VOLATOLOMICS ANALYSIS OF LUNG AND COLON CANCER USING TERAHERTZ AND INFRARED SPECTROSCOPY

AHMAD ZULHILMI BIN ARSHAD

UNIVERSITI TEKNOLOGI MALAYSIA

VOLATOLOMICS ANALYSIS OF LUNG AND COLON CANCER USING TERAHERTZ AND INFRARED SPECTROSCOPY

AHMAD ZULHILMI BIN ARSHAD

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy (Biomedical Engineering)

Faculty of Bioscience and Medical Engineering
Universiti Teknologi Malaysia

.

SEPTEMBER 2016

A special dedication to my parents,
Arshad Mustapa & Rohana Hassan

To my beloved siblings,
Herman, Anis, Faiz, Hisyam & Tasya

To my beloved best friends,
Sarhan, Sya, Krik, Arep, Azani,
Razak, Fauzi, Syamil

Thank you for everything.

~ipsa scientia potestas est~
"KNOWLEDGE ITSELF IS POWER"

...with love and care

a.zulhilmi

ACKNOWLEDGEMENT

Bismillahirrahmanirrahim… In the name of Allah S.W.T., the most Gracious, Merciful and Knowledgeable. All this work is belonging to Him, the owner of all knowledge and creatures.

I would like to express my sincere gratitude to my supervisor Associate Professor Dr. Yusof Munajat for his mentoring and guidance. He is untiring enthusiasm, support, advice and guidance throughout my study. He has encouraged me to reach my full potential as a research scientist and to be an independent and critical thinker. He provided me with many academic opportunities on campus to widen my horizons. Without him, this study would not have been possible to finish.

I also would like to acknowledge all my co-supervisors, Dr. Raja Kamarulzaman Raja Ibrahim, Dr. Salehhuddin Hamdan and Dr. Nasrul Humaimi Mahmood, for their advice, time, critical feedback and input. I would like to thanks Dr. Noradzimah Johdi and Mr. Hanif Zulkhairi from UKM Medical Molecular Biology Institute (UMBI) for facilitating collaborative work with us. Not to forget the Photonics Research Centre (PRC), Universiti Malaya for providing Terahertz time domain spectroscopy instrument to analyse the samples.

I would also like to thanks everyone who helped with experiments, talking through ideas and being resourceful and to my colleagues in the Physics Departments, Faculty of Sciences, and Department of Biotechnology & Medical Engineering, Faculty of Biosciences and Medical Engineering, for making the campus enjoyable

and intellectuality stimulating. Do not forget the member of the Applied Optics Research lab, Kak Sara, Hamdi, Ayu, Farha, Wani and Elham.

Special thanks to Razak, Fauzi, Azani, Rizal, Hafizi, Sham and Aidil for their valuable discussions and suggestions in finishing my study. I extend many thanks to my best friend group since secondary school for their support, care and motivation through my difficulties and sorrows over a decade. I love them all.

Finally, yet importantly, I would like to say a heartfelt thank you to my family. Thanks to my parents for their endless support, love and faith in me over the years. Also, for Malaysia Ministry of Higher Education (MOHE) for providing MyBrain15 (MyPhD) scholarship throughout my three years and half of studies. This study is funded by two Fundamental Research Grant Scheme (FRGS - grant no: 4F178 and 4F648) and one UTM Research University Grant (RUG Tier 1: 06H63). Thank you.

ABSTRACT

Terahertz and infrared spectroscopy are effective analytical spectroscopic techniques to identify and study the conformation and molecular interaction of the biomolecules. It has a huge potential in cancer diagnosis because these spectroscopies are non-invasive technique and do not require labelling for tissues and cells. Volatolomics analysis is a technique to analyse the volatile organic compounds (VOCs) emitted and released by human metabolites, which are not limited to breathe analysis. VOCs that are released by cancerous cells can be one of the bio-diagnostics techniques to diagnose cancer. Although studies on breath analysis have been widely carried out, the study of the volatolomics analysis by using Fourier transform infrared spectroscopy (FTIR) and Terahertz time-domain spectroscopy (THz-TDS) is still new. Both FTIR and THz-TDS instruments are installed with a gas cell sampling tools by absorption technique to analyse and detect the key species released from the VOCs. Lung cancer (NCL-H1299) and colon cancer (COLO320DM) cell lines are uas samples to identify the key species of each of the cancerous cells. The experiment has been verified and validated by comparing with control samples such as normal lung (MRC-5) cell lines, normal colon (CCD112CoN) cell lines, empty flask, air from the culture media and normal lab air. All the samples have been cultured into different sealed flasks for 24 to 120 hours, before the VOCs are collected and transferred into the gas cells to analyse using FTIR and THz-TDS. Hydrogen chloride and benzamide have been identified as key species for lung and colon cancer, respectively. These findings have been verified and validated by using residual gas analyser (RGA), gas chromatography – mass selective detector (GC-MSD), and confirmed by earlier literatures. A chemometric statistical analysis also has been applied to this study to extract the important information of the biochemical data from the VOCs with the greatest discriminative power and highest precision. These findings demonstrate the potential use of FTIR and THz-TDS as clinical tools through the volatolomics analysis. In addition, more work is needed if it is to be applied in clinical practice.

ABSTRAK

Spektroskopi terahertz dan inframerah merupakan teknik spektroskopik analitikal yang efektif dalam mengenalpasti dan mengkaji struktur interaksi molekul bagi sesuatu biomolekul. Ia merupakan potensi yang besar dalam proses diagnosis kanser kerana teknik spektroskopik ini adalah tidak invasif dan tidak memerlukan pelabelan untuk tisu dan sel. Analisis volatolomik pula merupakan satu teknik untuk menganalisis sebatian organik yang mudah meruap (VOCs) yang terhasil daripada proses metabolisme manusia, yang mana tidak terhad kepada analisis pernafasan sahaja. VOCs yang dihasilkan oleh sel kanser boleh menjadi salah satu teknik bio-diagnostik sel kanser. Walaupun kajian mengenai analisis pernafasan telah banyak dijalankan, tetapi kajian analisis volatolomik dengan mengunakan spektroskopi infra merah transformasi Fourier (FTIR) dan spektroskopi Terahertz domain masa (THz-TDS) masih baru. Kedua-dua instrument FTIR dan THz-TDS telah dipasangkan pada satu alat persampelan sel gas melalui teknik penyerapan untuk menganalisa dan mengesan spesies petunjuk daripada VOCs yang dilepaskan. Titisan sel-sel bagi kanser paru-paru (NCL-H1299) dan kanser kolon (COLO320DM) digunakan di dalam kajian ini untuk mengesan spesies petunjuk bagi setiap kanser. Ujikaji yang dijalankan telah diverifikasi dan divalidasi dengan membandingkan sampel terkawal seperti titisan sel paru-paru normal (MRC-5), sel kolon normal (CCD112CoN), udara kelalang kosong, udara daripada medium kultur dan udara persekitaran makmal. Semua sampel titisan sel telah dikultur melalui kelalang-kelalang yang kedap yang berbeza selama 24 jam hingga 120 jam, sebelum VOCs dikumpul dan dipindahkan ke sel-sel gas untuk dianalisis menggunakan FTIR dan THz-TDS. Hidrogen klorida dan benzamida telah dikenalpasti sebagai spesies petunjuk bagi kanser paru-paru dan kanser kolon. Penemuan ini telah diverifikasi dan divalidasi dengan menggunakan penganalisis gas sisa (RGA), kromatografi gas – pengesan jisim terpilih (GC-MSD) dan disahkan oleh literature terdahulu. Satu statistikal analisis kemometri juga diterapkan untuk kajian ini bagi mengekstrak maklumat penting data biokimia daripada VOCs dengan kuasa diskrimitif terbesar dan kepersisan tertinggi. Hasil kajian ini menunjukkan potensi penggunaan FTIR dan THz-TDS sebagai peralatan klinikal menerusi analisis volatolomik. Di samping itu, kajian lanjut masih diperlukan jika ia ingin diaplikasikan di dalam amalan klinikal.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATIONS

THz-TDS	-	Terahertz time-domain spectroscopy
FTIR	-	Fourier transform infrared
VOCs	-	Volatile organic compounds
GC-MS	-	Gas chromatography – mass spectrometer
GC-MSD	-	Gas chromatography – mass selective detector
PCA	-	Principal component analysis
PLS	-	Partial least square
RGA	-	Residual Gas Analyser

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Overview

This introductory chapter illustrates the core of this study, including the background which stimulates this research work, the motivation for the focus of the work which highlighted the importance of the volatolomics analysis used in disease and cancer diagnosis, the problem statement, aim and objectives, scope, and significance of the study. This chapter is structured to introduce the huge potential of cancer detection through volatolomics analysis using Fourier transform infrared spectroscopic and Terahertz time-domain spectroscopy technique.

1.2 Background of the Study

Cancer is a major cause of mortality in this world with more than half a million deaths by the year 2013 in the United States alone, and the number of cancer cases are increasing every year, especially in the low and mid-income countries [1]. The imbalanced socio-economics from these countries led to lack of awareness, expertise and equipment to diagnose the cancer accurately, in short duration and cost effective manners.

In general, cancer can be diagnosed by invasive and non-invasive techniques. The invasive techniques, such as biopsy and endoscopy, involve making an incision in the body to gain access to the target area. The non-invasive techniques, such as imaging and laboratory testing, do not involve surgical procedures. There also are alternative techniques to detect and identify the cancer, such as breath analysis [2], chemometric analysis [3], bio-fluids analysis [4]s and others.

Fourier transform infrared spectroscopy (FTIR) and Terahertz time-domain spectroscopy (THz-TDS) based technique in cancerous tissue diagnostic are dependent on terahertz and infrared spectral analysis between healthy and cancerous tissue samples. However, spectrum broadening is the most challenging to determine [5,6]. The broadening character of the terahertz and infrared spectrum in particular recorded from liquid or solid samples can mask and also interfere with other cancerous tissue constituents, including water spectrum thus affecting the measurement resolution. Detection of volatile organic compounds (VOCs) produced by cancerous cell can be a better option as the terahertz and infrared spectrum of the gas exhibit less broadening character.

This approach allows for a better terahertz and infrared spectrum of key products of the VOCs to be identified [7,8]. This key product then can be used as a fingerprint for that particular cancer type. Other diagnostic tools have not been able to yield the biochemical information to identify the key species of the cancer. Even so, the current techniques to detect the cancer have many disadvantages, such as high cost, many procedure, contamination or side effects. The proposed techniques using FTIR and THz-TDS complement the other techniques in assisting the physician to diagnose the cancer effectively.

In this study, the development of cancer fingerprints uses FTIR and THz-TDS is to identify the key species of volatile organic compounds (VOCs). This approach will utilize the gas released from cancerous cell, which contains VOCs, and infrared light absorbance to monitor specific absorbance patterns which produced the following changes in chemical compound species: for example, 6-aldehydes, isoprene, n-butyl

acetate, and n-propyl propionate released by hepatocellular carcinoma cell using GC-MS [9].

1.3 Motivation of the Work

This study involves three key aspects: (i) developing a system to capture and measure the volatile organic compounds released by cancerous cells, (ii) measuring and identifying the key species, and (iii) verifying and validating the key species of the cancer. The following sub-section highlight the significance of the study and the importance of the current work.

1.3.1 Why is volatolomics analysis used in cancer diagnosis?

Volatolomics analysis is the examination of the volatile organic compounds (VOCs) released by all metabolites from living things for the presence of certain compounds to determine the presence of cancers or diseases of the human body. Volatolomics analysis is not limited to breath analysis, but it is covered the VOCs released from breath, sweat, skin, urine, faeces and vaginal secretions. Volatolomics analysis has huge potential in detection and identification of diseases and cancer diagnosis [10,11], especially when it is involved in the end products of cellular processes of the human as well as a non-destructive technique.

There are many advantages by using Volatolomics analysis to diagnose the cancerous or diseases samples compared to other conventional methods; for example, this analysis technique is a non-invasive method which may reduce the risks and be less harmful to the patient and personnel. Furthermore, the results can also analyze and appear immediately if we have an established database of the diseases.

1.3.2 Why should a volatolomics analysis system be developed?

This volatolomics analysis technique will assist physicians and medical experts to diagnose the cancer or other diseases effectively. The non-destructive sample collection technique will help some patients who have problems with conventional sampling and diagnostics technique.

1.4 Problem Statement

The VOCs released by cancerous cells can be one of the bio-diagnostics techniques to diagnose the cancer. Previous works on VOCs detection released by cancerous cells have been performed by using a few analytical instruments, such as gas chromatography – mass spectrometer (GS-MS), electronic nose, proton transfer reaction mass spectrometry (PTR-MS), selected ion flow tube – mass spectrometry (SIFT-MS) and ion mobility spectrometry (IMS). However, each of these analytical instruments had their limitations, such as the need to change the filters, cannot measure in real time and simultaneously, need sample preparations, not effective and time consuming. Fourier transform infrared spectroscopy (FTIR) and Terahertz time-domain spectroscopy (THz-TDS) with gas absorption sampling techniques can overcome this limitation. The key species from VOCs released by cancerous cells can be identified and obtained from the literatures and National Institute of Science and Technology (NIST) [12] databases. The key species will be verified with other gas recognition technique and validated with the literature. Then, the samples are analysed by using chemometric statistical analysis technique for the highest discrimination and precision of results.

1.5 Objectives

The aim of this study was to carry out a detailed study of the application of Fourier transform infrared spectroscopy (FTIR) and Terahertz time-domain spectroscopy (THz-TDS) as potential diagnostics tools for clinical use for detection of cancer through volatolomics analysis. The study shows the application of FTIR and THz-TDS as a method to characterise biochemical differences that detect and distinguish the volatile organic compounds released by cancerous cells. This is based on the FTIR and THz-TDS analytical instruments measurements and statistical analysis of lung and colon cancer cell lines as well as normal cell lines and control experiment.

The study aims to address the following objectives:

a) To develop a technique to capture and measure the volatile organic compounds (VOCs) from lung and colon cancer cell lines
b) To measure and identify the key species of the samples using Fourier transform infrared spectroscopy (FTIR) and Terahertz time-domain spectroscopy (THz-TDS)
c) To analyse the samples using chemometric analysis and validate the key species

1.6 Scope of Study

This study is focused on identification of two types of cancer fingerprint, such as lung and colon cancer, through release and uptake of volatile organic compounds by cell lines by using Fourier transform infrared spectroscopy (FTIR) and Terahertz time-domain spectroscopy (THz-TDS).

This study consists of four parts of science and mathematics areas. Firstly, the concept of physics radiation of infrared and terahertz for detection of the samples. Second is biomedical samples, such as cancerous cell lines, to be detected and identified by the analytical instruments. Thirdly, the chemical compounds need to be identified as key species of the particular cancer from the volatile organic compounds (VOCs). Fourth is the mathematical statistical analysis of the sample for the highest discrimination and accuracy of the samples by using chemometric analysis.

1.7 Significance and Original Contributions of This Study

Detection and identification of cancer fingerprints are very important and have a high impact to the community. In this world of health and medical practice, fast techniques, cost-effective and accurate detection and identification of diseases and cancer is very crucial to assist the medical practitioner. The cancer also affects human health and causes the most human mortality every year, worldwide. In addition, research on identification of cancer fingerprint through release and uptake of volatile organic compounds using analytical spectroscopy instruments such as FTIR and THz-TDS is still new and not established yet.

The spectroscopic analytical instruments, such as FTIR and THz-TDS, with a combination of gas cell sampling tools and the analytical capabilities of this combination were demonstrated by simultaneous in vitro gas monitoring and detection of cancerous cells. Furthermore, assistance of chemometric analysis technique will provide the highest discriminative power and highest precision of the result.

The THz-TDS with gas analysis technique is a preliminary study to identify the cancer key species through volatile organic compounds (VOCs). The terahertz spectroscopy technique can capture the signal directly, simultaneously and effective

from the samples. All of the key species will be saved into a database and can be used as cancer identifier for patient using mobile THz-TDS – gas analyzer in the near future.

1.8 Thesis Structure and Organization

Chapter 2 of this thesis reviews the literature highlighting applications of FTIR and THz-TDS in biomedicine, cancer identification and volatolomics analysis. This includes detailed discussion on spectroscopic studies in various diseases in human tissues, cells and bio-fluids. This is followed by an outline of the methodology and materials used for analysis and identification of cancerous cells in Chapter 3. This chapter also covers system optimization and data analysis. The results are presented in Chapter 4 with discussion as the data was analysed. This chapter is divided into three sections, presenting and discussing results from a) cell lines analysis, b) detection of key species, and c) verification and validation. The conclusions drawn from the work will follow in Chapter 5 with suggestions for future work.

1.9 Summary

This chapter summarizes the foundation of this study to make sure this study will be achieved within the prescribed scope. Two types of cancerous cells will be investigated in this study, namely colon and lung cancer. Fourier transform infrared spectroscopy (FTIR) and Terahertz time-domain spectroscopy (THz-TDS) is used as analytical instruments. Chemometric analysis is also used to refine and to determine the highest discrimination and accuracy of the results.

REFERENCES

1. J, F., I, S., M, E., R, D., et al., GLOBOCAN 2012 v1.0: Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11, Lyon, France, 2013.
2. Dent, A.G., Sutedja, T.G., Zimmerman, P. V., Exhaled breath analysis for lung cancer. *J. Thorac. Dis.* 2013, 5.
3. Madsen, R., Lundstedt, T., Trygg, J., Chemometrics in metabolomics-A review in human disease diagnosis. *Anal. Chim. Acta* 2010, 659, 23–33.
4. Ollesch, J., Drees, S.L., Heise, H.M., Behrens, T., et al., FTIR spectroscopy of biofluids revisited: an automated approach to spectral biomarker identification. *Analyst* 2013, 138, 4092–102.
5. Hayat, A., Ginzburg, P., Orenstein, M., Measurement and model of the infrared two-photon emission spectrum of GaAs. *Phys. Rev. Lett.* 2009, 103.
6. Balan, E., Delattre, S., Roche, D., Segalen, L., et al., Line-broadening effects in the powder infrared spectrum of apatite. *Phys. Chem. Miner.* 2011, 38, 111–122.
7. Li, Y., Wang, J.D., Spatially resolved investigation of remote sensing FTIR for the quantitative determination of air toxic VOCs. *Spectrosc. Spectr. Anal.* 2003, 23, 885–887.
8. Dai, J., Xie, X., Zhang, X.C., Detection of broadband terahertz waves with a laser-induced plasma in gases. *Phys. Rev. Lett.* 2006, 97.
9. Mochalski, P., Sponring, A., King, J., Unterkofler, K., et al., Release and uptake of volatile organic compounds by human hepatocellular carcinoma cells (HepG2) in vitro. *Cancer Cell Int.* 2013, 13, 72.
10. Amann, A., Miekisch, W., Schubert, J., Buszewski, B., et al., Analysis of exhaled breath for disease detection. *Annu. Rev. Anal. Chem. (Palo Alto. Calif).* 2014, 7, 455–82.
11. Braun, P.X., Gmachl, C.F., Member, S., Dweik, R.A., Bridging the

Collaborative Gap : Realizing the Clinical Potential of Breath Analysis for Disease Diagnosis and Monitoring – Tutorial 2012, 12, 3258–3270.

12. Commerce, U.S.S. of, The NIST WebBook 2016.
13. Broza, Y.Y., Zuri, L., Haick, H., Combined Volatolomics for Monitoring of Human Body Chemistry. *Sci. Rep.* 2014, 2–7.
14. Wolkoff, P., Nielsen, G.D., Organic compounds in indoor air—their relevance for perceived indoor air quality? *Atmos. Environ.* 2001, 35, 4407–4417.
15. Król, S., Zabiegała, B., Namieśnik, J., Monitoring VOCs in atmospheric air I. On-line gas analyzers. *TrAC Trends Anal. Chem.* 2010, 29, 1092–1100.
16. NCI, N.C.I., Understanding Cancer Series: Cancer. *Natl. Institue Heal.* 2005, 6.
17. Siegel, R., Naishadham, D., Jemal, A., Cancer Statistics , 2013 2013, 63, 11–30.
18. Ministry of Health Malaysia, National Cancer Registry Report, Ministry of Health Malaysia, Malaysia, 2011.
19. Hesketh, R., Introduction to Cancer Biology, Cambridge University Press, New York, 2013.
20. Dunning, M.B., Fischbach, F., Common Laboratoty & Diagnostic Tests, Lippincott Williams & Wilkins, Philadelphia, 2011.
21. Schnell, Z.B., Van Leeuwen, A.M., Kranpitz, T.R., Davis's Comprehensive Handbook of Laboratory and Diagnostic Tests with Nursing Implications, F.A. Davis Company, Canada, 2003.
22. Wilson, D.D., Manual of Laboratory & Diagnostic Tests, McGraw-Hill, United States of America, 2008.
23. Ward, E., Ph, D., Ries, L.A.G., Wingo, P.A., et al., Annual Report to the Nation on the Status of Cancer , 1975 – 2001 , with a Special Feature Regarding 2004, 4251, 675–690.
24. Sridhar, S., Karnani, N., Connell, D.W., Millington, K.A., et al., Increased Risk of Mycobacterium tuberculosis Infection in Household Child Contacts Exposed to Passive Tobacco Smoke. *Pediatr. Infect. Dis. J.* 2014, 33, 1303–1306.
25. Weitberg, A.B., Cancer of the Lung: From Molecular Biology to Treatment Guidelines, 2002.
26. Risby, T.H., Solga, S.F., Current status of clinical breath analysis. *Appl. Phys. B* 2006, 85, 421–426.

27. Tittel, F.K., Current status of midinfrared quantum and interband cascade lasers for clinical breath analysis. *Opt. Eng.* 2010, 49, 111123.
28. Amann, A., Smith, D., Breath Analysis for Clinical Diagnosis and Therapeutic Monitoring, World Scientific Publishing, Singapore, 2005.
29. Lourenço, C., Turner, C., Breath Analysis in Disease Diagnosis: Methodological Considerations and Applications. *Metabolites* 2014, 4, 465–498.
30. Giovannucci, E., Physical Activity, Obesity, and Risk for Colon Cancer and Adenoma in Men. *Ann. Intern. Med.* 1995, 122, 327.
31. Willett, W., The search for the causes of breast and colon cancer. *Nature* 1989, 338, 389–394.
32. Giovannucci, E., Insulin and colon cancer. *Cancer Causes Control* 1995, 6, 164–179.
33. Gerson, C., Healing Colon, Liver and Pancreas Cancer, The Gerson Institute, California, 2002.
34. Zimmermann, D., Hartmann, M., Moyer, M.P., Nolte, J., Baumbach, J.I., Determination of volatile products of human colon cell line metabolism by GC/MS analysis. *Metabolomics* 2007, 3, 13–17.
35. Frame, G.M., A collaborative study of 209 PCB congeners and 6 Aroclors on 20 different HRGC columns. *Fresenius. J. Anal. Chem.* 1997, 357, 714–722.
36. Kim, K.-H., Jahan, S.A., Kabir, E., A review of breath analysis for diagnosis of human health. *TrAC Trends Anal. Chem.* 2012, 33, 1–8.
37. Cao, W., Duan, Y., Breath analysis: potential for clinical diagnosis and exposure assessment. *Clin. Chem.* 2006, 52, 800–11.
38. Phillips, M., Cataneo, R.N., Ditkoff, B.A., Fisher, P., et al., Volatile markers of breast cancer in the breath. *Breast J.* 2003, 9, 184–91.
39. Olopade, C.O., Zakkar, M., Swedler, W.I., Rubinstein, I., Exhaled pentane levels in acute asthma. *Chest* 1997, 111, 862–5.
40. Paredi, P., Kharitonov, S. a, Barnes, P.J., Elevation of exhaled ethane concentration in asthma. *Am. J. Respir. Crit. Care Med.* 2000, 162, 1450–4.
41. Montuschi, P., Corradi, M., Ciabattoni, G., Nightingale, J., et al., Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensate of asthma patients. *Am. J. Respir. Crit. Care Med.* 1999, 160, 216–20.
42. Corradi, M., Majori, M., Cacciani, G.C., Consigli, G.F., et al., Increased

exhaled nitric oxide in patients with stable chronic obstructive pulmonary disease. *Thorax* 1999, 54, 572–5.

43. Phillips, M., Cataneo, R.N., Cummin, A.R.C., Gagliardi, A.J., et al., Detection of Lung Cancer With Volatile Markers in the Breath. *Chest J.* 2003, 123, 2115–2123.
44. Pyo, J.S., Ju, H.K., Park, J.H., Kwon, S.W., Determination of volatile biomarkers for apoptosis and necrosis by solid-phase microextraction-gas chromatography/mass spectrometry: a pharmacometabolomic approach to cisplatin's cytotoxicity to human lung cancer cell lines. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* 2008, 876, 170–4.
45. Kaji, H., Hisamura, M., Saito, N., Murao, M., Evaluation of volatile sulfur compounds in the expired alveolar gas in patients with liver cirrhosis. *Clin. Chim. Acta* 1978, 85, 279–284.
46. Novak, B.J., Blake, D.R., Meinardi, S., Rowland, F.S., et al., Exhaled methyl nitrate as a noninvasive marker of hyperglycemia in type 1 diabetes. *PNAS* 2007, 104, 15613–15618.
47. Phillips, M., Sabas, M., Greenberg, J., Increase pentane and carbon disulfide in breath of patients with schizophrenia. *J. Clin. Pathol.* 1993, 46, 861–864.
48. Phillips, M., Boehmer, J.P., Cataneo, R.N., Cheema, T., et al., Prediction of heart transplant rejection with a breath test for markers of oxidative stress. *Am. J. Cardiol.* 2004, 94, 1593–4.
49. Studer, S.M., Orens, J.B., Rosas, I., Krishnan, J. a, et al., Patterns and significance of exhaled-breath biomarkers in lung transplant recipients with acute allograft rejection. *J. Heart Lung Transplant.* 2001, 20, 1158–66.
50. Phillips, M., Basa-Dalay, V., Bothamley, G., Cataneo, R.N., et al., Breath biomarkers of active pulmonary tuberculosis. *Tuberculosis (Edinb).* 2010, 90, 145–51.
51. Grushka, E., Grinberg, N., Advances in Chromatography, vol. 52, CRC Press, Florida, 2014.
52. Jennings, W., Shibamoto, T., Qualitative Analysis of Flavor and Fragrance Volatiles, Academic Press, New York, 1980.
53. Demtröder, W., Laser Spectroscopy: Basic Concepts and Instrumentation, vol. 53, Springer Science+Business Media New York, New York, 1989.
54. Chen, X., Shao, Z., Marinkovic, N.S., Miller, L.M., et al., Conformation

transition kinetics of regenerated Bombyx mori silk fibroin membrane monitored by time-resolved FTIR spectroscopy. *Biophys. Chem.* 2001, 89, 25–34.

55. Kno, H., Huber, S., IR spectroscopy of small and weakly interacting molecular probes for acidic and basic zeolites. *J. Chem. Soc.* 1998, 94.
56. Wolkers, W.F., Hoekstra, F. a., In situ FTIR assessment of desiccation–tolerant tissues. *Spectroscopy* 2003, 17, 297–313.
57. Ahmad, M.S., Mirza, B., Hussain, M., Hanif, M., et al., ATR-FTIR spectroscopy detects alterations induced by organotin(IV) carboxylates in MCF-7 cells at sub-cytotoxic/-genotoxic concentrations. *PMC Biophys.* 2008, 1, 3.
58. Mourant, J.R., Yamada, Y.R., Carpenter, S., Dominique, L.R., Freyer, J.P., FTIR spectroscopy demonstrates biochemical differences in mammalian cell cultures at different growth stages. *Biophys. J.* 2003, 85, 1938–47.
59. Llabjani, V., Jones, K.C., Thomas, G.O., Walker, L. a, et al., Polybrominated diphenyl ether-associated alterations in cell biochemistry as determined by attenuated total reflection Fourier-transform infrared spectroscopy: a comparison with DNA-reactive and/or endocrine-disrupting agents. *Environ. Sci. Technol.* 2009, 43, 3356–64.
60. Zendehdel, R., Masoudi-Nejad, A., Mohammadzadeh, J., H Shirazi, F., Cisplatin Resistant Patterns in Ovarian Cell Line Using FTIR and Principle Component Analysis. *Iran. J. Pharm. Res. IJPR* 2012, 11, 235–40.
61. Zendehdel, R., Masoudi-Nejad, A., H Shirazi, F., Patterns Prediction of Chemotherapy Sensitivity in Cancer Cell lines Using FTIR Spectrum, Neural Network and Principal Components Analysis. *Iran. J. Pharm. Res. IJPR* 2012, 11, 401–10.
62. Vitorino, P.G., Alves, J.D., Magalhães, P.C., Magalhães, M.M., et al., Flooding tolerance and cell wall alterations. *Pesq. agropec. bras.* 2001, 1027–1035.
63. Romeo, M.J., Quinn, M. a, Burden, F.R., McNaughton, D., Influence of benign cellular changes in diagnosis of cervical cancer using IR microspectroscopy. *Biopolymers* 2002, 67, 362–6.
64. Salman, A., Erukhimovitch, V., Talyshinsky, M., Huleihil, M., Huleihel, M., FTIR spectroscopic method for detection of cells infected with herpes viruses. *Biopolymers* 2002, 67, 406–12.

65. Mostaço-Guidolin, L.B., Bachmann, L., Application of FTIR Spectroscopy for Identification of Blood and Leukemia Biomarkers: A Review over the Past 15 Years. *Appl. Spectrosc. Rev.* 2011, 46, 388–404.

66. Mordehai, J., Ramesh, J., Huleihel, M., Cohen, Z., et al., Studies on Acute Human Infections Using FTIR Microspectroscopy and. *Infect. Stud. Using Nov. Opt. Technol.* 2004, 73, 494–502.

67. Shen, Y.C., Davies, a G., Linfield, E.H., Taday, P.F., et al., Determination of Glucose Concentration in Whole Blood using Fourier-Transform Infrared Spectroscopy. *J. Biol. Phys.* 2003, 29, 129–33.

68. Krafft, C., Codrich, D., Pelizzo, G., Sergo, V., Raman and FTIR microscopic imaging of colon tissue: a comparative study. *J. Biophotonics* 2008, 1, 154–69.

69. Journal, I., Pharmacology, C., Anti-inflammatory and anti-cancer activities of essential oils and their biological constituents *. *Int. J. Clin. Pharmacol. Ther.* 2011, 49, 93–95.

70. Ramesh, J., Salman, A., Mordechai, S., Argov, S., et al., FTIR Microscopic Studies on Normal , Polyp , and Malignant Human Colonic Tissues. *Subsurf. Sens. Technol. Appl.* 2001, 2, 99–117.

71. Peng, G., Hakim, M., Broza, Y.Y., Billan, S., et al., Detection of lung, breast, colorectal, and prostate cancers from exhaled breath using a single array of nanosensors. *Br. J. Cancer* 2010, 103, 542–51.

72. Gao, T., Feng, J., Ci, Y., Human breast carcinomal tissues display distinctive FTIR spectra: implication for the histological characterization of carcinomas. *Anal. Cell. Pathol.* 1999, 18, 87–93.

73. Sokolov, V. V, Frank, A., Minimally invasive and ex vivo diagnostics of breast cancer tissues by fiber optic evanescent wave Fourier transform IR (FEW-FTIR) spectroscopy. *SPIE* n.d., 3250, 140–147.

74. Szarawara, E., Trybalska, B., Cichocin, M., Stoch, A., et al., FTIR monitoring of the growth of the carbonate containing apatite layers from simulated and natural body fluids. *J. Mol. Struct.* 1999, 512, 287–294.

75. Rettig, R., Virtanen, S., Composition of corrosion layers on a magnesium rare-earth alloy in simulated body fluids. *J. Biomed. Mater. Res. A* 2009, 88, 359–69.

76. Stoch, J., Szaraniec, J., Trybalska, B., Broz, A., FTIR absorption – reflection study of biomimetic growth of phosphates on titanium implants. *J. Mol. Struct.*

2000, 555, 375–382.

77. Brogden, K. a, Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nat. Rev. Microbiol.* 2005, 3, 238–50.

78. Gasper, R., Dewelle, J., Kiss, R., Mijatovic, T., Goormaghtigh, E., IR spectroscopy as a new tool for evidencing antitumor drug signatures. *Biochim. Biophys. Acta* 2009, 1788, 1263–70.

79. Hynes, A., Scott, D. a, Man, A., Singer, D.L., et al., Molecular mapping of periodontal tissues using infrared microspectroscopy. *BMC Med. Imaging* 2005, 5, 2.

80. Kirley, M.P., Booske, J.H., in:, *2015 IEEE Int. Vac. Electron. Conf.*, IEEE, Beijing, 2015, pp. 1–2.

81. Alhuwaidi, S., Zubair, K., Song, H., Shellman, Y., et al., in:, *2015 IEEE Biomed. Circuits Syst. Conf.*, IEEE, Georgia, USA, 2015, pp. 1–4.

82. Song, H.-J., Nagatsuma, T., Handbook of Terahertz Technologies: Devices and Applications, CRC Press, Florida, 2015.

83. Zhenwei, Z., Kejia, W., Yong, L., Zhuoyong, Z., et al., Non-destructive detection of pigments in oil painting by using terahertz tomography. *Sci. China* 2015, 58, 1–2.

84. Stedwell, C.N., Polfer, N.C., in:, Polfer NC, Dugourd P (Eds.), *Laser Photodissociation Spectrosc. Mass-separated Biomol. Ions*, Springer Science+Business Media New York, Switzerland, 2013, pp. 1–21.

85. Choudhury, B., Menon, A., Jha, R.M., Active Terahertz Metamaterial for Biomedical Applications, Springer Science+Business Media New York, New York, 2016.

86. Liu, W., Lu, C., Jiang, Z., Sun, P., in:, *Proc. SPIE*, vol. 9655, SPIE, Jeju, Korea, 2015, p. 96551G.

87. Jen, C.Y., Richter, C., Sample thickness measurement with THz-TDS: Resolution and implications. *J. Infrared, Millimeter, Terahertz Waves* 2014, 35, 840–859.

88. Lin, H., Withayachumnankul, W., Fischer, B.M., Mickan, S.P., Abbott, D., Gas recognition with terahertz time-domain spectroscopy and spectral catalog: a preliminary study. *Proc. SPIE* 2007, 6840, 68400X–68400X–9.

89. Zhang, X.C., Xu, J., Introduction to THz wave photonics, 2010.

90. Wilmink, G.J., Grundt, J.E., in:, *J. Infrared, Millimeter, Terahertz Waves*, vol.

32, 2011, pp. 1074–1122.

91. Mittleman, D.M., Jacobsen, R.H., Neelamani, R., Baraniuk, R.G., Nuss, M.C., Gas sensing using terahertz time-domain spectroscopy. *Appl. Phys. B-Lasers Opt.* 1998, 67, 379–390.
92. Kawase, K., Iwasaki, A., Shibuya, T., in:, *CLEO 2011 Laser Sci. to Photonic Appl.*, 2011, pp. 1–3.
93. Lewis, R.A., in:, *Proc. IEEE*, vol. 95, 2007, pp. 1641–1645.
94. Davies, A.G., Burnett, A.D., Fan, W., Linfield, E.H., Cunningham, J.E., Terahertz spectroscopy of explosives and drugs. *Mater. Today* 2008, 11, 18–26.
95. National Institute of Information and Communications Technolog, THz Database 2016.
96. Yasuda, H., Hosako, I., Measurement of terahertz refractive index of metal with terahertz time-domain spectroscopy. *Jpn. J. Appl. Phys.* 2008, 47, 1632–1634.
97. Laboratory, T., THz database 1998.
98. Laman, N., Harsha, S.S., Grischkowsky, D., Melinger, J.S., High-Resolution Waveguide THz Spectroscopy of Biological Molecules. *Biophys. J.* 2008, 94, 1010–1020.
99. Http://webbook.nist.gov/chemistry/thz-ir/, Terahertz Spectral Database 2016.
100. University, P.S., Terahertz Signal Propagation Database 2015.
101. Braakman, R., Gas-Phase Terahertz Spectroscopy and the Study of Complex Interstellar Chemistry 2010, 2010.
102. Neese, C.F., Medvedev, I.R., Plummer, G.M., Frank, A.J., et al., Compact submillimeter/terahertz gas sensor with efficient gas collection, preconcentration, and ppt sensitivity. *IEEE Sens. J.* 2012, 12, 2565–2574.
103. Danylov, A., THz laboratory measurements of atmospheric absorption between 6% and 52% relative humidity. *Submillimeter-Wave Technol. Lab. Univ. Massachusetts Lowell* 2006, 1–7.
104. Andersen, J., Heimdal, J., Mahler, D.W., Nelander, B., Larsen, R.W., Communication: THz absorption spectrum of the CO2-H2O complex: observation and assignment of intermolecular van der Waals vibrations. *J. Chem. Phys.* 2014, 140, 091103.
105. Saito, S., Syouji, A., Sakai, K., Fukunaga, K., et al., Broadband terahertz time-domain spectroscopic system with photoconductive antennas. *2007 Jt. 32nd Int. Conf. Infrared Millim. Waves 15th Int. Conf. Terahertz Electron. Vols 1 2* 2007,

475–476.

106. Guo, T., Chemical Analysis of Exhaled Breath by Means of Terahertz Rotational Spectroscopy. Wright State University, 2014.
107. Gerecht, E., Douglass, K.O., Plusquellic, D.F., Chirped-pulse terahertz spectroscopy for broadband trace gas sensing. *Opt. Express* 2011, 19, 8973–84.
108. Jiang, Y., Zhou, F., Wen, X., Yang, L., et al., Terahertz absorption spectroscopy of benzamide, acrylamide, caprolactam, salicylamide and sulfanilamide in the solid state. *Downloads.Hindawi.Com* 2013, 2014, 1–24.
109. Honary, S., Ebrahimi, P., Ghasemitabar, M., Preparation of Gold Nanoparticles for Biomedical Applications Using Chemometric Technique 2013, 12, 295–298.
110. Winterhalder, M., Zumbusch, A., Beyond the borders - Biomedical applications of non-linear Raman microscopy. *Adv. Drug Deliv. Rev.* 2015, 89, 135–144.
111. Taylor, P., Combined, C.S., Analysis of Olive Fruit Essential Oil : Application of Gas Analysis of Olive Fruit Essential Oil : Application of Gas Chromatography-Mass Spectrometry Combined with Chemometrics 2013, 2912, 37–41.
112. Jalali-heravi, M., Arrastia, M., Gomez, F.A., How Can Chemometrics Improve Micro fl uidic Research? *Anal. Chem.* 2015, 87, 3544–3555.
113. Sciences, N., Chemometric Classification of Citrus Juices of Moroccan Cultivars by Infrared Spectroscopy 2015, 2015, 137–142.
114. Wei, S.K., Raja Ibrahim, R.K., Lani, M.N., Abd Razak, S.B., Munajat, Y., in:, *Proc. 4th Int. Sci. Postgrad. Conf. 2016*, UTM Press, Johor Bahru, 2016, pp. 314–323.
115. Savanoriu, A., Terahertz spectrometers. *Ekspla* 2015, 1–14.
116. Gander, W., Hřebíček, J., Solving Problems in Scientific Computing Using Maple and MATLAB, Springer, New York, 2004.
117. Xiao, G., Dong, D., Liao, T., Zheng, L., A Novel Measurement Method of the Emission Rules of Greenhouse Gases from Fertilized Soil Based on Fourier Transform Infrared Spectrometry with Long Optical Path. *Spectrosc. Lett.* 2015, 48, 572–577.
118. Arshad, A.Z., Munajat, Y., Kamarulzaman, R., Ibrahim, R., et al., Volatolomics Analysis using FTIR Spectroscopy for Breast Cancer Identification in vitro,

IEEE, Kuala Lumpur, Malaysia, 2014.

119. Sellakumar, A.R., Snyder, C.A., Solomon, J.J., Albert, R.E., Carcinogenicity of formaldehyde and hydrogen chloride in rats. *Toxicol. Appl. Pharmacol.* 1985, 81, 401–406.

120. Albert, R.E., Sellakumar, A.R., Laskin, S., Snyder, C.A., et al., Gaseous Formaldehyde and Hydrogen Chloride Induction of Nasal Cancer in the Rat 1,2. *J NCI* 1982, 68, 597–603.

121. Chen, C.P., Missett, B., Yom, S.S., Nasal Cavity and Paranasal Sinus Cancer. *Cancer* 2010.

122. Shu-Chen, C., Ruey-Hong, W., Li-Jie, S., Ming-Chih, C., Huei, L., Exposure to Mosquito Coil Smoke May be a Risk Factor for Lung Cancer in Taiwan. *J. Epidemiol.* 2008, 18, 19–25.

123. Fagan, P., Moolchan, E.T., Pokhrel, P., Herzog, T., et al., Biomarkers of tobacco smoke exposure in racial/ethnic groups at high risk for lung cancer. *Am. J. Public Health* 2015, 105, 1237–1245.

124. Sethi, M., Fanayan, S., Mass Spectrometry-Based N-Glycomics of Colorectal Cancer. *Int. J. Mol. Sci.* 2015, 16, 29278–29304.

125. Protection, D. of E., Bureau of Environmental Cleanup and Brownfields Division of Storage Tanks REGULATED SUBSTANCES LIST, vol. 2014, 2014.

126. Agency, U.S.E.P., Consolidated List of Chemicals Subject to the Emergency Planning and Community Right- To-Know Act (EPCRA), Comprehensive Environmental Response , Compensation and Liability Act (CERCLA) and Section 112 (r) of the Clean Air Act, vol. 112, 2015.

127. Qi, B.C., Aldrich, C., Biosorption of heavy metals from aqueous solutions with tobacco dust. *Bioresour. Technol.* 2008, 99, 5595–5601.

128. Wang, L.K., Chen, J.P., Hung, Y.-T., Shammas, N.K., Heavy Metals in the Environment: Advances in Industrial and Hazardous Wastes Treatment Series, Taylor & Francis Group, Florida, 2009.