International Journal of Early Childhood Special Education (INT-JECSE)
DOI:10.9756/INTJECSE/V14I5.702 ISSN: 1308-5581 Vol 14, Issue 05 2022

DIAGNOSING COLON CANCER BY USING LASER INDUCED FLUORESCENCE (LIF) SPECTROSCOPY

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ABSTRACT
Cancer is a serious disease in which cancer cells divide out of control, resulting in uncontrolled growth and spread. LIF (laser fluorescence spectroscopy) as a potential cancer diagnostic technique. As a non-invasive tool for cancer diagnostics, a complete analytical investigation of an in vitro fiber-optic-based compact sensor LIF for point monitoring of tissue auto fluorescence is provided. The effects of two key sensor design parameters, excitation-collecting geometry and excitation wavelength, on tissue spontaneous fluorescence response and malignancy resolution, were studied. An attempt was made to establish the best sensor configuration for developing a diagnostic technique that would improve the accuracy of fluorescence cancer diagnosis by enhancing a tiny spectral difference.

Keywords: *Laser-induced fluorescent spectroscopy, Auto-fluorescence, cancer diagnosis.*

1. INTRODUCTION

Optical spectroscopy has long been used to gain a basic understanding of the physical, chemical, and biological processes that occur in biomaterials. Over the last 20 years, laser-induced auto-fluorescence, which was first detected by Stokes4 and later recognized as a possible diagnostic tool by Stubel5, has been widely explored and has emerged as a viable technique for biomedical diagnostics [1]. Molecules/atoms are stimulated to higher electronic energy states via laser absorption and then glow at emission wavelengths, which are distinct from the excitation wavelength and are longer (red-shifted). [2] It has been used to analyze a variety of materials in vitro and in vivo6,7, ranging from specific biochemical species (e.g. NADH, tryptophan) to human and animal tissues, because the related signal is strong and has a good signal to noise ratio. As a result, the achievable sensitivity increases[3]. This technique can investigate the biochemical and morphological changes that occur as tissue converts from normal to malignant in a rapid, non-invasive, and quantifiable manner. The spectral properties of measured fluorescence reflect the changed tissue architecture and bio-characteristics of native fluorophores associated with malignant transformations[4].aser produced fluorescence has so emerged as a promising tool6-9 in recent years, and great progress has been made in tissue fluorescence spectroscopy for malignancy. However, finding an accurate, sensitive, and quick approach for distinguishing between normal and cancerous tissue is difficult. The measurement of continuous wave (CW) fluorescence emission spectra of tissue when illuminated at a single excitation wavelength was the focus of several early studies[5]. The differences in the emission spectra of normal and sick tissue were subsequently investigated in order to try to optically diagnose the disease process. Variations in tissue structure and fluorescent species concentration were blamed for the spectrum discrepancies [6]. The spectral contrast between normal and malignant tissue, on the other hand, is exceedingly important and a crucial concern for the successful treatment of cancer In these studies, the diagnosis remained exceedingly poor. Demos et al15, for example, were able to achieve a maximum fluorescence intensity contrast of 0.4 to 0.65 using their method. Because the fluorescence signal from a photo-sensitizer is often higher in strength than auto-fluorescence,[7] Nadeau et al16 used aminolaevulinic acid (ALA) as a photo-sensitizer medication to obtain high signal contrast, despite its own drawbacks and potentially serious side effects. They were able to achieve a fluorescence contrast of less than 5 in the optical spectrum of malignant and normal tissue. In addition, all of these experiments used bulky, expensive, and complicated equipment, such as pulsed nitrogen or dye lasers and time gated enhanced CCD or diode array detectors. [8]Another key aspect of tissue fluorescence diagnostics is that it is not just dependent on the concentration and distribution of fluorophore (s) in the tissue. In tissue fluorescence measurement, the lighting and collecting geometries of the excitation and emitted light are also important. Furthermore, as a constitutive bio-species, most tissues contain numerous fluorophores [9]. The existence of each of these integral fluorophores has a different effect on the composite tissue spectrum, depending on how intensely the excitation light excites these fluorescent species. As a result, using a single wavelength for optical tissue diagnosis has limitations in terms of fluorescence analysis and optical identification for illness prediction. Different types of excitation may be induced by using different wavelengths of light.fluorophore to fluoresce, resulting in different spectral.[6,12].Tissue spectra profiles/intensities with improved ability to distinguish between diseased and non-cancerous tissue. This necessitates the use of multiple wavelength excitation to provide greater spectral resolution and tissue type separation for early cancer detection. This research presents a thorough investigation of a laser-induced fluorescence (LIF)-based optical fiber sensor that can distinguish between normal and cancerous tissue at an early stage[10]. The sensor has been tweaked to improve the accuracy of cancer diagnosis by enhancing the slight but persistent spectral difference between normal and malignant tissue. [11]

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2- **EXPERIMENTS**

2-1 Sample Preparation

2-1-1 Preparation of Serum-Free Medium

Liquid RPMI-1640 medium was prepared from powdered RPMI-1640 medium according to the Gibco product manual as the following:

From the RPMI-1640 powdered medium, 10.43 g was dissolved in approximately 900 ml of DDW in a volumetric flask.[15] The other components include: 2 g sodium bicarbonate powder or according to need and 1.25 ml from gentamycin stock solution had been added with continuous stirring. The volume was completed by DDW to one liter and the pH of the medium adjusted to 7.4. [13] Sterilization was done by 0.4 and 0.2 µm Millipore filters subsequently. After the end of the procedure, 5 ml of the medium was incubated at 37 ºC in a sterile flask for 4 days with daily examination for signs of bacterial and fungal contamination. It was considered sterile only in case of no signs of contamination during the four days of incubation. Then the medium was stored at 4ºC until use. [14,16]

2-1-2 Preparation of Serum-Medium

Serum-medium was prepared with the addition of 10% FBS.

2-1-3 Preparation 6f Colonic Prostate PC3 and Madin-Darby Canine Kidney (MDCK) Cell Lines For Cytotoxicity Assays (Meleady and O'Connor, 2006):

prostate PC3and MDCK cell lines in frozen vials were obtained from Tissue Culture Laboratory in the College of Medicine / University of Babylon.

2.2 Sensor Configuration

The figure (1-1) shows the arrangement of the LIF system and as an indication of each part of it:

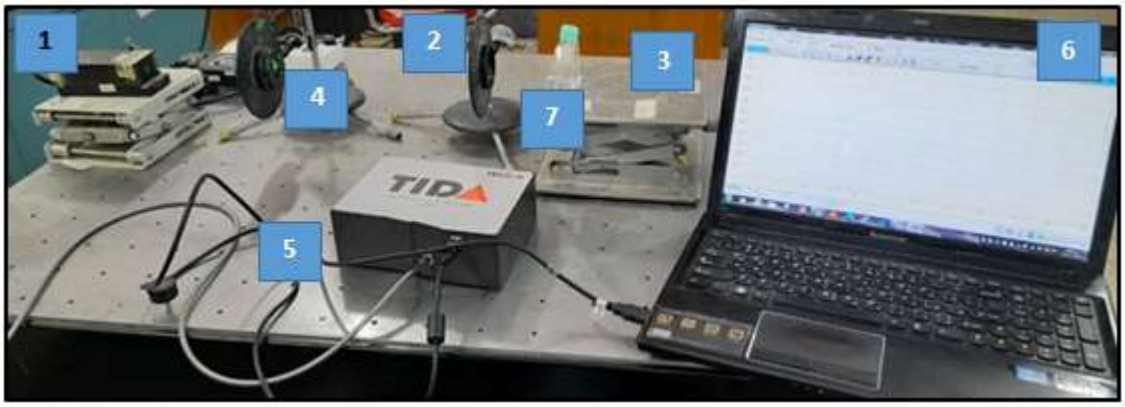


Fig. (1-1) Parts of the LIF technology system

The Main Parts Are

1- A CW diode laser source, with a basic wavelength (473 nm), for sample irradiation.
2- A light lens used to illuminate the laser pulse on the surface of the sample with a focal length (f =10 cm).
3- A surface for placing the sample for the purpose of examination. The distance between the laser and the sample (10cm).
4- Aperture to control the diameter of the laser beam.
5- The optical fiber holder is divided into circular angles (360°), to control the placement of the optical fiber at the best angle for receiving the plasma spectrum.
6- A computer to run the spectrum analyzer software that shows the data as a graph of wavelength as a function of intensity. Hence, the possibility of storing detailed data for the spectrum.
7 - The sample under study and targeted by diode laser and located under the arm carrying it.

3- RESULTS ANDCONCLUSION

1- Effect of Excitation Wavelength to Diagnosis the Colon Cancer Cells

Figure (1-2) shows the effect of excitation wavelengths (473, 532, 403) nm to diagnosis the colon cancer cells in fluorescence spectrum.

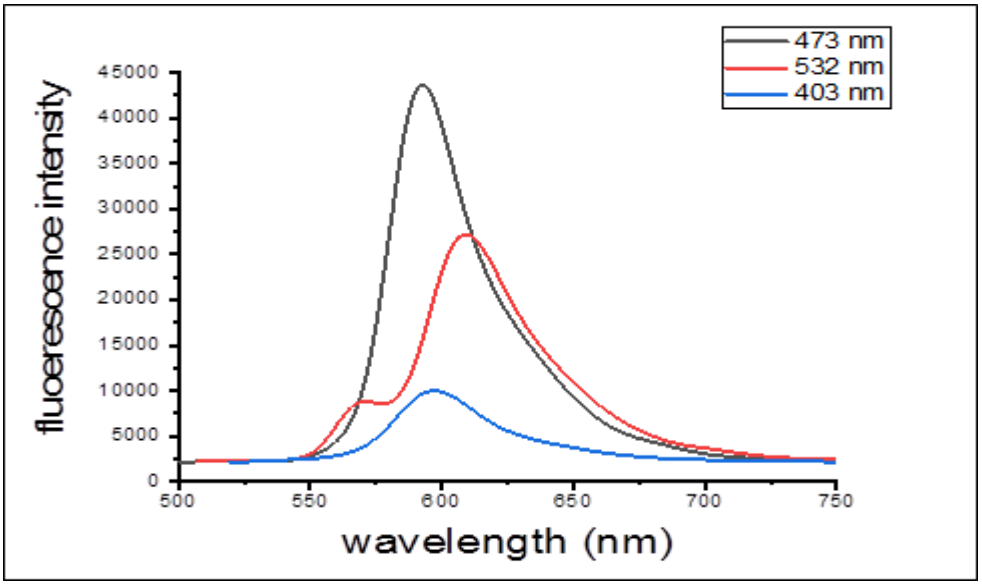


Fig. (1-2): Fluorescence spectracancerous cell at excitation wavelengths (473, 532 , 403) nm

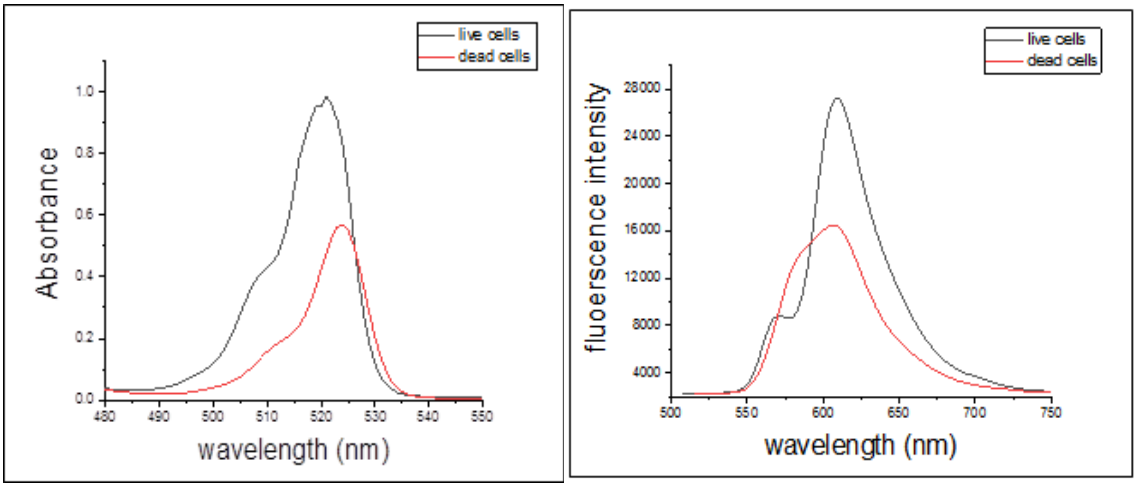
In this figure, the difference in diagnosis using the LIF technique using three different wavelengths (473,532,403) nm is discussed and explained. Where it is expected that the use of different wavelengths will lead to the formation of different spectral intensities of the spectra of tissues to verify the response of cancer cells to the wavelength used, and this reflects the spectral contents similar to the wavelength used in excitation. From the figure above , that notice the difference between the fluorescence intensities at different wavelengths due to the amount of proteins present in the contents of the colon cancer cell, and This is due to the difference in the characteristic emission wavelengths, corresponding to the different constituting bio-species (fluorophores) that got excited with each excitation wavelength. The blue line represents the emission spectrum obtained at wavelength 403 nm, the red line represents the emission spectrum obtained at wavelength 532 nm, and the black line represents the emission spectrum at wavelength 473 nm. In the case of excitation at wavelength 473 nm, a maximum emission spectrum for colon cancer sample was observed equal to (43680) in contrast to the case of excitation at wavelength 403 nm in which the peak of the emission spectrum is very small equal to (9990). In the emission spectra, there is a component that fluoresces at wavelengths 532 nm and 473 nm, but stops contributing to emission at wavelength 403 nm. The spectral shapes were not completely identical at all wavelengths used in the diagnosis, and the difference in the intensity of emission is due to the presence of many biochemical components in the cell sample this agreement with (reference).The values are shown as in the table below:

Table (1-1): Results of Fluorescence spectracancerous cell at excitation wavelengths (473, 532 , 403) nm

Laser source	λ_{max} (nm)	intensity
473 (nm)	600	43680
532 (nm)	609	27220
403 (nm)	597	9990

2- Comparison of the fluorescence and Absorption for live and Dead Colon Cancer Cells

Figure (1-3) shows the comparison between live and dead cells of colon cancer cells by absorption and fluorescence spectra:



(a)(b)

Fig.(1-3): a) Absorption of live and dead cell colonic Cancer b) Fluorescence of live and dead cell colonic Cancer

Figure (1-3) illustrates the comparison with the absorption and fluorescence spectra of live and dead cells of colon cancer diagnosed using the LIF technique.

In this figure the difference between live and dead cells of colon cancer cells is discussed and explained. When preparing these cells by the method of tissue culture of cells, these cells live for four hours at most, and then die due to different conditions. Where it was possible to clarify the difference and diagnose these cells using laser induced fluorescence technology.

Figure a represents the absorbance, the red line represents the absorbance of dead cells and the black line represents the absorbance of living cells for colon cancer. It was observed that the absorbance of living cells (0.957) at a wavelength of 522 nm was higher than the absorbance of dead cells (0.571) at a wavelength of 524 nm . In living cells, the reason for the high absorbance is due to the presence of cancer cells in addition to other chemicals present in the cancer cell formation compound. As for dead cells, the absorbance mostly refers to the chemical compounds found in the compound, where the absorbance of dead cells is very low. This also applies to the fluorescence spectra shown in figure b. The red line represents the fluorescence spectrum of dead cells and the black line represents the fluorescence spectrum of living cells. It was noticed that the fluorescence spectrum of live cells (27220) at a wavelength of 610 nm is higher than the fluorescence spectrum of dead cells (16500) at a wavelength of 607 nm, and this is due to the accuracy of the LIF technique in the diagnosis. The values are shown as in the table below:

Table (1-2): Results of Absorption and Fluorescence of live and dead cell colonic Cancer

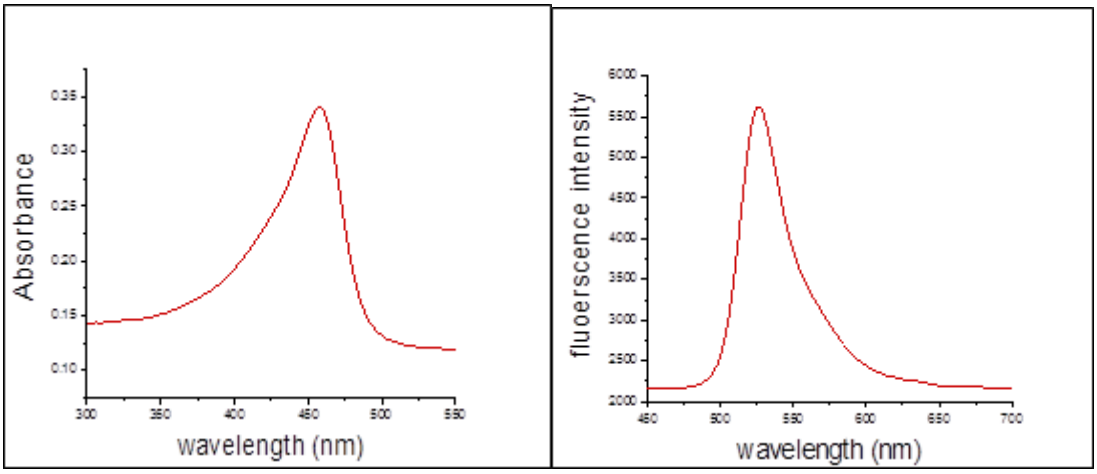
Material	λ_{max} (nm)	Absorbance
Live cells	526	0.295
Dead cells	529	0. 506

Material	λ_{max} (nm)	Fluorescence
Live cells	526	0.295
Dead cells	529	0. 506

3-Absorption and Fluorescence of thecolon Cancer Cells culture without TiO_2 NPs treatment

This part show and discuss the absorbance and fluorescence spectra behavior of the colon cancer cell before treatment with the TiO_2 NPs. As well as knowing the behavior of the absorption and fluorescence spectra of each chemical compound that was used in the tissue culture of cells that include rpmi 1640 medium and trypsin in order to distinguish them from the absorption and fluorescence spectra of colon cancer cell.

4- Absorptionand Fluorescence of Rpmi 1640 Medium



(a)(b)

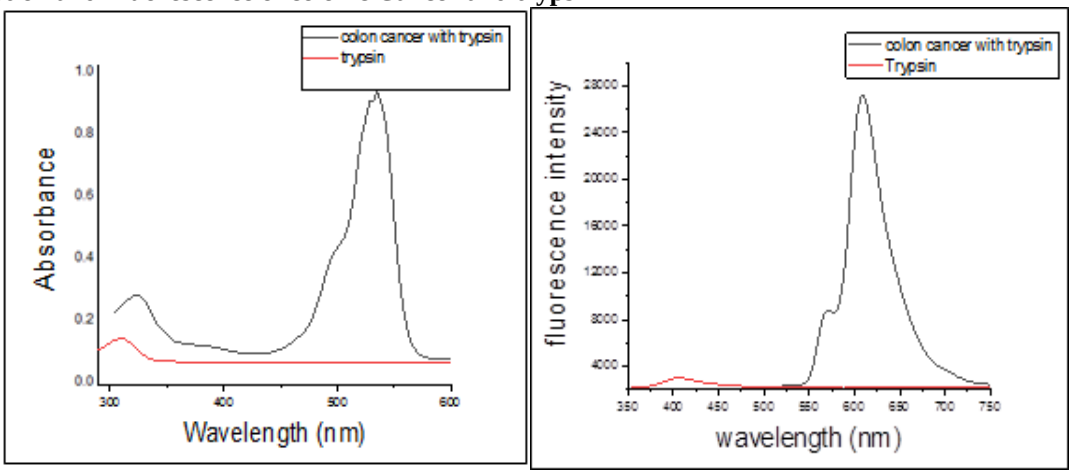
Fig.(1-4): a)Absorption of rpmi 1640 medium, b)Fluorescence of rpmi 1640 medium

rpmi 1640 medium represents the major component of all cell culture cultures as it is the food medium for tumor cells, so it can negatively affect the fluorescence assay by causing high levels of fluorescence to the media. To evaluate the effect of this medium on the fluorescence results at diagnosis, the fluorescence and absorption spectra of the rpmi 1640 medium were calculated. Where the highest absorption of the rpmi 1640 medium is (0.329) at the wavelength (452 nm) and the highest fluorescence of the medium (5620) at the wavelength (526 nm), as these results showed a relationship between the wavelengths of the radiation of the medium with wavelengths of fluorescence from the cancer cell, and the difference in intensity and wavelength was observed.The values are shown as in the table below

Table (1-3): Results of Absorption and Fluorescence of rpmi 1640 medium

	λ_{max} (nm)	Maximum peak
Absorbance	452	0.329
Fluorescence	526	5620

5- Absorption and Fluorescence of colonic Cancer and trypsin



(a) (b)

Fig. (1-5): a) Absorption of colonic Cancer and trypsin , b) Fluorescence colonic Cancer and trypsin

Often used in proteomic research, Trypsin is an enzyme that breaks down proteins to separate or detach cells from the culture vessel. This process is important when cells need to move from vessel to vessel, and when complete, the cells will become suspended and appear rounded. Since trypsin is one of the chemical compounds found in colon cancer cells, the fluorescence and absorption spectra of trypsin were calculated and compared with the fluorescence and absorption spectra of colon cancer cells.

The figure (a), the red line represents the absorbance spectrum of trypsin (0.261) at a wavelength (326 nm) and the black line represents the absorbance spectrum of colon cancer cells with trypsin (0.957) at a wavelength (522nm). In figure (b) , the red line represents the fluorescence spectrum of trypsin (2990) at a wavelength (409nm) and the black line represents the fluorescence spectrum of colon cancer cells with trypsin (27220) at a wavelength (610nm). The values are shown as in the table below:

Table (1-4): Results of Absorption and Fluorescence of colonic Cancer and trypsin

Material	λ_{max} (nm)	A
Colon cancer with trypsin	522	0.957

Trypsin	326	0. 261

Material	**λ_{max} (nm)**	**intensity**
Colon cancer with trypsin	610	27220
Trypsin	409	2990

CONCLUSIONS

A thorough investigation of the effects of various excitation wavelengths on tissue fluorescence properties and diagnostic response is presented. The sensor, which consists of a single illumination centered fiber and six ring fiber probe, was found to significantly improve sensitivity, as well as cancer diagnosis and resolution capability. It did, however, depend on the wavelength of stimulation. The use of a sensor with a 473nm excitation wavelength increased sensitivity significantly, allowing for substantially improved cancer detection and resolution. As a result, this operational sensor configuration was considered to be the best combination for achieving the highest spectral discrepancy and disease resolution for biomedical applications, as well as the best for diagnostic analysis. This research also shows that auto-fluorescence spectroscopy can be improved to better distinguish between cancerous and non-cancerous states. When the findings of this method were compared to those described in the literature, they showed great agreement and a higher resolution capacity.

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