Sudan University of Science and Technology College of Graduate Studies

Evaluation of Using Fourier Infrared

Spectroscopy and Laser – Raman in Bladder

Malignancy Diagnosis

تقويم استخدام تحويلات فوريير للأطياف في المنطقة

تحت الحمراء و مطيافية ليزر – رامان في تشخيص

سرطان المثانة

A thesis submitted in fulfillment of the requirements for the philosophy degree in laser applications in physics

By: Essam Elgeily Ahmed Ali

Supervised By : Prof. Dr. Nafie A. AL Muslet

Abstract

Bladder cancer is one of the most common cancers in Africa. It takes several days to reach a diagnosis using histological examinations of specimens obtained by endoscope, which increases the medical expense.

Spectroscopic analysis of bladder cancer tissues has received considerable attention due to its sensitivity to biochemical variations in the samples.

The present study investigated the use of FTIR and laser Raman spectrometer as a diagnose tools of bladder cancer.

Fourty two bladder samples were collected from 21 patients during surgery from different hospitals without any pretreatment.

FTIR, with a ceramic source, was used to differentiate between normal and cancerous bladder tissues via the change in the spectra of these samples.

The investigations detected obvious spectroscopic change in the proteins (1650, 1550 cm⁻¹), lipids (2925, 2850 cm⁻¹) and nucleic acid (1080, 1236 cm⁻¹).

With FT Raman spectrometer supplied by Nd:YAG laser, as an excitation source, some of the samples were studied and a significant difference between the normal and cancerous bladder tissues were found around Raman shifts of 1650 cm⁻¹, 1440 cm⁻¹, 1270 cm⁻¹ and 1080 cm⁻¹.

Abstract

Bladder cancer is one of the most common cancers in Africa. It takes several days to reach a diagnosis using histological examinations of specimens obtained by endoscope, which increases the medical expense.

Spectroscopic analysis of bladder cancer tissues has received considerable attention due to its sensitivity to biochemical variations in the samples.

The present study investigated the use of FTIR and laser Raman spectrometer as a diagnose tools of bladder cancer.

Fourty two bladder samples were collected from 21 patients during surgery from different hospitals without any pretreatment.

FTIR, with a ceramic source, was used to differentiate between normal and cancerous bladder tissues via the change in the spectra of these samples.

The investigations detected obvious spectroscopic change in the proteins (1650, 1550 cm⁻¹), lipids (2925, 2850 cm⁻¹) and nucleic acid (1080, 1236 cm⁻¹).

With FT Raman spectrometer supplied by Nd:YAG laser, as an excitation source, some of the samples were studied and a significant difference between the normal and cancerous bladder tissues were found around Raman shifts of 1650 cm⁻¹, 1440 cm⁻¹, 1270 cm⁻¹ and 1080 cm⁻¹.

The comparison between the two techniques showed that Raman spectroscopy holds much promising as a rapid, accurate, nondestructive method, and easy to use as an alternative method for identification and diagnosis of bladder cancerous tissues.

المستخلص

سرطان المثانة يعتبر من أكثر أنواع السرطانات شيوعاً في القارة الأفريقيا، يأخذ التشخيص العادى للمرض عده ايام للوصول لفحص النماذج التي يحصل عليها الطبيب بواسطة المنظار ثم يرسلها الى معمل التحليلات المرضية مما يزيد النفقات المالية للتشخيص.

التحليل الطيفى لأنسجة سرطان المثانة بدأ يحظى باهتمام كبير في الاونة الاخيرة بسبب تحسسه للاختلافات الكيماوية الحاصلة في الخلايا الحيوية في الخلايا المرض.

نتحرّی الدراسه الحالیة امکانیة استعمال تحویلات فورییر للاشعة تحت الحمراء و تحویلات فورییر لرامان، کأدوات تشخیصیة لسرطان المثانة.

في هذه الدراسة تم فحص 42 عينة جمعت من 21 مريض أثناء جراحة المثانة من مستشفيات مختلفة قبل اجراء اى معالجة.

تحويل فوريير للاشعه تحت الحمراء الذي له مصدر خزفي، أستعمل ليفرق بين أنسجة المثانة الطبيعية والسرطانية حيث تم تحديد فروقات طيفية بين النوعين من الانسجة في: البروتين (1650، 1550 سم $^{-1}$) ، والدهون (2850 , 2850 سم $^{-1}$) و الحامض النووي (1080، 1236 سم $^{-1}$).

كذلك تم استخدام مطياف رامان الذي يستخدم ليزر Nd:YAG كمـصدر الثارة لدراسة عدد من هذه العينات حيث سجلت اختلافات طيفية هامة بين أنسجة المثانة الطبيعية والسرطانية وتم تحديد ازاحات رامان حول المناطق 1650 سم $^{-1}$ ، 1440 سم $^{-1}$ ، 1270 سم $^{-1}$ و1080 سم $^{-1}$.

وبالمقارنة بين الطريقتين، نستخلص أن تحويلات فوريير لرامان يمكن اعتمادها كتقانة واعدة جدا في تشخيص سرطان المثانة وذلك لانها تقانة دقيقة وسريعة ، غير إتلافيه، وسهلة الاستعمال كبديل للطرق التقليدية في تشخيص التغيرات السرطانية لانسجة المثانة.

Contents

| Subject | Page |
|-----------------------------------------------------|-------|
| Holy Quran | i |
| Acknowledgment | ii |
| Dedication | iv |
| Abstract by English | V |
| Abstract by Arabic | vii |
| Contents | ix |
| List of Figures | xiii |
| List of Tables | xviii |
| Chapter One Introduction and Basic Concepts | 1 |
| 1-1 Introduction | 1 |
| 1-2 Quantum Nature of Light | 3 |
| 1-3 The Electromagnetic Spectrum | 4 |
| 1-4 Absorption and Emission Processes | 6 |
| 1-5 Fluorescence | 9 |
| 1-6 Phosphorescence | 12 |
| 1-7 Laser Fundamentals | 14 |
| 1-7-1 Types of Laser | 15 |
| 1-7-1-1 Gas Lasers | 16 |
| 1-7-1-2 Solid State Lasers | 17 |
| 1-7-1-3 Diode Lasers | 18 |
| 1-8 Laser Spectroscopy | 19 |
| 1-9 Fourier Transform Infrared Spectrometers (FTIR) | 21 |
| 1-9-1 Michelson Interferometer | 22 |
| 1-9-2 Fourier Transformation | 23 |

| 1-10 Optical Spectrometers | 24 |
|-----------------------------------------------------------------------------|----|
| 1-11 Infrared Spectroscopy | 26 |
| 1-12 Raman Spectroscopy | 27 |
| 1-13 Aim of the Work | 29 |
| Chapter Two Infrared and Raman Spectroscopy | 30 |
| 2-1 Introduction | 30 |
| 2-2 The Origins of the Infrared Spectrum | 30 |
| 2-3 Normal Modes of Molecule Vibration | 31 |
| 2-4 The IR Device Structure | 38 |
| 2-5 Raman Scattering in Spectroscopic Investigation | 41 |
| 2-5-1 Raman Sample illumination | 42 |
| 2-6 NIR Spectrometers | 44 |
| 2-6-1 FT NIR Spectrometers | 45 |
| 2-6-2 Scanning Grating Spectrometers | 46 |
| 2-6-3 Diode Array Spectrometers | 46 |
| 2-6-4 Filter Spectrometers | 46 |
| 2-6-5 LED Spectrometers | 47 |
| 2-6-6 AOTF Spectrometers | 47 |
| 2-7 Raman Spectrometers | 48 |
| 2-7-1 Raman Grating Spectrometer with Single Channel Detector | 49 |
| 2-7-2 FT Raman Spectrometers with NIR Excitation | 52 |
| 2-8 Vibrational Spectroscopy Sensitivity to Structures Biological Molecules | 53 |
| 2-8-1 Nucleic Acids | 54 |
| 2-8-2 Proteins | 55 |
| 2-8-3 Lipids | 57 |

| 2-8-4 Carbohydrates | 57 |
|--------------------------------------------------------------------------------|-----|
| 2-9 Raman Spectroscopy for Cancer Diagnosis | 58 |
| 2-10 Literature Survey | 62 |
| Chapter Three | 67 |
| Experimental Part | 07 |
| 3-1 Introduction | 67 |
| 3-2 FTIR Spectrometer | 67 |
| 3-2-1 FTIR- 430 Spectrometer usage | 70 |
| 3-2-2 Output recording | 70 |
| 3-2-3 Sample Holder | 71 |
| 3-3 FT Raman System | 71 |
| 3-4 Materials and Methods | 73 |
| 3-4-1 Tissue Preparation | 73 |
| 3-4-2 Sampling for FTIR | 76 |
| 3-4-3 Procedure of FTIR Sampling | 77 |
| 3-4-4 Sampling for FT Raman Spectroscopy | 77 |
| 3-4-5 Procedure of FT Raman Sampling | 78 |
| Chapter Four | 79 |
| Results and Discussion | |
| 4-1 Introduction | 79 |
| 4-2 FTIR Spectra for a Normal Bladder and Cancerous Tissues | 79 |
| 4-2-1 Intensity Ratio of Proteins (amide I/II) | 104 |
| 4-2-2 Intensity Ratio of Lipids (I ₂₉₂₅ /I ₂₈₅₀) | 105 |
| 4-2-3 Intensity Ratio of Nucleic Acids (I ₁₀₈₀ /I ₁₂₃₆) | 106 |
| 4-3 FT Raman Spectra for a Normal Bladder and Cancerous Tissues | 107 |

| 4-3-1 Raman Shifts for Normal and Cancerous Bladder Tissues | 117 |
|-----------------------------------------------------------------------------------|-----|
| 4-3-2 Comparison between Raman Intensity for Normal and Cancerous Bladder Tissues | 119 |
| 4-4 Comparison between the Results of FTIR and FT Raman Spectrometers | 120 |
| 4-5 Conclusions | 122 |
| 4-6 Future Work | 123 |
| References | 124 |