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Sensing and differentiation between normal flora and pathogenic of *E.coli* Bacteria using 410 nm diode laser

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Abstract: Background: Optical biosensors offer excellent properties and methods for detecting bacteria when compared to traditional analytical techniques. It allows direct detection of many biological and chemical materials. Bacteria are found in the human body naturally non-pathogenic and pathologically, as they are found in other living organisms. One of these bacteria is Escherichia coli (E. coli) which are found in the human body in its natural and pathogenic form. E.coli bacteria cause many diseases, including Stomach, intestines, urinary system infections, and others**. The aim of this study**: is sensing and differentiation between normal flora and pathogenic E.coli**. Material and method:** The optical biosensor constructed of a multi-mode – no core- multi mode optical fibre that differentiates between pathogenic and non-pathogenic bacteria of E.coli by measuring the changing for light intensity using source of light 410nm laser diode. Multi-mode - no core - multi-mode optical fibre (MM-NOC-MM) connected to the OSA analyser (HR2000) by means of an adapter and finally connected to a computer to show the results**. Results**: The intensity of the transmitted light recorded in the case of pathogenic bacteria is less than the intensity of the transmitted light recorded in the case of non-pathogenic bacteria**. Conclusion**: these results were obtained because of the ideal and better choice of the wavelength of the laser used with its absorption E.coli bacteria**.**

Introduction

Normal flora are the microorganisms that live on the surface or inside another living organism (human or animal) or inanimate object without causing disease ((Wang et al., 2017)). Sometime it is called commensal because of their permanent presence on body surfaces even if covered by epithelial cells and are even exposed to the external environment (e.g., respiratory and gastrointestinal tract, genital, hair, etc.) ((Dekaboruah et al., 2020)).Normal flora plays an important role in immunity and inflammation. Significance of the normal Flora for their host is very important. They directly influences the anatomy, physiology, immunology, even susceptibility to true pathogenic organisms, and even morbidity-mortality of the host; in short terms, it affects the homeostasis of their host (Best et al., 2019). Microbial normal flora has spatio-temporal involvement that differs individually, regional body niche, age, geographical location, health condition, diet and also by type of host ((Lloyd-Price et al., 2016)) **.**

Pathogenic bacteria: The oldest bacterial pathogens in microbiological strain collections date from the 1890s, soon after medical bacteriology was introduced Pathogenic microorganisms cause various

infectious diseases and even death. Despite early triumphs over infectious diseases with the development of vaccines and antibiotics, new and multidrug-resistant pathogens are continuously emerging (Yoo & Lee, 2016).

***Escherichia coli* (*E. coli*)** *Escherichia coli* is a facultative anaerobic microorganism found in the gastrointestinal tract of warm-blooded animals, with which it maintains a mostly symbiotic relationship. *E. coli* has also been found in soil, water, and sediments not directly influenced by sewage discharges. Besides commensal strains, there exist also pathogenic variants of *E. coli*, capable of causing either intestinal or extra intestinal diseases. Pathogenic strains were probably derived from commensal strains following the horizontal acquisition of chromosomal and extrachromosomal genes and operons, as well as gene loss (Tallon et al., 2005; Whitman et al., 2006; Tenaillon et al., 2010) Some *E. coli* strains can cause a wide variety of intestinal and extra-intestinal diseases, such as diarrhea, urinary tract infections, septicemia, and neonatal meningitis (Clermont et al.,2000) Phylogenetic analyses have shown that *E.coli* strains fall into four main phylogenetic groups (A, B1, B2, and D) and that virulent extra-intestinal strains belong mainly to group B2 and B1, to a lesser extent, to group D, whereas most commensal strains belong to group A. These studies have also given us a better understanding of how pathogenic strains acquire virulence gene. E.coli is one of the most important and prevalent bacterial types in causing urinary tract infections in women, and it constitutes more than 80% of pathogens because it is a natural plant inside the human body. From sticking to the epithelial lining of the urinary tract, these bacteria have the ability to survive and gather for a long time inside the urinary tract of the host to be able to avoid the body's immune response and resistance to antibiotics and make it capable of causing repeated infections of the urinary tract(Journal, 2014). **Biosensor:** A biosensor is a device that contains a biological sensing element that is closely associated or integrated with a transducer. The common aim is to produce a digital electronic signal that is proportional to the concentration of a particular chemical or set of chemicals. ((Shatti & Al-ameri, 2021)) The seemingly bizarre connection of two opposing disciplines combines the specificity and sensitivity of biological systems with the computing power of the microprocessor. This emerging technology transcends many traditional academic frontiers and offers a powerful new tool that threatens to radically change the way we think about analytical science. (Pandey and Malhotra 2019) In general, biosensors consist of a bio receptor, the recognition element responsible for capturing the target analyse, and a transducer, the properties of which are modified by the binding of the analyse as shown in figure (1). (Peltomaa et al. 2018) The biological component of a biosensor used for molecular detection consists of highly specialized macromolecules or complex systems with corresponding selectivity and sensitivity. Biosensors can be classified according to the bio components used for detection. (Hossain and Mansour 2019).

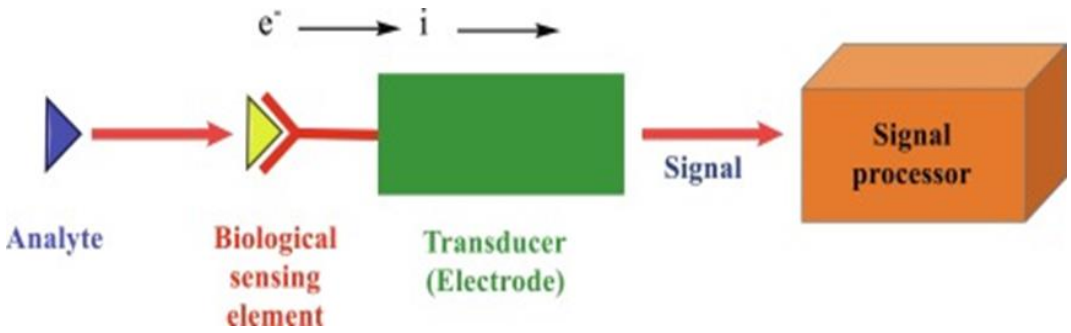

Fig. 1: Biosensor system detection ((Saleh, 2020)

Types of Biosensors: Biosensor is classified according to:
1. Bio-elemnt (Molecular, Cell-based, Tissue-based)
2. Transducer (Optical, Mechanical, Electrochemical)
3. Principle of operation (Fluorescence, Surface plasma resonance, Absorbance\reflectance, Piezoelectric, Surface acoustic wave, Cantilever resonance frequency, Amperometric, Potentiometric, Impedimetric (Alageedi & Alameri, 2019) as shown in the figure (2).

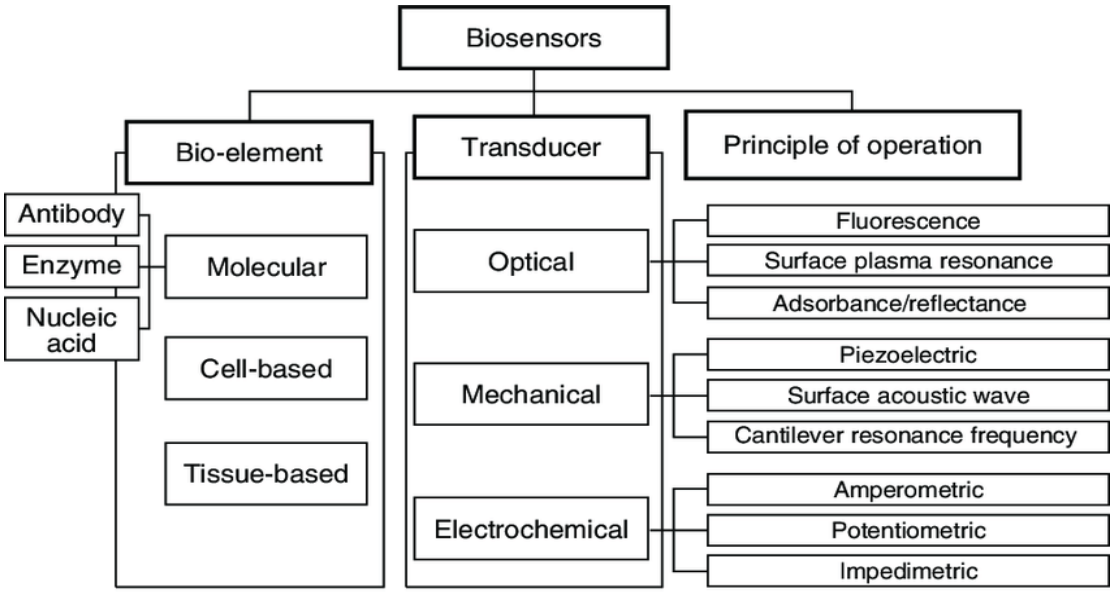

Fig. 2: Types of biosensors (Salam & Alabd, 2019)

In general, optical sensors can be distributed into four classes based on changing in light parameters such as polarization modulation intensity modulation, wavelength modulation, and phase modulation.(Alameri et al. 2020)

Basic Design of the Optical Biosensor consisting of:

A. Light source (LED, laser, and other types).
B. An Optical fibre.
C. The sensing element (translating the measured into optical signal).
D. Optical detector and electronic processing (spectrum analyzer, oscilloscope, etc.).
optical systems have: been an important target by many researchers. Because, it has been applied in many fields. Optical fibres are uses in different applications like fibre sensing, spectroscopic analysis, optical fibre laser, and optically filtering. (Kareem & Mansour, 2022)

Material and Method: Samples were collected from a group of patients in Al-Diwaniyah Teaching Hospital with diarrhea, intestinal infections and urinary tract infections, as well as from a group of healthy people. The probes were taken, sterilized, and taken to the laboratory. They were cultured on special and differential culture media and incubated at 37°C for 18-24 h.

Subsequently, biochemical and antibiotic sensitivity tests were performed as well as the Vitec 2 device to confirm the diagnosis

Table(1): showing biochemical test for E.coli bacteria

Lactose fermenting MacConkey agar	Blood agar	Gram stain	oxidase	catalase	urea's	indole	Methyl red	citrate
+	**variable**	**- ve**	**-**	+	-	+	+	**-**

The Optical Biosensor Setup:

1- multimode- no core - multimode optical fibre 40cm in length was considered as the conventional optical fiber, a segment about 3 cm in length was made in the middle of the fibre(no core fibre) using a cutter to make .finally using hydrofluoric acid 40% for 20 min to etching the clading so that the diameter of the optical fiber after etching becomes 75 μm by viewing and measuring it under a microscope ding (A small drop of the bacterial solution is placed on it to detect and differentiate the bacteria) .

2- The whole fibre (40) cm was put on the plate using a adhesive.

3- The two ends of fiber were connected with an adapter device

An adapter is used for connecting the optical fiber with the laser from one side and the spectrum analyzer (OSA) from the other side. standard type connectors such as FC, SC, ST, LC, MTRJ, or SMA

4- First end connected with laser source (diode laser) power supply. The light source had been used in this experiment was Blue laser with λ= 410nm and output power = 40 mw .This source of laser has a power supply which is stable at all the time of use it. Selecting this light source was because the absorption spectra of the bacteria samples covered this wavelength

5- The second end connected with spectrometer (ocean HR2000) and computer to obtain signal of intensity as shown in figure An optical spectrum analyzer (ocean optics HR2000) with (0.065nm) resolution was used to display

transmission interference spectrum of the sensor.

The spectrometer type (ocean optics HR2000), have the following characteristics:

Operating in the wavelength range from 200nm to 1100nm

-Resolution are 0.065nm of the high wavelength

-At full signal, the signal-to-noise – ratio are 250:1

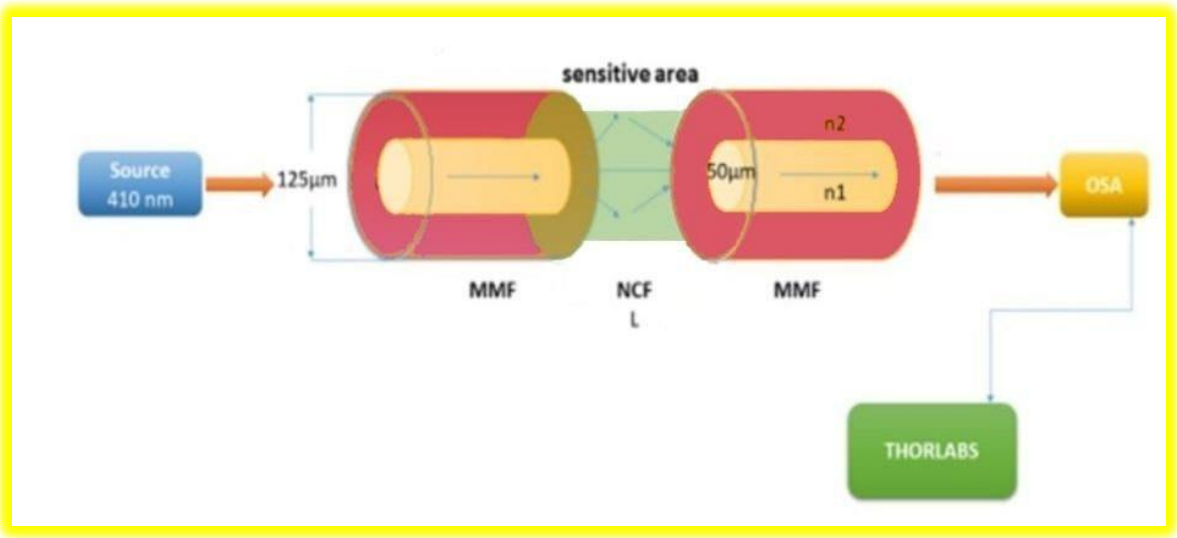

Fig. 3: Basic Elements of a Fiber Optic Sensor(Lang et al., 2019)

Statistical analysis:

The data were analysed using the Microsoft Excel 2021 and Statistical Package for Social Sciences (SPSS IBM-version 26.0) software. The results reported in this study were expressed as mean ± SD (Standard Deviation) and frequencies were expressed as percentages on tables and graphs. Chi-square, independent T-test, and ANOVA were used to examine the degree of significance. Probability values (p-value) less than 0.05 were regarded as significantly different while probability values less than 0.01 were regarded as highly significant .

Result and discussion:

Biological measurement The positive and negative biochemical tests that were performed on the bacterial colonies of E.coli can be seen as shown in Table (1). The laser intensity of normal and pathogenic E.coli was measured by the sensor MM.NOC.MM as shown in Table (2, 3). Through the shape of the peaks, we can see the unique shape of the peak of laser intensity according to the type of fibre used, the absorption of bacteria to laser wavelength and the laser source used(410nm). Through the study, it was found that the laser intensity measurement of pathogenic E.coli bacteria is less than that of non-pathogenic E.coli bacteria due to the higher absorption of pathogenic bacteria because it contains virulence factors, as shown in the figure(4) and figure(5)

Table 2 (pathogenic E.coli): Show results of sample intensity, Peak wavelength nm (air), FWHM(pm)air).

Sample no	Intensity	Peak wavelength nm (air)	FWHM (pm)air
Source	1.000000	406.0802002	820.1606058
1	0.6932	406.5158	447.0406
2	0.6301	406.5158	772.9235
3	0.7026	406.5158	662.4109
4	0.7295	406.5158	587.2161
5	0.6036	406.5158	671.4633
6	0.8087	406.7337	661.1219
7	0.6848	406.5158	658.2355
8	0.7995	406.5158	634.9289
9	0.6937	406.5158	700.9178
10	0.7523	406.5158	661.4069

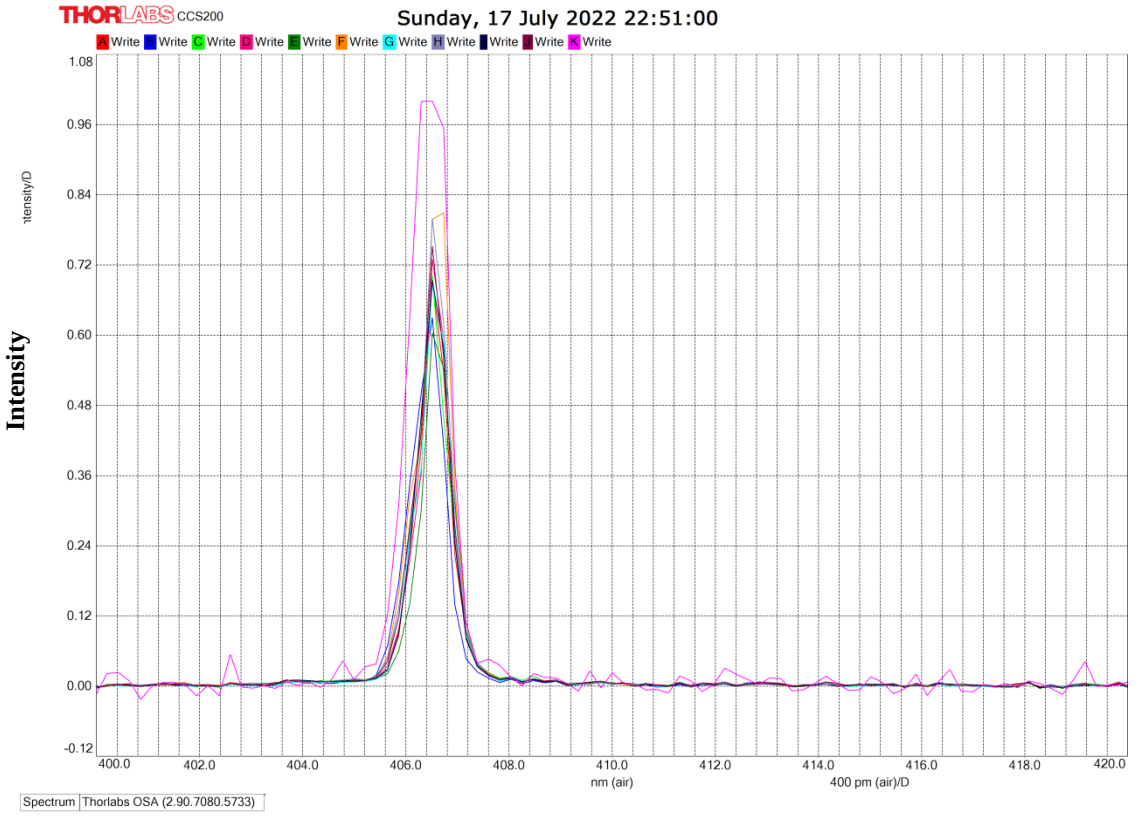

Fig. 4: show intensity peak in pathogenic E.coli

Table 3: (E.coli normal flora): Show results of sample, intensity, Peak wavelength nm (air), FWHM(pm)air.

Sample no	Intensity	Peak wavelength nm (air)	FWHM (pm)air
source	1.000000	406.0802002	820.1606058
1	0.8381	406.5158	882.4570
2	0.8229	406.5158	791.3645
3	0.9287	406.5158	634.3673
4	0.9134	406.5158	684.9534
5	0.8962	406.7337	706.9323
6	0.9063	406.5158	739.3869
7	0.8386	406.5158	767.2167
8	0.9340	406.5158	717.5493
9	0.8728	406.5158	812.7075
10	0.9125	406.2980	886.8341

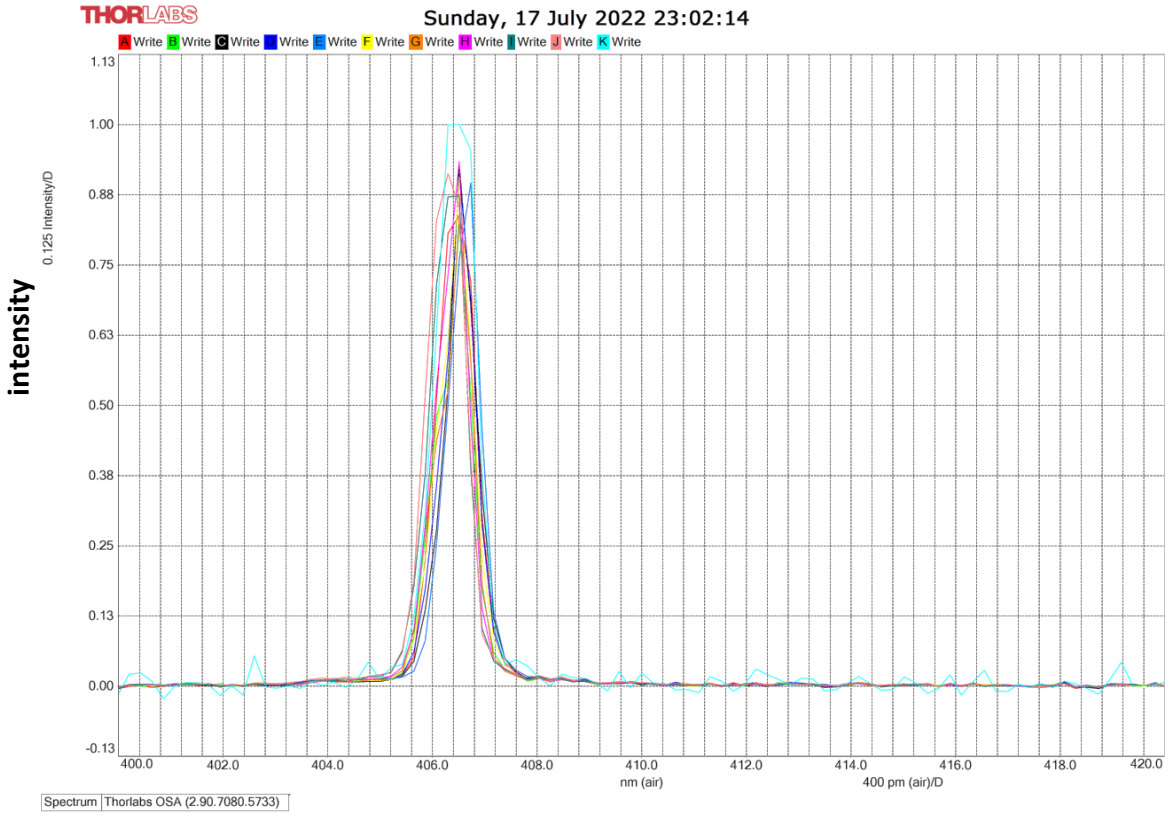

Fig. 5: show) in Normal flora *E.coli*

sample 1.... Sample 2.... Sample 3.... Sample4.... Sample 5.... Sample 6....sample7

sample 8....sample 9.... Sample 10.... Sample(source)

Conclusion:

The use of optical biosensor multimode – no core - multimode fibre with a 410nm laser source to sensing and differentiate between normal and pathogenic bacteria of E.coli . Through the study, we found that laser intensity (410nm) in state the normal flora bacteria higher than intensity laser (410nm) in state the pathogenic bacteria because the pathogenic bacteria contain many virulence factors(higher absorption). The biosensor is more accurate, quick to diagnose and less expensive**.**

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الاستشعار والتفريق بين البكتريا الطبيعية والممرضة للاشريكيه القولونية باستخدام ليزر ديود 410 نانومتر

ثائر فرحان عكاب الخزاعي ليلى محمد حسن العامري

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الخلاصة

توفر المستشعرات الحيوية الضوئية خصائص وطرقًا ممتازة للكشف عن البكتيريا عند مقارنتها بتقنيات التحليل التقليدية. يسمح بالكشف المباشر عن العديد من المواد البيولوجية والكيميائية. توجد البكتيريا في جسم الإنسان بشكل طبيعي غير مسببة للامراض و انواع اخرى مسببة للامراض ، كما توجد في الكائنات الحية الأخرى. واحدة من هذه البكتيريا هي Escherichia coli (E. coli) والتي توجد في جسم الإنسان بشكلها الطبيعي والممرض. تسبب بكتيريا الإشريكية القولونية العديد من الأمراض ، بما في ذلك أمراض المعدة والأمعاء والتهابات الجهاز البولي وغيرها. الهدف من هذه الدراسة هو الاستشعار والتفريق بين بكتيريا الإشريكية القولونية الطبيعية والممرضة. المواد والطرق: المستشعر الحيوي البصري المصنوع من ألياف بصرية متعددة الأوضاع - بدون نواة - متعددة الأوضاع تميز بين البكتيريا المسببة للأمراض وغير المسببة للأمراض للإشريكية القولونية عن طريق قياس التغير في الشدة لطول موجة الليزر باستخدام مصدر ضوء ليزر 410 نانومتر. ألياف بصرية متعددة الأوضاع - لا نواة – متعددة الاوضاع (MM-NOC-MM)متصلة بمحلل OSA (HR2000) عن طريق محول وأخيراً متصلة بجهاز كمبيوتر لإظهار النتيجه. النتائج: شدة الضوء المرسل المسجل في حالة البكتيريا المسببة للأمراض أقل من شدة الضوء المرسل المسجل في حالة البكتيريا غير الممرضة .الخلاصة: تم الحصول على هذه النتائج بسبب الاختيار الأمثل والأفضل لطول موجة الليزر المستخدم مع بكتيريا الإشريكية القولونية.