules, check the state of the biological system. This interaction is tracked by monitoring changes in the optical properties of the fluorescent probe. Lots of fluorescence spectroscopy measurement methods, such as laser-induced fluorescence" LIF", have been used in recent years [3].The absorption and dispersion of electromagnetic radiation by noble metal is greatly enhanced due to intense electrical fields at the surface. These specific features give the ability to create new optically active reagents for the simultaneous imaging of molecular cancer [4].

Related work

For sensitive detection and in vivo imaging of cancer cells taking advantage of their spontaneous ability to supply silver nano clusters (NCs) with high emission of near-infrared fluorescence by intracellular reduction of innocuous silver salts, a completely unique strategy is documented. [5]

Data from various experimental and clinical trials has shown important advantages of fluorescent imaging in targeted surgery with preoperative molecular diagnostic screening. a number of the researchers mentioned this method as "molecular imaging-guided surgery." Fluorescent imaging may help enhance intra operative staging and permit for more radical cyto reduction, detect deep tumor lesions in special organs, highlight tumor margins, better map metastases of the lymph gland , and intra operatively recognize important normal structures [6].FI(FLOURESENCE IMAGING)

systems are doubled since in addition to those under active monitoring, it are often extended to high-risk patients. This technology promises to spotlight lesions that aren't easily recognized by traditional imaging or physical inspection, enabling the identification of diseases at an earlier stage of growth. there's also a unbroken need for creative, cost-effective imaging approaches to scale back healthcare inequalities and therefore the global burden of cancer worldwide .[7]

As it allows use of non-ionizing radiation, optical imaging is completely secure. In addition, the method is quick, inexpensive, and allows anatomical and functional imaging in real-time. For non-invasive identification and visualization of tumors, optical imaging could be applied, enhancing the way cancer is treated and tracked. In addition, Image-guided surgery can help surgeons locate a tumor in real time and determine the scope of surgery during surgery. The main optical imaging technologies, imaging systems, and fluorescent probes currently available in the field of cancer research are the subject of this chapter. In addition, several studies are listed in which optical imaging for early detection of cancer, staging procedures, surgical guidance and monitoring of treatment are applied [8]. Laser-induced fluorescence (LIF) is an effective analysis tool with high sensitivity, smaller sampling consumption, short testing time and in situ testing advantages. It has also been one of the most commonly used spectroscopic approaches in recent years for in vivo diagnosis of cancer. There is a clear discussion of the possible impact factors for cancer diagnostics and the resulting suitability of the process for various applications. Meanwhile the technological merits and limitations of the cancer diagnosis LIF technology are also evaluated. In addition, on its active theory and effect comparison, various

exogenous fluorophores, endogenous fluorophores, and fluorophores synthesized in the tissue are contrasted. LIF's technological potential for further growth and future use[9]. A promising approach for deep-tissue high-resolution optical imaging in vivo is that the second near-infrared window (NIR-II, 1000-1700 nm), primarily thanks to the reduced dispersion of photons across biological tissues. For in vivo fluorescence imaging within the long-wavelength NIR range (1500-1700 nm, NIR-IIb), semiconducting single-walled carbon nanotubes with large diameters were used here. 3-4 μm large capillary blood vessels at a depth of around 3 mm might be resolved with this imaging agent [10] . To quantify fluorescence lifetime, optical imaging systems can be adjusted to measure fluorescence intensity. The lifetime of fluorescence is intrinsic, The property of the inner and outer fluorophores that relates to the typical time that the fluorophores remain until emitting light within the excited state. Advances in laser technology and devices have contributed to the development of time-resolved imaging techniques in small animals for full-body cancer imaging. Growing interest in development new fluorescence lifetime imaging (FLI) systems for tumor imaging has supported this trend [11-14] . Together with the right form of microscopy, in vivo fluorescence imaging using different fluorophores and/or fluorescent proteins enables in vivo visualization of the actions and function of cancer cells, as well as the microenvironment of the tumor. Using two photon excitation microscopy, cancer can be diagnosed in vivo without optical biopsy [15] . Laser detection of pieces of lung tissue excised from patients

Confocal microscopy and spectroscopy scanning. The cellular morphology and tissue structure, also because the pathology of

stained images, were demonstrated by the auto fluorescence images. supported the spectra analysis, most patients displayed distinguishing fluorescence from normal tissues in tumor tissues. Auto fluorescence imaging and spectroscopy also can be a possible tool to assist diagnose lung cancer [16]. microscopy and photography have gained particular attention in recent years. this is often thanks to the supply of fluorescent proteins, dyes, and samples that enable organic phenomenon, protein function, protein-protein interactions, and an outsized number of cellular processes to be non-invasively studied. At an equivalent time, there's an increasing list of fluorescent imaging techniques that give microscopic resolutions and video-rate scans, or techniques that operate at resolutions On the other side of the resolution spectrum, as a molecular imaging tool for small-animal whole-body tissue interrogations, macroscopic fluorescence imaging is gaining momentum. It has long been recognized that light in the far-red and far-red will spread through several centimeters of tissue.

Close-infrared (NIR) [17] . LIFS research showed its ability to differentiate between normal and malignant bone cells due to intracellular fluorophores, predominantly NADH, which showed cell metabolic activity. a considerable decrease in fluorescence spectra with malignancy was observed with normalized intensity. additionally to differences in strength, the R1 ratio has been shown to increase due to spectral shape changes in both normal and malignant cells [18] .

Fluorescence Terminology

Fluorescence is a mechanism from the photon emission that occurs from electronic excited states during molecular relaxation. These photonic processes involve transitions of polyatomic fluorescent molecules between electronic and vibrational states (fluorophores). Figure (1) provides a convenient

representation of the excited state configuration and related transitions. External light of a specific wavelength is used in fluorescence imaging to excite the target fluorescent molecule. The fluorescent molecule emits a photon of lower energy with a better wavelength upon excitation. CCD sensor camera system will subsequently detect this emitted photon.

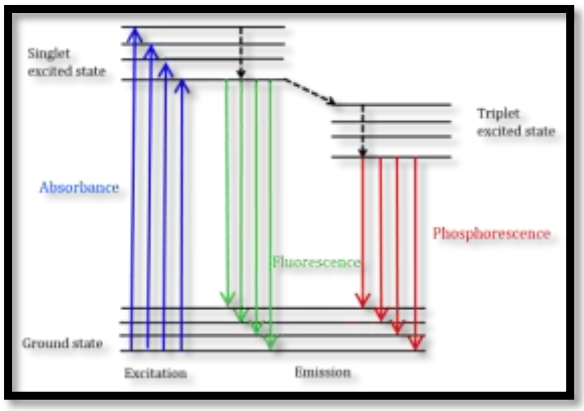


Fig (1): Transitions giving rise to absorption and fluorescence emission spectra

Fluorescence Imaging system

Optical imaging system simply consist of laser source (532nm) , a sensitive (CCD- genetic) camera and spectrum analyzer (Ocean-4000) used to image and analyzed emitted photons. Between emission and detection, photons usually have to propagate through a certain amount of tissue. The propagation of photons through tissue is determined by several optical parameters, including absorption and scattering as shown in figure (2).

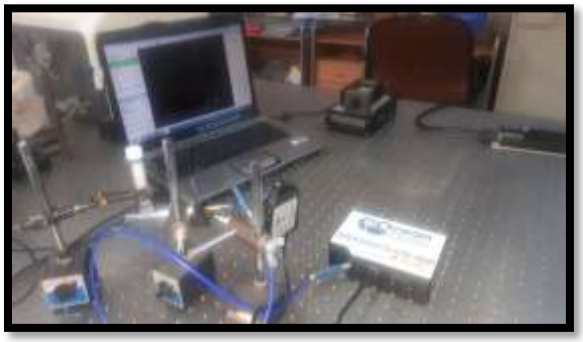


Fig (2): Fluorescence imaging system

Results and Discussion:

Ag synthesis

Optical and electronic properties of nano materials are governed by the very fact that their sub-nanometer dimensions are like the Fermi wavelengths of conduction electrons, resulting in molecule-like features like discrete size-dependent fluorescence. Silver nanoparticle's had been synthesis by using a pure silver target (99.98%) exposed to

Nd: YAG laser (1joule , 1 Hz,100 pulse , spot size 0.6mm) , energy density was (353.85 J/cm2) and time of ablation (1µs). due to their ultra-small size, biocompatibility, non-toxicity, good photo stability and high NIR fluorescence figure (3). Furthermore, Ag Nps generate large fluorescence intensities, often of an order of magnitude above traditional fluorophores.

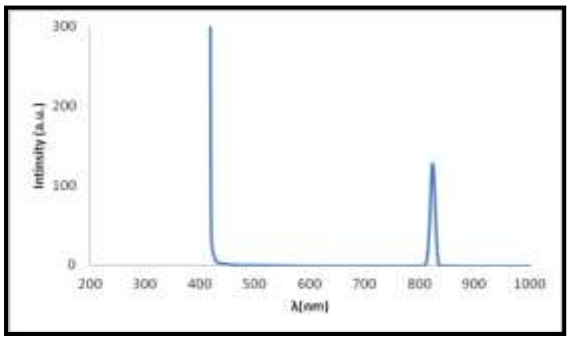
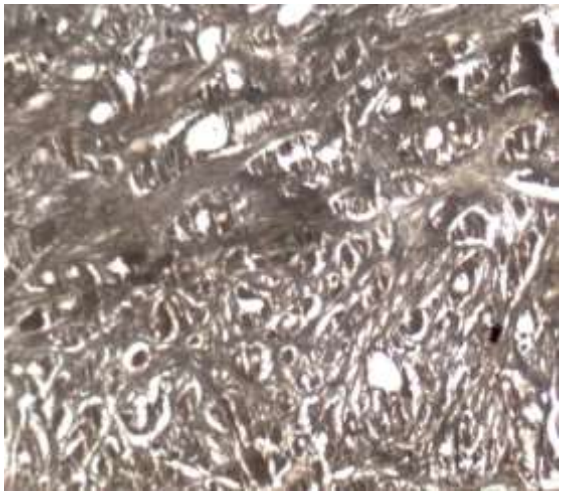
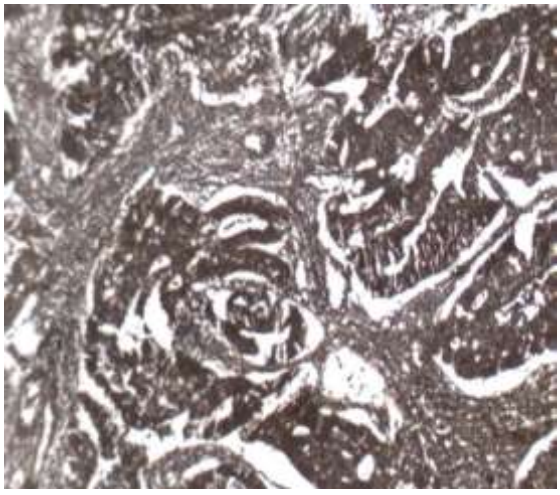


Fig (3): fluorescent spectrum for Ag nanoparticles solution

Microscope images

A microscope is used to observe and study the surface of the tissue with 50 times magnification, through which the tumor area is determined for later visualization by fluorescence spectroscopy as shown in figure (4) for two different tissues (1) breast cancer, (2) endometrial cancer. Two image has different morphology and shows normal and un normal cells within tissues.





(1) **(2)**

Fig (4): microscope images for two different tissues (1) breast cancer, (2) endometrial cancer

Beam profile images

charged-coupled device or beam profiler may be a diagnostic device for beam characterization which may measure the entire optical intensity profile of a beam, i.e., not only the beam radius but also the detailed shape. Beam profilers are used to measure beam quality figure (5).

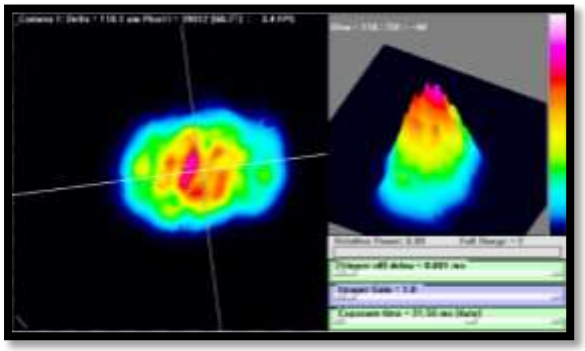


Fig (5): Intensity profiles of a Gaussian beam (right) and a multi-mode laser beam (left)

Beam quality monitoring with appropriate beam diagnostics are often important for several laser applications. A resolution (given by the pixel size) of the order of 5 µm is feasible with both CCDs camera. Most cameras are Very light sensitive. The beam then has got to be attenuated (see below) before hitting the camera. the camera records a beam profile because it occurs at another location (the imaged plane). This also allows good the recorded beam profile could also be displayed on a display screen, possibly alongside measured parameters like shown in figure (6) that represent green laser profile parameters that used for optical imaging.

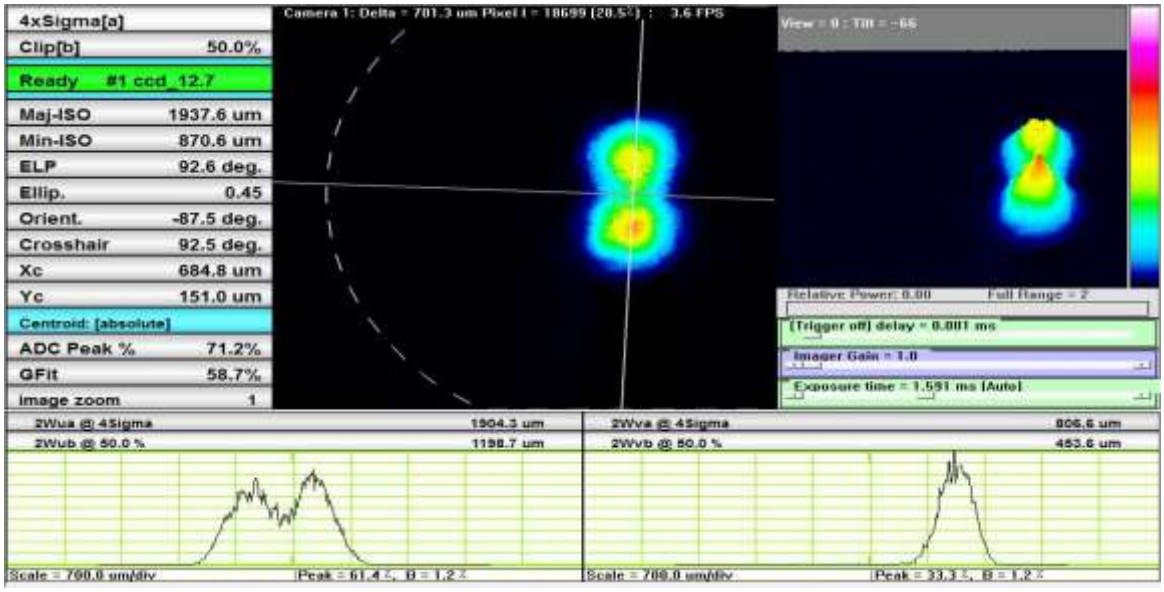
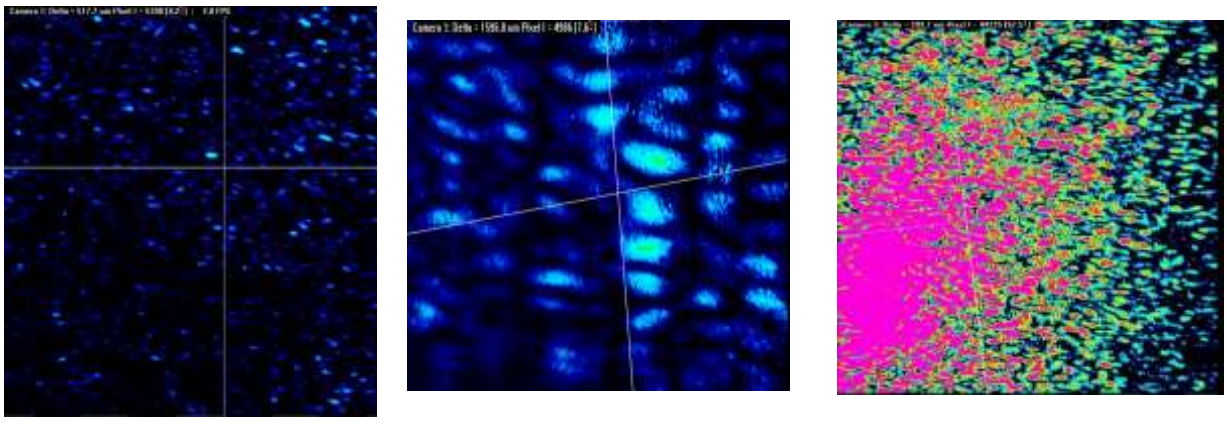


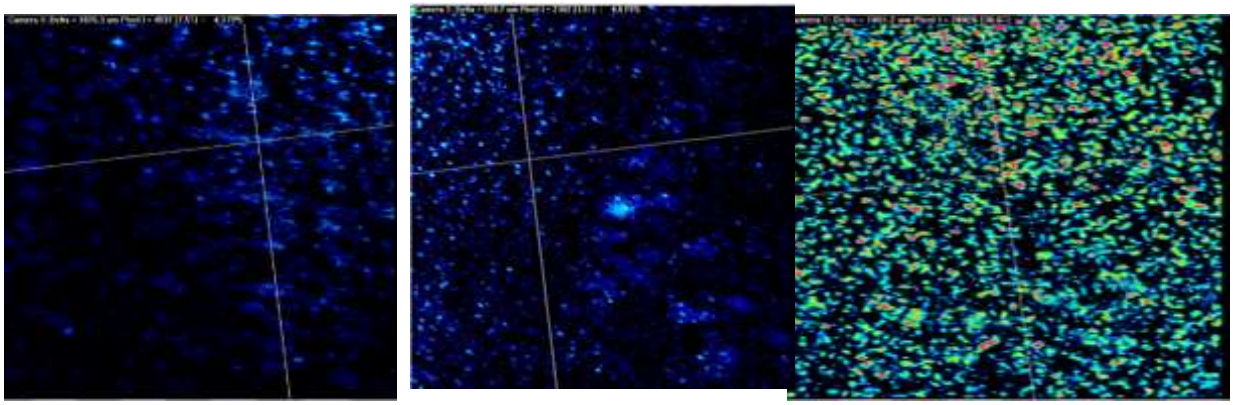
Figure (6) green laser beam profile image parameters (pixel size =701.1 µm, tilt= -60 , peak= 61.4)

Cancer tissues which had supplied from (Radioisotope laboratory), without any additives or dyes. Beam profile images for breast cancer and for endometrial cancer tissue shows different morphology for cancer tissue that show great visualize as a florescence image for un normal cell as in (a), more brightness with addition of Ag nanoparticles as in (b) and (c) figure (7) and (8).



(a) (b) (c)

Fig (7): beam profile images for breast cancer tissue were (a) tissue (1) without Ag (b) tissue (1) with Ag (c) color image for (b)



(a) (b) (c)

Fig (8): beam profile images for endometrial cancer tissues were (a) tissue (1) without Ag (b) tissue (1) with Ag (c) color image for (b)

spectrum analyzer (Ocean-4000)

The excitation light spectrum is shown with (532 nm) in Figure (9)

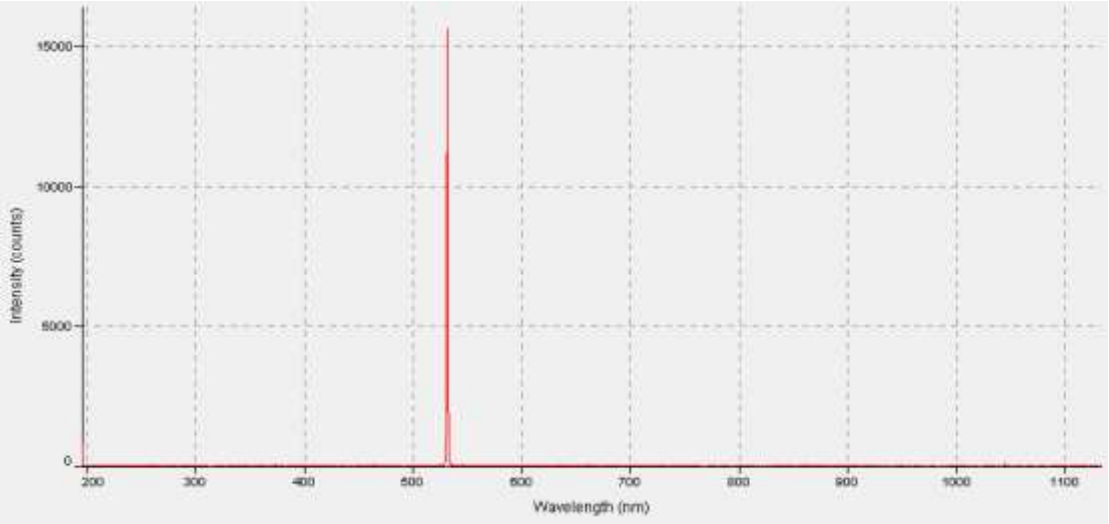


Fig (9): green laser spectrum

laser induced-fluorescence spectra for endometrial cancer tissue with two The fluorescence peak in nanometers (abnormal cell density peak), shown in Figure 10. It is often observed that after excitation, two peaks at 970 and 1070 nm were observed as abnormal cells with a significant decrease in fluorescence amplitude. This decrease is often

explained in terms of biochemical and microstructure changes due to cancer cell. Same manner for breast cancer tissue there is two florescent peaks (980nm ,1085 nm) with high amplitude, this can explain to the different structure and thickness for breast cancer tissue as figure (11) .

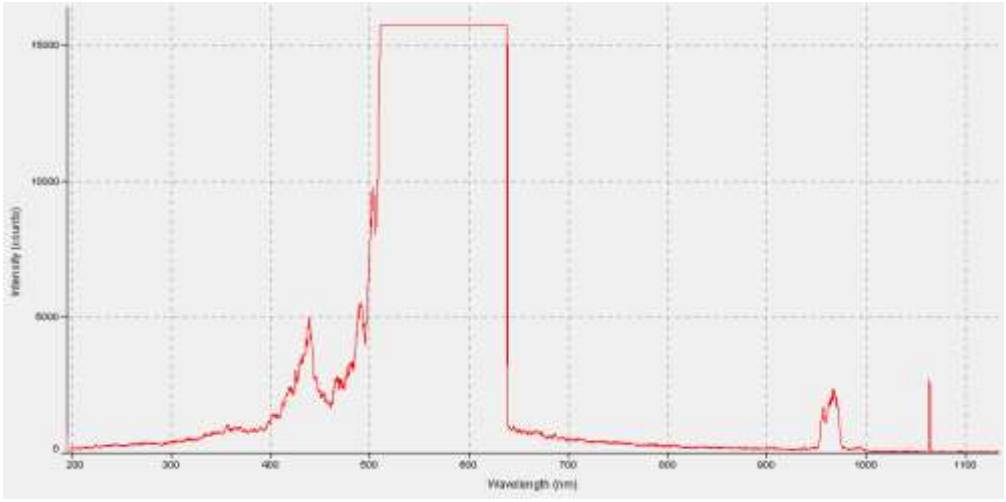


Fig (10): fluorescence spectrum spectra for endometrial cancer tissue

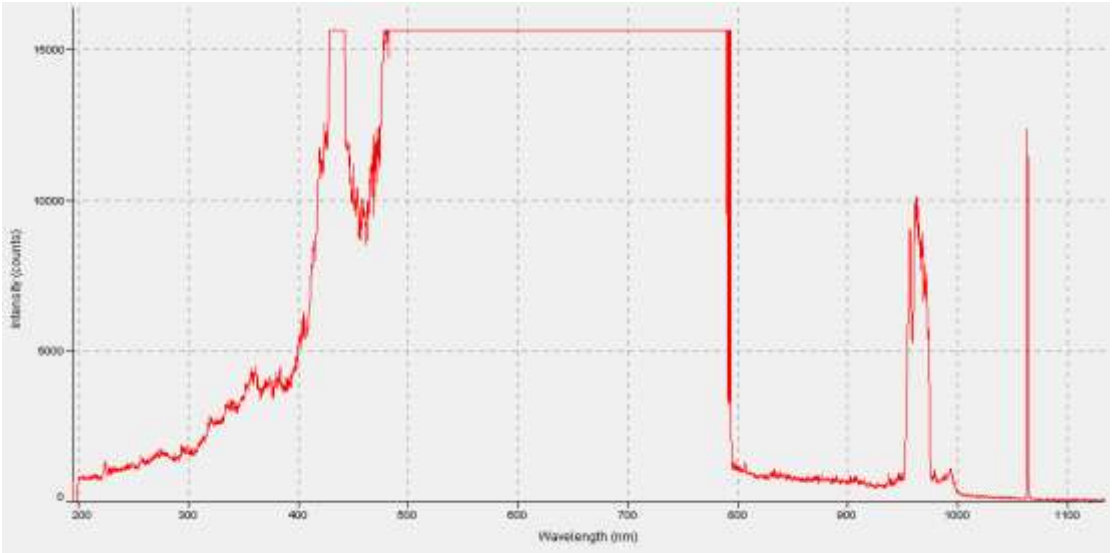


Fig (11): fluorescence spectrum for breast cancer tissue

Conclusions:

1- In this research the Laser-Induced Fluorescence technique demonstrated its ability to for Imaging Cancer Cells.

2- Optical imaging is taken into account safe because it makes use of non-ionizing radiation. Moreover, the technique is fast, cheap.

3- Intensity, a significant decrease in fluorescence spectra and intensity variations for two type of cancer tissues due to changes in spectral shape.

4-Information is obtained by measuring the photons came from a specific target of interest. Photons can be emitted either as a result of a chemical (bio) reaction or due to excitation of fluorescent molecules.

5- Silver nano materials are already widely used as fluorescence probes.

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