

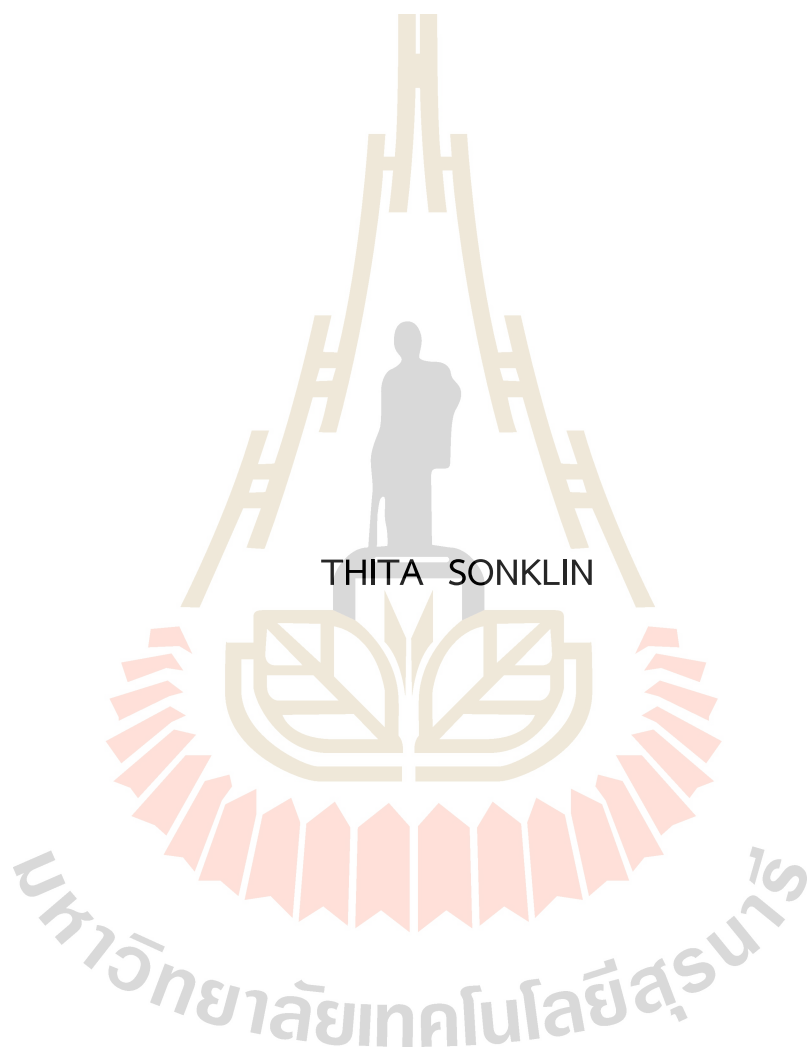
การศึกษาเพียโซอิเล็กทริกไบโอเซ็นเซอร์สำหรับเครื่องตรวจจับ
มะเร็งต่อมลูกหมาก



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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิศวกรรมศาสตรดุษฎีบัณฑิต
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THE INVESTIGATION OF PIEZOELECTRIC BIOSENSOR
FOR PROSTATE CANCER DETECTOR

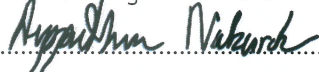


A Thesis Submitted in Partial Fulfillment of the Requirements for the
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Suranaree University of Technology has approved this thesis submitted in
partial fulfillment of the requirements for the Degree of Doctor of Philosophy

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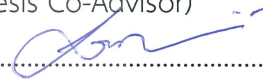
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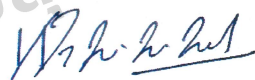
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ฐิตา ช่อนกลิ่น : การศึกษาเพียโซอิเล็กทริกไบโอเซ็นเซอร์สำหรับเครื่องตรวจจับมะเร็งต่อมลูกหมาก (THE INVESTIGATION OF PIEZOELECTRIC BIOSENSOR FOR PROSTATE CANCER DETECTOR)

อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร.สุดเขตต์ พจน์ประไพ, 188 หน้า.

คำสำคัญ: เพียโซอิเล็กทริกเซ็นเซอร์/ไบโอเซ็นเซอร์/สารบ่งชี้โรคมะเร็ง/ควอตซ์คริสตัลไมโครบาลานซ์ /สารบ่งชี้มะเร็งต่อมลูกหมาก/เซ็นเซอร์คลื่นเสียงพื้นผิว/การตรวจจับสารบ่งชี้

การตรวจจับการเพิ่มขึ้นของตัวบ่งชี้ทางชีวภาพโรคมะเร็ง (Cancer biomarker) ในเซรั่มในร่างกายมนุษย์ มีความสำคัญอย่างมากสำหรับการพยากรณ์ การวินิจฉัยโรคและการรักษาโรคมะเร็ง สารบ่งชี้มะเร็งต่อมลูกหมาก หรือ Prostate-specific antigen (PSA) เป็นสารที่นิยมแพร่หลายในทาง การแพทย์สำหรับการคัดกรองมะเร็งต่อมลูกหมาก รวมถึงในการตรวจสุขภาพประจำปีของเพศชาย การคัดกรอง PSA จึงเป็นบทบาทสำคัญในการตรวจพบมะเร็งต่อมลูกหมากในระยะเริ่มต้นและช่วยใน เพิ่มประสิทธิภาพของการรักษาในขั้นถัดไป อย่างไรก็ตาม วิธีการดั้งเดิม เช่น เคมีคลินิก (Clinical chemistry) และเครื่องมือตรวจเคมีภูมิคุ้มกัน (Immunochemistry instruments) ที่ใช้ปัจจุบันใน โรงพยาบาล เป็นเครื่องมือที่มีประสิทธิภาพมาก แต่ก็มีค่าใช้จ่ายสูงและต้องใช้บุคลากรที่มีความ เชี่ยวชาญพิเศษในการควบคุมเครื่องมือ จึงทำให้เกิดข้อจำกัดในการใช้งานในสถานพยาบาลที่มี ทรัพยากรจำกัด ดังนั้น การพัฒนาเครื่องมือทางเลือกที่สามารถคัดกรองในเบื้องต้นได้ ผู้ป่วยสามารถ เข้าถึงได้ง่าย รวมถึงมีประสิทธิภาพในการตรวจจับตัวบ่งชี้ทางชีวภาพ เช่น เครื่องมือไบโอเซ็นเซอร์ จึง กลายเป็นสิ่งจำเป็นที่จะช่วยให้ก้าวผ่านข้อจำกัดต่าง ๆ เหล่านี้ ไบโอเซ็นเซอร์เพียโซอิเล็กทริก (Piezoelectric biosensor) ชนิดควอตซ์คริสตัลไมโครบาลานซ์ (Quartz crystal microbalance, QCM) เป็นหนึ่งในไบโอเซ็นเซอร์ที่มีความโดดเด่น ไม่ว่าจะเป็นความสามารถในการตรวจจับตัวบ่งชี้ ทางชีวภาพโดยไม่ต้องใช้ฉลาก (Label-free biomarker detection) หรือจะเป็นความสามารถใน การตรวจจับอย่างรวดเร็ว เนื่องจากมีความไวต่อการตรวจจับที่สูงและประกอบควบรวมกับชุด อิเล็กทรอนิกส์ได้ไม่ยาก จึงทำให้ไบโอเซ็นเซอร์ QCM เป็นเครื่องมือตัวเลือกที่ถูกนำมาพัฒนาและ ประยุกต์ใช้สำหรับการตรวจคัดกรองโรคมะเร็ง นอกจากนี้การควบรวมนาโนเทคโนโลยี (Nanotechnology) กับไบโอเซ็นเซอร์ ก็ถือเป็นจุดมุ่งหมายสำคัญในการพัฒนาไบโอเซ็นเซอร์เหล่านี้ ให้มีขีดความสามารถก้าวไปอีกขั้น เนื่องจากวัสดุนาโนเทคโนโลยีช่วยเพิ่มสัญญาณในการตรวจจับ ทำ ให้ตรวจจับไบโอมาร์กเกอร์ต่าง ๆ โดยสามารถช่วยแยกแยะค่าสัญญาณที่วัดได้ที่มีความเข้มข้นน้อย ใน เวลาการตรวจวัดที่เหมาะสมและเพิ่มประสิทธิภาพในผลลัพธ์ของการรักษาทางการแพทย์ในขั้นต่อไป ของผู้ป่วย

การวิจัยนี้มุ่งเน้นการพัฒนาไบโอเซ็นเซอร์เพียโซอิเล็กทริกที่ไม่ใช้ฉลากสำหรับการตรวจจับตัวบ่งชี้ทางชีวภาพโรคมะเร็งต่อมลูกหมาก ชนิดสาร PSA โดยได้มีการสร้างโครงสร้างนาโนแบบต่าง ๆ ด้วยเทคนิค การชุบทางไฟฟ้า (Electrodeposition) โดยแบบใช้กระแสไฟฟ้าตรง (Direct-current (DC) electroplating) และ กระแสไฟฟ้าสลับ (Pulse current electrodeposition, PCD) เพื่อเพิ่มความไวในการตรวจจับสาร PSA ด้วยการพัฒนาการสร้างโครงสร้างนาโนบนพื้นผิวอิเล็กโทรดของ QCM โดยเน้นไปถึงการปรับปรุงพื้นผิวจากพื้นผิวดั้งเดิม แต่ไม่ทำให้การไหลดมวลของอนุภาคนาโนลงบนพื้นผิว QCM เซ็นเซอร์เพิ่มมากขึ้นเกินช่วงที่เครื่องมือไม่สามารถวัดได้ และช่วยเพิ่มประสิทธิภาพของไบโอเซ็นเซอร์ QCM โดยได้มีการศึกษาถึงความสามารถของไบโอเซ็นเซอร์ QCM ต่อการตรวจจับความเข้มข้นของ PSA ในระดับต่าง ๆ และการพัฒนาอนุภาคนาโนเคลือบบนด้วยลงบนพื้นผิว QCM เซ็นเซอร์ ได้รับการตรวจสอบด้วยเทคนิคต่าง ๆ อาทิ เช่น Wettability test X-ray diffraction (XRD) Fourier-transform infrared spectroscopy (FTIR) Field-emission scanning electron microscopy (FE-SEM) Atomic force microscopy (AFM) X-ray photoelectron spectroscopy (XPS) และ Cyclic voltammetry (CV) และรวมถึงศึกษาการใช้สารเคมีในการปรับปรุงพื้นผิวไบโอเซ็นเซอร์ QCM ให้เหมาะสมกับการตรวจจับตัวบ่งชี้ทางชีวภาพโรคมะเร็ง PSA อีกด้วย นอกจากนี้ ยังมีการศึกษาเพิ่มเติมเกี่ยวกับการพัฒนาไบโอเซ็นเซอร์เพียโซอิเล็กทริกอีกชนิดที่เป็นอุปกรณ์เพียโซอิเล็กทริกชนิดพื้นผิวเสียง (Surface acoustic wave device, SAW) จากวัสดุ Zinc oxide (ZnO) โดยการควบคุมโครงสร้างจุลภาคของ ZnO ให้เหมาะสมสำหรับการนำไปประกอบเป็นอุปกรณ์ SAW เพื่อใช้สำหรับการตรวจจับตัวบ่งชี้ทางชีวภาพโรคมะเร็งในอนาคต

ซึ่งผลการศึกษาในครั้งนี้แสดงให้เห็นว่าไบโอเซ็นเซอร์ QCM ที่พัฒนาขึ้น มีความสามารถในการตรวจจับสารโมเลกุลตัวบ่งชี้มะเร็งต่อมลูกหมากผ่านตรวจจับตัวบ่งชี้ทางชีวภาพโรคมะเร็งชนิด PSA โดยการเพิ่มพื้นที่ผิวสัมผัสของเซ็นเซอร์ผ่านการสร้างโครงสร้างจากอนุภาคทองคำ (Gold, Au) นอกจากนี้ การผลิตอุปกรณ์ ZnO-SAW ได้แสดงให้เห็นถึงการรวมกันของวิธีการทดลองและผลการจำลองในการผลิตอุปกรณ์ SAW ความสอดคล้องระหว่างผลลัพธ์จากการจำลองและการทดลอง โดยให้ข้อมูลที่เป็นประโยชน์เกี่ยวกับการสร้างอุปกรณ์และการนำมาซึ่งการศึกษาในขั้นต่อไปและนำไปสู่การสร้างเครื่องมือในการตรวจจับตัวบ่งชี้ทางชีวภาพของโรคต่าง ๆ ในอนาคตอีกด้วย

สาขาวิชา วิศวกรรมวัสดุ

ปีการศึกษา 2566

ลายมือชื่อนักศึกษา.....

ลายมือชื่ออาจารย์ที่ปรึกษา.....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

THITA SONKLIN : THE INVESTIGATION OF PIEZOELECTRIC BIOSENSOR FOR
PROSTATE CANCER DETECTOR.

THESIS ADVISOR : ASSOC. PROF. SOODKHET POJPRAPI, Ph.D., 188 PP.

Keywords: piezoelectric sensor/biosensor/cancer biomarker/quartz crystal
microbalance/prostate specific antigen/surface acoustic wave sensor/
biomarker detection

Detecting overexpressed unique cancer biomarkers in serum holds significant promise for cancer prognosis, diagnostics, and therapies. Prostate-specific antigen (PSA) serves as a prominent biomarker, widely employed in medical settings for prostate cancer screening and routine health check-ups in men. PSA screening plays a crucial role in early prostate cancer detection and assessment of treatment efficacy. However, conventional methods such as clinical chemistry and immunochemistry instruments, while effective, are costly and demand specialized expertise, thus limiting their utilization in resource-limited settings. Consequently, the development of accessible and efficient screening tools, such as biosensors, becomes imperative. Among these, the piezoelectric quartz crystal microbalance (QCM) biosensor stands out for label-free biomarker detection capabilities, particularly in tumor screening, owing to its rapid detection, high sensitivity, and compatibility with cost-effective electronics. Nanotechnology-based biosensors play a pivotal role in precisely detecting these biomarkers, enabling timely interventions and enhancing patient outcomes. Exploring a signal amplification strategy with nanotechnology would be invaluable for developing a highly effective biosensing platform.

This study aims to develop a label-free QCM biosensor for detecting PSA biomarker. Various nanostructures were fabricated using electrodeposition techniques, including direct-current (DC) electroplating and pulse current electrodeposition (PCD), to heighten sensitivity while minimizing nanoparticle mass loading. The performance of the developed QCM biosensor was evaluated across various PSA concentrations, with nanostructures characterized using techniques such as Wettability test, X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), Field-emission

scanning electron microscopy (FE-SEM), Atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), and Cyclic voltammetry (CV). Furthermore, the study explores the development of a Zinc oxide surface acoustic wave device (ZnO-SAW) alongside the QCM biosensor, aiming to optimize fabrication and effectively control its performance for future cancer biomarker detection. The results demonstrated that the developed QCM biosensor enhances detection efficiency by augmenting binding sites for biomolecules through increased surface area, demonstrating promising sensitivity for early diagnosis and screening applications. Moreover, the successful fabrication of a two-port SAW device on ZnO films with Au/Ti delay line electrodes underscores the integration of experimental and simulation approaches in precise device fabrication and performance prediction. The consistency between simulation and experimental results validates the research, providing insights into SAW device fabrication and operation, with implications for enhancing biosensing capabilities across various biomarkers for other diseases.



School of Materials Engineering

Academic Year 2023

Student's Signature.....

Advisor's Signature.....

Co-Advisor's Signature.....

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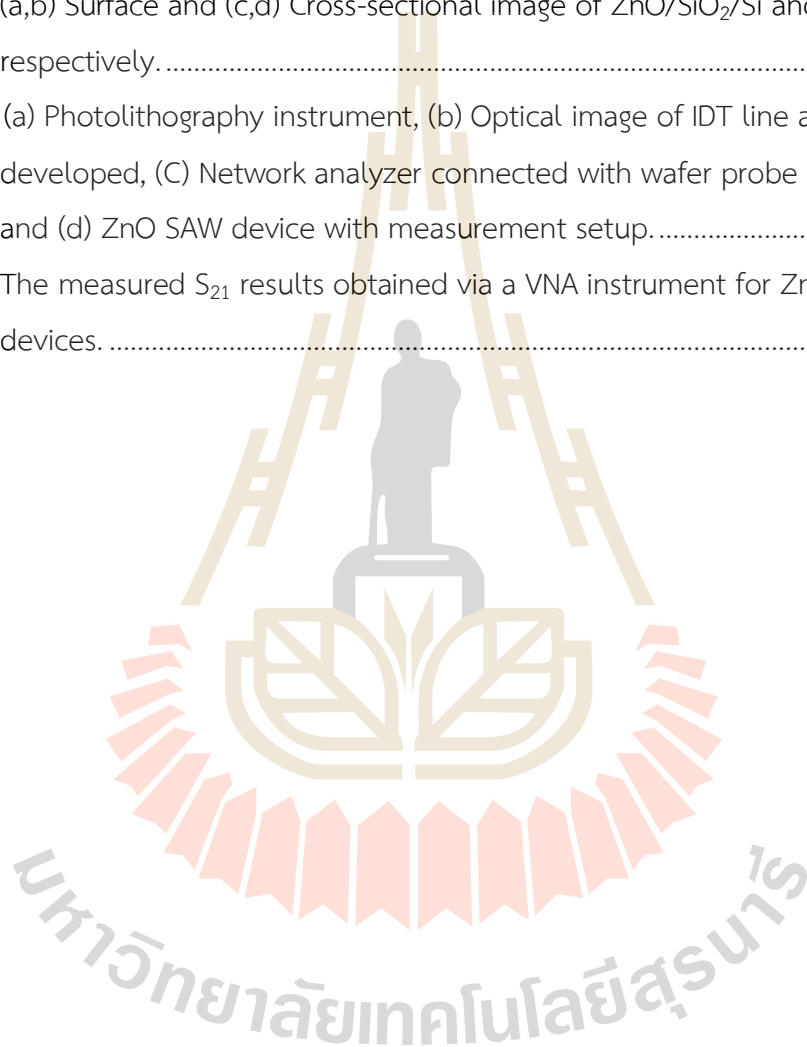
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LIST OF ABBREVIATIONS

AC	=	Alternating voltage
AFM	=	Atomic force microscopy
AuNP	=	Gold nanoparticle
AuNS	=	Gold nanospike
CA	=	Contact angle
CV	=	Cyclic voltammetry
DC	=	Direct-current
DRE	=	Digital rectal examination
FEM	=	Finite Element Method
FE-SEM	=	Field-emission scanning electron microscopy
FT-IR	=	Fourier-transform infrared spectroscopy
GLAD	=	Glancing angle deposition
HFF-QCM	=	High Fundamental Frequency
IDT	=	Interdigital transducer
LOD	=	Limit of detection
PCa	=	Prostate cancer
PCD	=	Pulse current electrodeposition
PSA	=	Prostate-specific antigen
QC	=	Quartz crystal
QCM	=	Quartz crystal microbalance
RF	=	Radio frequency
RMS	=	Root mean square
S11	=	Reflection parameter
S21	=	Transmission parameter
SAM	=	Self-assembled monolayer
SAW	=	Surface acoustic wave
UV-Vis	=	Ultraviolet-visible spectroscopy

LIST OF ABBREVIATIONS (Continued)

VNA	=	Vector network analyzer
XPS	=	X-ray photoelectron spectroscopy
XRD	=	X-ray Diffraction
ZnO	=	Zinc oxide
ZnO-SAW	=	Zinc oxide surface acoustic wave device



CHAPTER I

INTRODUCTION

1.1 Rationale and Background

Prostate cancer (PCa) is the most prevalent malignancy diagnosed in men worldwide, with 1.41 million cases reported by World Health Organization in 2020. Approximately 1 in 8 men will be diagnosed with PCa during their lifetime, and older men have a higher risk for PCa, with ~99% of patients aged >50 years (Tan, Xu, Zhong, & Wang, 2021). It is estimated that 1 in 41 men die from PCa. Early diagnosis and screening for malignant growths significantly improve prognosis, survival rates, and overall understanding of personal well-being. Biomarker detection at an early stage enables prompt initiation of treatment, resulting in increased effectiveness of drug therapy, reduced risk, and decreased patient mortality (Malode & Shetti, 2022). Prostate-specific antigen (PSA) is among the biomarkers utilized in hospitals and clinics for PCa screening and health check-ups. In 1994, the US Food and Drug Administration authorized PSA as a marker for the detection of malignant prostate growth (Cary & Cooperberg, 2013). PSA can be detected in males' blood, urine, and saliva (Chang, Gadzinski, & Nyame, 2021). In clinical practice, a serum PSA concentration is essential for guiding patient care in various situations, spanning from initial detection of PCa to post-treatment monitoring. Having a reliable point-of-care (POC) test for prostate cancer screening allows for PSA testing and subsequent decision-making to occur in a single clinical encounter. Furthermore, the POC allows for immediate discussions about the next steps, such as whether to proceed with a digital rectal examination (DRE), transrectal ultrasound (TRUS), positron emission tomography/computed tomography (PET/CT) scan, magnetic resonance imaging (MRI), or other diagnostic procedures (Meyer & Gorin, 2019).

Nowadays, current medical instruments for detecting PSA have proven to be highly beneficial. However, they have certain limitations due to their sophisticated nature, time-consuming operation, slow response time, high cost, laborious

procedures, and lack of portability for POC scenarios. To overcome these challenges, biosensors present several advantages, such as cost-effectiveness, high sensitivity, ease of use, portability, real-time analysis, and the capability to offer POC diagnosis. Various types of biosensors, including electrochemical, optical, surface plasmon resonance, and piezoelectric (Akbari Jonous et al., 2019; Choi et al., 2013; Damborska et al., 2017; Malode & Shetti, 2022), have been developed with a specific focus on detecting PCa biomarkers, aiming to enhance the effectiveness of cancer tumor screening, monitoring, and diagnosis.

Among these biosensors, piezoelectric quartz crystal microbalance (QCM) biosensor has been reviewed as an attractive device for label-free biomarker applications, particularly in tumor screening, monitoring, and diagnosis. This is because the QCM offers fast detection, high sensitivity, and the ability to be integrated with cost-effective electronic devices, making it a viable alternative POC to traditional methods. The QCM consists of a specialized quartz crystal (QC) oriented for precision cutting, along with metallic electrodes. The orientation of the QC cut plays a crucial role in its piezoelectric properties. The QCM functions as a high-resolution mass sensing technique. It can operate under various conditions, including liquid, gas, and vacuum environments (Bhattacharya, Rathore, & Majumdar, 2018; Li et al., 2022). The principle of QCM relies on the piezoelectric effect, which exhibits an indirect effect. When an external electric field is applied to the QC, it induces mechanical stresses within the QC. An alternating voltage (AC) creates mechanical oscillations in the direction perpendicular to the QC surface, and a resonance frequency is recorded when the crystal is placed in an oscillation circuit (Lim, Saha, Tey, Tan, & Ooi, 2020; M. Pohanka, 2018). The QCM measures mass variation per unit area by detecting changes in the frequency of a QC resonator. The introduction or removal of a small mass influences the resonance frequency. When target analytes such as antibody, antigen, enzyme, nucleic acid (DNA, RNA), and tissues are captured by the recognition elements on the QCM sensor electrode, it induces a change in the mass and/or viscoelastic properties of the QCM, resulting in a shift in its resonance frequency. The resonance frequency shift and mass sensitivity of the QCM can be determined using Sauerbrey's equation, in which the resonance frequency shift is proportional to adding mass (m) and inversely

proportional to the surface area of the working electrode. To enhance the sensitivity of a QCM electrode (N. Asai et al., 2019), either of these three methods can be employed: (1) reducing the thickness of the QC, (2) enlarging the surface area for attachment of a greater quantity of biomolecule, and/or (3) incorporating nanoparticles on the sensor electrode. Several researchers have reported that the combination of nanotechnology with QCM electrodes enhances the capabilities of biosensing platforms by increasing the biomolecule binding sites and signal amplification.

Nanotechnology has garnered significant attention in 21st-century technology, and researchers are actively exploring its innovative applications, especially in medical research. Nanotechnology-based diagnostic methods are being investigated as potential tools for real-time, convenient, and cost-effective cancer diagnosis and detection (Chen, Zhang, Liu, Zhang, & Zhou, 2018). Nanomaterials offer an opportunity to advance early diagnosis, improve patient prognoses, and enhance treatment monitoring, ultimately resulting in improved patient outcomes. The use of nanomaterials underscores the potential for progress and improved patient care. Currently, cancer diagnostics are gradually shifting from invasive and complex procedures toward the adoption of highly sensitive point-of-care liquid biopsies (Kemp & Kwon, 2021), where nanomaterials have demonstrated remarkable effectiveness in detecting cancer biomarkers. A key advantage of using nanomaterials in cancer detection is their large surface area-to-volume ratio compared to bulk materials. This characteristic allows nanomaterial surfaces to be densely coated with antibodies and other small molecules capable of identifying specific cancer molecules, thereby enhancing assay specificity and sensitivity (Huang et al., 2018; Zhang, Li, Gao, Chen, & Liu, 2019).

Various nanomaterials, including nanotubes, nanorods, and nanoparticles, are being studied for use in immunological sensing systems. For instance, Pohanka et al. (Miroslav Pohanka, 2021) developed an approach that involved using Gold (Au) nanoparticles-conjugated antibody to improve the sensitivity of QCM detection of PSA, resulting in a simple, selective, and fast detection method that is suitable for POC applications. Uludag et al. (Uludag & Tothill, 2012) described a sandwich hybridization assay based on antibody-modified nanoparticles amplification and signal enhancement

for POC immunosensor of PSA detection. Asai et al. (Naoto Asai, Shimizu, Shingubara, & Ito, 2018) modified the active surface area of QCM by introducing Anodic Aluminum Oxide nanostructures. This modification was done to enhance antibody-antigen interactions and improve mass sensitivity. Pirich et al. (Pirich, de Freitas, Torresi, Picheth, & Sierakowski, 2017) utilized a bacterial cellulose nanocrystal thin film-coated QCM electrode to increase sensitivity and create an adapted interface for the attachment of captured immunoglobulin G. In this regard, exploring a signal amplification strategy with nanotechnology would be valuable for the development of a highly effective QCM biosensing platform.

Gold (Au) has emerged as a remarkable class of nanomaterials, possessing properties that make them highly desirable for various applications, such as biomedicine and biosensors. These unique properties include controllable synthesis steps, low toxicity, and high biocompatibility (Hassan et al., 2022). The combination of the general properties of nanomaterials with the unique properties of Au enables them to offer an enhanced surface for the immobilization of biomolecules, making them exceptional candidates for the development of highly sensitive and selective biosensing platforms. In various sensor designs, Au has been used to facilitate the immobilization of recognition elements, amplify signal transduction, and improve the LOD for biomarkers.

In this thesis, the author studied a label-free QCM biosensor for the detection of PSA. Various sizes of nanostructures were fabricated using electrodeposition methods. Initially, direct current (DC) electroplating was studied to enhance the sensitivity of PSA detection. Subsequently, pulse current electrodeposition (PCD) was employed to create a distinct structure with minimal nanoparticle mass loading on the QCM electrode surface. The performance of a commercial QCM with the developed unique nanostructure was then evaluated for detecting PSA at different concentrations.

Additionally, the author intended to develop another piezoelectric biosensor. To enhance the sensitivity of such devices, it involves increasing the resonant frequency and minimizing both the base mass and surface area. However, QCM sensitivity is constrained by factors such as substrate thickness and low operating frequency. To overcome these constraints, Surface Acoustic Wave (SAW) can be

applied for this application due to higher operating frequencies and significantly reduced base mass. In this thesis, the author tried to develop and investigate a Zinc-Oxide (ZnO) thin film for a SAW device. The optimization of materials deposition was performed under various conditions, including GLAD and post-annealing temperature adjustments. The results of SAW obtained from simulation and experimentation were thoroughly examined.

1.2 Research Objectives

The critical goals of this dissertation are outlined as follows:

1.2.1 To investigate a piezoelectric biosensor platform for detecting PCa biomarkers.

1.2.2 To enhance the detection signal of the piezoelectric biosensor by integrating nanotechnology via the electrodeposition method. The influence of related chemicals will be examined to comprehend the biosensor's detection behavior.

1.2.3 To fabricate ZnO films for SAW devices and investigate the acoustic wave properties of the devices. The impact of GLAD and post-annealing temperature on uniformly piezoelectric ZnO films on Si-based substrates (Si and SiO₂/Si) will be explored. The ZnO-SAW results will be obtained through theoretical modeling using COMSOL Multiphysics and experimental fabrication.

1.3 Hypotheses

The objective of this dissertation is to utilize a piezoelectric QCM sensor for cancer detection and enhance its performance by incorporating nanomaterials. To achieve this, the author investigates the detection of PSA as a biomarker using a piezoelectric QCM sensor and examines signal enhancement under controlled nanomaterial conditions based on the following hypotheses:

1.3.1 PSA could be detected through resonance frequency shift measurements using the piezoelectric biosensor QCM.

1.3.2 The output frequency could be influenced by nanomaterials, leading to enhanced resonance frequency shifts detected via frequency shift measurements.

1.3.3 The size of nanomaterials can impact the sensor's performance in biomarker detection. QCM-based nanomaterials could provide greater resonance frequency shift signals than pristine QCM sensors.

1.3.4 Chemical treatments such as EDC/NHS and L-cysteine can influence the QCM electrode surface, thereby enhancing the efficiency of PSA biomarker detection.

1.3.5 The microstructure of ZnO for SAW devices could be affected by GLAD and annealing temperature.

1.3.6 SAW could be modeled via COMSOL Multiphysics, and the simulated results align well with experimental data.

1.4 Scope of Thesis

This thesis focuses on the development of a piezoelectric biosensor platform, including both QCM and SAW devices. The specific scope of this research is outlined as follows:

1.4.1 To detect PSA using a QCM biosensor and enhance the nanostructure loaded on the QCM electrode using the electrodeposition method. Different durations of direct current electroplating (30 and 60 minutes) and varying pulse electrodeposition percentages (20% and 80%) will be employed to create distinctive nanostructures.

1.4.2 The influence of related chemicals will be investigated to understand the behavior of QCM biosensor detection. Chemical treatments on the developed QCM electrode surface prior to PSA binding will be studied via wettability and functional group analysis for PSA detection.

1.4.3 To improve the fabrication process and achieve a uniform surface for ZnO piezoelectric films via sputtering techniques for fabricating SAW devices. GLAD and post-annealing techniques will be utilized. SAW devices will be studied through simulation and experimentation. Simulation will be conducted using COMSOL Multiphysics.

1.5 Usefulness of the Research

This research aims to achieve the following objectives by highlighting its significance:

1.5.1 To gain a comprehensive understanding of the PSA detection process of QCM biosensors and explore their frequency response for potential medical applications.

1.5.2 To establish a method for enhancing PCa detection through piezoelectric biosensor devices based on QCM and examine the impact of electrodeposition time and duty cycle on creating unique nanostructures.

1.5.3 To offer guidelines for fabricating SAW devices for medical applications using a combination of simulation and experimental approaches.

1.6 Outline of Thesis

The sections of this thesis are structured as follows:

Chapter 2 provides an overview of the current trends and challenges in medical applications, with a particular focus on piezoelectric biosensors. It begins by discussing the background and basic knowledge of biosensors, elucidating their properties in medical contexts. Specifically, attention is directed toward piezoelectric acoustic biosensors, such as QCM and SAW devices, which play pivotal roles in biomedical sensing applications.

Chapter 3 explores the potential of using QCM piezoelectric biosensors for PCa detection via the PSA biomarker. It presents the design and output performance of the QCM for PSA detection, investigates the frequency response in different nanostructures for sensitive detection of PSA, and details the impact of surface modification on the QCM electrode via DC electroplating. Various characterization techniques such as SEM, XRD, and AFM are utilized to investigate surface morphology, roughness, and crystal structure.

Chapter 4 introduces the PCD for enhancing the surface area for PSA biomarker detection. It describes the fabrication and characterization of gold nanostructures, electrode surface treatments, and immobilization steps of PSA. The various concentration of PSA was investigated on the developed QCM biosensor. The impact

of chemical treatments on the QCM electrode surface and the performance of the modified QCM biosensor for PSA detection are investigated through wettability characterization and identification of functional groups.

Chapter 5 develops uniformly growth of piezoelectric ZnO films on Si substrates using GLAD. It demonstrates the fabrication of SAW piezoelectric sensors and their construction from ZnO piezoelectric materials. The fabrication process using RF magnetron sputtering method at different glancing angles is described, and the influence of oblique angle deposition on growth direction, phase formation, nanostructure formation, and surface morphology is explored. Comprehensive characterization including surface roughness and crystallography using various techniques is detailed.

Chapter 6 provides the optimization of annealing treatment temperature for ZnO films in SAW applications. It underscores the crucial role of annealing in enhancing film quality by regulating factors such as crystallinity, orientation, morphology, and defects. The chapter presents the effect of annealing temperature under oxygen gas on the microstructural properties of ZnO films.

Chapter 7 demonstrates the deposition of ZnO on Si-based substrates (Si and SiO₂) with microstructure refinement through prolonged deposition times and reduced deposition power. A two-port SAW device on ZnO films with Au/Ti delay line electrodes for biomolecule binding is constructed. Experimental and simulation approaches for device fabrication and performance prediction are utilized. The influence of the sensing area on acoustic wave propagation using 2D and 3D frequency-domain Finite Element Method (FEM) analysis is investigated. Integration of experimental and simulation approaches for precise device fabrication and accurate performance prediction is performed.

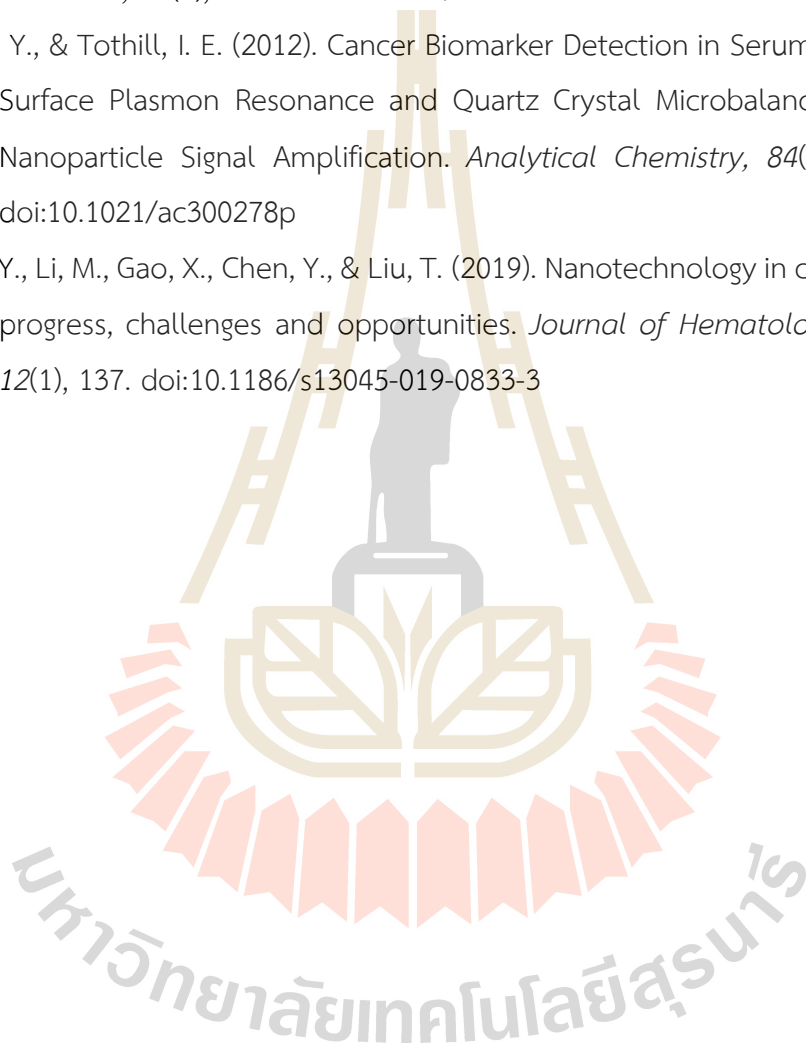
Finally, **Chapter 8** provides a summary of all the conclusions reached in this thesis, along with recommendations for further research.

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CHAPTER II

LITERATURE REVIEWS

2.1 Cancer: Current Trends and Challenges

Cancer originates from the transformation of healthy cells into tumor cells, progressing through various stages from a pre-cancerous lesion to a malignant tumor. According to the World Health Organization, cancer was a significant contributor to global mortality in 2020, responsible for nearly 10 million deaths, approximately one in six fatalities. Prominent among the types of cancer diagnosed in 2020 were breast, lung, colon, rectal, and PCa, as shown in Figure 2.1. Additionally, the WHO reported the top five cancers for cancer-related mortality, as illustrated in Figure 2.2.

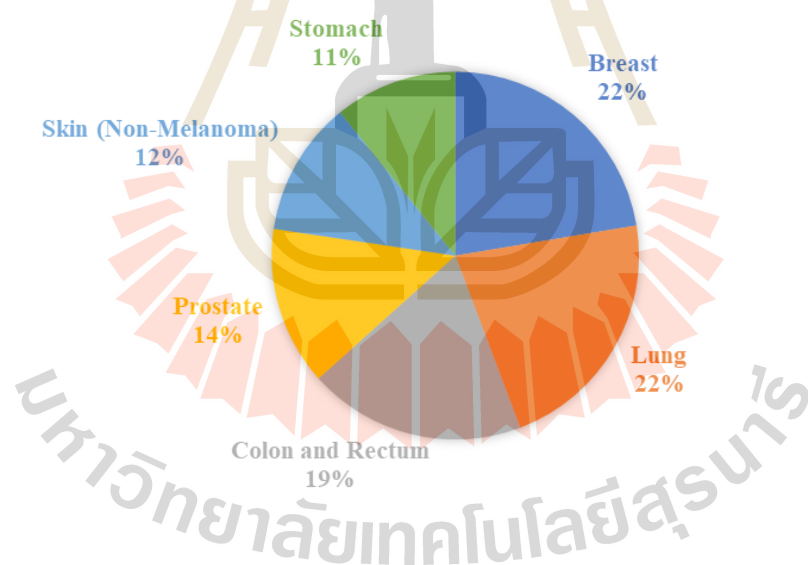


Figure 2.1 The most common types of cancer in terms of new cases reported in 2020 by WHO. (Source: <https://www.who.int/news-room/fact-sheets/detail/cancer>)

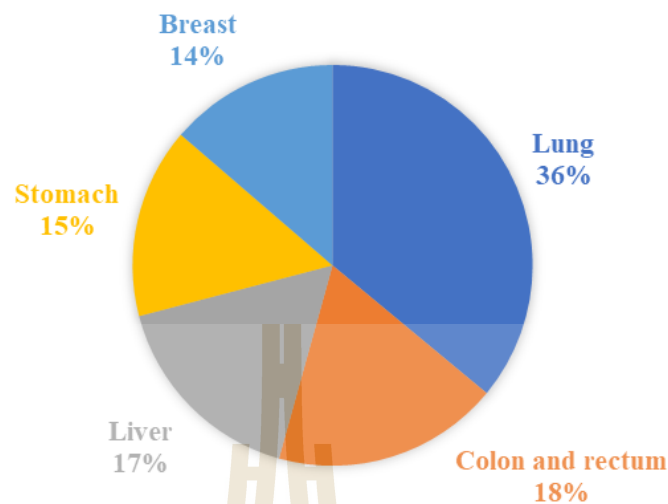


Figure 2.2 Top 5 cancers for cancer-related mortality in 2020 reported by WHO.
(Source: <https://www.who.int/news-room/fact-sheets/detail/cancer>)

Several approaches have been reported to reduce the mortality rate and incidence of new cancer patients. For instance, early detection, when coupled with effective treatment and comprehensive care for cancer patients, plays a crucial role in mitigating the overall impact of the disease. Early diagnosis followed by appropriate treatment significantly increases the likelihood of a successful cure for cancer (Al-Azri, 2016; Crosby et al., 2022). Several strategies play a role in reducing cancer mortality and identifying new cancer patients, including:

(1) Early detection

Early detection aims to identify consequential cancer or precancerous changes at the earliest time point at which intervention could improve survival or reduce morbidity (Crosby et al., 2022). Furthermore, risk stratification of patients would assist in effectively identifying individuals at high risk, thereby facilitating prevention and early detection efforts (Harvard Public Health https://www.hsph.harvard.edu/magazine/magazine_article/the-cancer-miracle-isnt-a-cure-its-prevention/).

(2) Early diagnosis

Diagnosing cancer at an earlier stage through early diagnosis programs involves supporting prompt help-seeking among symptomatic individuals and facilitating timely access to diagnosis and treatment, leading to early treatment and optimized outcomes (Koo et al., 2021).

(3) Screening

Screening aims to identify individuals with findings suggestive of a specific cancer or pre-cancer before they have developed symptoms. Cancer screening recommendations have significantly reduced mortality from certain cancers, such as cervical and colorectal, while demonstrating more modest decreases in mortality for others (Loud & Murphy, 2017).

(4) Treatment

Accurate cancer diagnosis is paramount to ensure suitable and efficient treatment, as each cancer type demands a tailored therapeutic approach. Cancer treatment involves employing surgery, radiation, medications, and other therapies to either cure the cancer, reduce its size, or halt its progression.

(5) Palliative Care

Palliative care aims to alleviate symptoms and suffering caused by cancer, focusing on improving the quality of life for patients and their families. It provides comfort and support, especially in regions where a significant proportion of patients are in advanced stages of cancer with little chance of cure.

2.1.1 Cancer Detection: Approaches and Current methods

Table 2.1 There are several diagnostic methods available for cancer detection in hospitals, as outlined below (source: <https://www.cancer.gov/about-cancer/diagnosis-staging/diagnosis>):

Diagnostic method	Resources from patient	Method	Main benefit
Blood Chemistry Test	Patient blood	Measures the amounts of certain substances released into the blood by organs and tissues.	Routine health check-up
Complete Blood Count (CBC)	Patient blood	Measures the number of red blood cells, white blood cells, and platelets.	Routine health check-up
Cytogenetic Analysis	Tissue, blood, bone marrow, or amniotic fluid	Identifies changes in chromosomes in patient samples.	Diagnose cancer, planning treatment
Immunophenotyping	Blood or bone marrow sample	Uses antibodies to identify cells based on the types of antigens or markers on the surface of the cells.	Diagnose and monitor blood cancers
Liquid Biopsy	Blood	Test done on a blood sample to look for cancer cells or pieces of DNA from tumor cells sometimes released into the blood.	Early detection of cancer, planning treatment
Sputum Cytology	Sputum	Looks for abnormal cells in sputum, mucus, and other matter brought up from the lungs by coughing.	Diagnose lung cancer

Table 2.1 There are several diagnostic methods available for cancer detection in hospitals, as outlined below (source: <https://www.cancer.gov/about-cancer/diagnosis-staging/diagnosis>):

Diagnostic method	Resources from patient	Method	Main benefit
Tumor Marker Tests	Body substances	Measures substances produced by cancer cells or other cells of the body in response to cancer.	Diagnose cancer, decide on treatment, measure treatment effectiveness
Urinalysis	Urine	Describes the color of urine and measures its contents.	Help diagnose kidney cancer, bladder cancer, and rarer urothelial cancers.
CT Scan	Pictures of organs from different angles	Uses an x-ray machine linked to a computer to create detailed 3-D images of the inside of the body.	Used to diagnose cancer
MRI	Pictures of body in slices	Uses a powerful magnet and radio waves to take pictures of the body in slices.	Used for detailed imaging of the body
Nuclear Scan	Uses radioactive material	Takes pictures of the inside of the body using radioactive material.	Used for bone scans

Table 2.1 There are several diagnostic methods available for cancer detection in hospitals, as outlined below (source: <https://www.cancer.gov/about-cancer/diagnosis-staging/diagnosis>):

Diagnostic method	Resources from patient	Method	Main benefit
Biopsy	Tissue or fluid	Removes a sample of abnormal tissue for diagnosis and treatment planning.	Essential for diagnosis and treatment planning
Ultrasound	Sound waves echo off tissues	Creates pictures of areas inside the body using echoes from sound waves.	Used for imaging various body parts
X-rays	Radiation	Creates pictures inside the body using low doses of radiation.	Used for imaging various body parts

2.1.2 Cancer Biomarkers

According to the National Cancer Institute, a biomarker is “a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or a condition or disease”. Biomarkers typically differentiate an affected patient from a person without the disease (Henry & Hayes, 2012). They may be used to assess how well the body responds to the treatment of the disease or condition, which is referred to as a molecular marker or a signature molecule (Chiu & Yen, 2023). Biomarkers may manifest in various forms, including antibody proteins, microbial indicators, RNAs, DNAs, lipid-based agents, metabolic compounds, and proteinaceous moieties. Changes in their levels, structural aspects, functional behavior, or pharmacological actions are correlated with initiation, progression, and regressive aspects of particular diseases and how patients’ bodies respond to these (Ahmad, Imran, & Ahsan, 2023). In cancer research, cancer biomarkers are chemicals released

by tumors that specifically indicate their presence. Cancer biomarkers denote the inherent characteristics of malignant growth cells (Malode & Shetti, 2022). Early detection of cancer enhances treatment outcomes and survivors' quality of life. Biomarkers, such as proteins and nucleic acids, in combination with advanced biosensor technologies, enable precise detection even at low levels, thereby improving screening and diagnosis (Wahab et al., 2023). The examples of some biomarkers derived from human organs are shown in Figure 2.3.

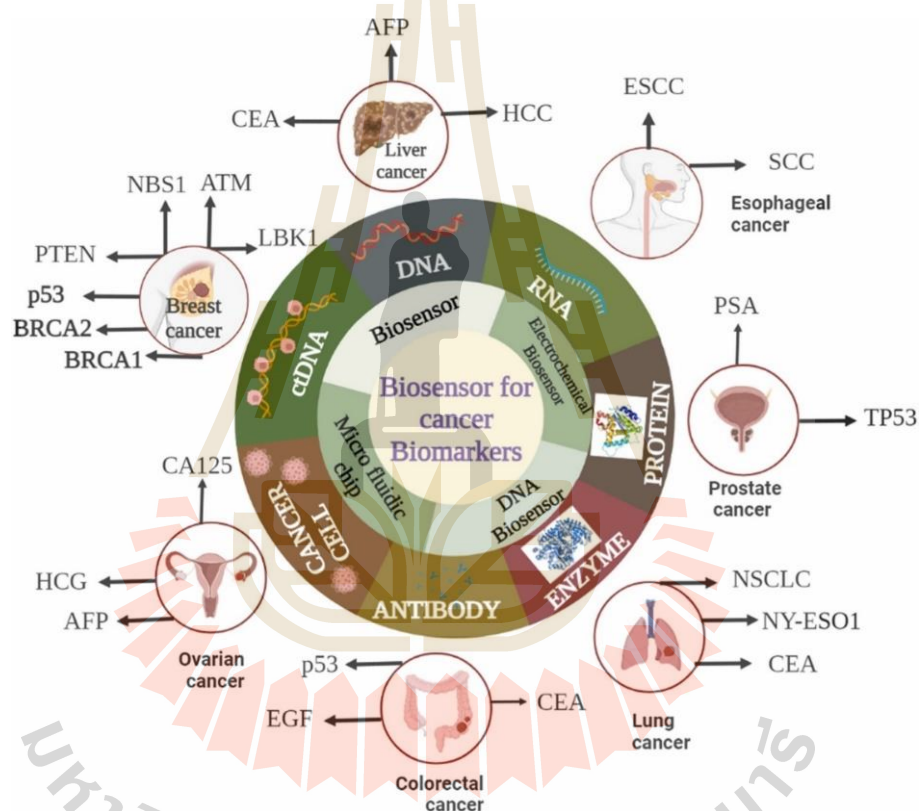


Figure 2.3 A list of biomarkers derived from human organs (Wahab et al., 2023), such as PSA, primarily originate from their respective organs. Elevated levels of PSA in the blood may indicate potential PCa or other prostate-related conditions.

Tumor markers can be used for one of five purposes (Dasgupta & Wahed, 2014):

(1) Screening a healthy population or a high-risk population for the probable presence of cancer.

- (2) Diagnosis of cancer or a specific type of cancers.
- (3) Evaluating prognosis in a patient.
- (4) Predicting the potential response of a patient to therapy.
- (5) Monitoring recovery of a patient receiving surgery, radiation, or chemotherapy

2.1.2.1 Prostate-Specific Antigen

PCa is a widespread malignancy worldwide and continues to pose a significant healthcare challenge. The most widely used tumor marker for PCa in the clinical setting is PSA (Dasgupta & Wahed, 2014). PSA, first discovered by Hara in 1971, It was synthesized from prostate epithelial cells and secreted into the semen, where it constitutes a major component of seminal plasma, as seen in Figure 2.4. PSA is a 34-kDa, 240-amino-acid glycoprotein belonging to the kallikrein family of serine proteases, specifically known as kallikrein-related peptidase 3 (KLK3). Its expression is predominantly confined to normal and malignant prostate epithelial cells in men. Consequently, PSA serves as a pivotal biomarker for PCa screening, recurrence detection post-definitive therapy, and treatment response assessment in metastatic disease settings. The standard reference value for PSA is below 4.0 $\mu\text{g/L}$, exhibiting a sensitivity of 63%-93% and a specificity of 75%-95% (Tang, Cui, Zhang, & Zhang, 2019). Elevated PSA levels, with a cutoff value of 4.0 ng mL^{-1} and a threshold level of 10 ng mL^{-1} , may indicate early-stage PCa. However, PSA levels can also signify benign prostatic hypertrophy, or prostatitis. Despite the discovery of various PCa markers, PSA remains the principal biomarker for both early diagnosis and disease monitoring. In the early 1990s, it was first demonstrated that the PSA blood test could be used as a first-line screening test for PCa (Catalona et al., 1991), which led to the approval of the PSA test by the United States Food and Drug Administration (FDA) as an aid to the early detection, staging, and treatment monitoring of PCa (Alqudah, Al-Samman, Matalgah, & Abu Farhah, 2022; Catalona, 2014).

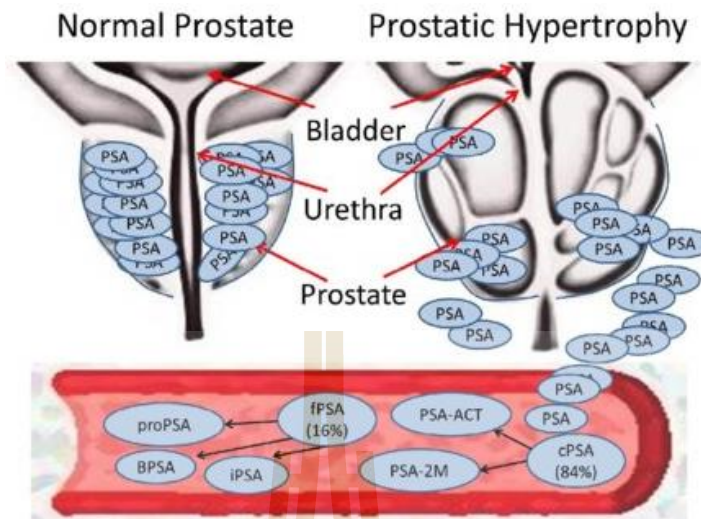


Figure 2.4 PSA is released from the prostate gland following perturbations of the cellular microenvironment. (O'Reilly, 2017)

2.2 Biosensor: Principle and Their Applications

Over the decade, biosensors have been widely used to detect cancer biomarkers, such as electrochemical, optical, and piezoelectric (Hong et al., 2022; Li et al., 2022; C. Lino et al., 2022). The superior characteristics of these biosensors included their portability, sensitivity, response accuracy, reproducibility, high specificity, and repeatability (Akgönüllü, Özgür, & Denizli, 2022; Malode & Shetti, 2022; Narita et al., 2021). The use of specific and sensitive analytical biosensing devices for early-stage cancer detection is considered the future of clinical advancements (Iqbal et al., 2022).

A biosensor, also known as a biological sensor, is a self-contained bioanalytical system that integrates biological molecules that determine illness-related signs in biological fluids for disease monitoring and detection, with a physical-chemical transducer electronic amplifier and display platform that can receive, respond, exhibit and converts a biological signal into a quantified and processable signal. The first generation of biosensor technology was pioneered by Leland C. Clark, Jr in 1956 (Bhalla, Jolly, Formisano, & Estrela, 2016).

A typical biosensor, as depicted in Figure 2.5, comprises several components:

(1) Analyte: This refers to the substance being targeted for detection, such as glucose, ammonia, alcohol, antigen, virus, toxins, or target DNA.

(2) Biorecognition element (also known as a bioreceptor): this molecule specifically identifies the target substrate (i.e., analyte) such as enzymes, cells, aptamers, DNA, and antibodies.

(3) Transducer: Its role is crucial in translating the biological interaction into a measurable form. This energy conversion process, known as signalization, typically results in either optical or electrical signals. Transducers can broadly be classified as electrochemical, optical, thermal, electronic, or gravimetric, light, heat, pH changes, plant or animal tissue reactions, or microbial product emissions depending on their operating principle.

(4) Electronics: This component processes the transduced signal, readying it for display. It involves intricate electronic circuitry that performs tasks such as signal conditioning, including amplification and conversion of signals from analog to digital. These processed signals are then quantified by the biosensor's display unit.

(5) Display: Comprising both hardware and software elements, the display presents the biosensor's results in a user-friendly format. The output signal displayed can take various forms, including numeric, graphic, tabular, or image-based, depending on the end user's requirements (Bhalla et al., 2016; Gergeroglu, Yildirim, & Ebeoglugil, 2020; Iqbal et al., 2022).

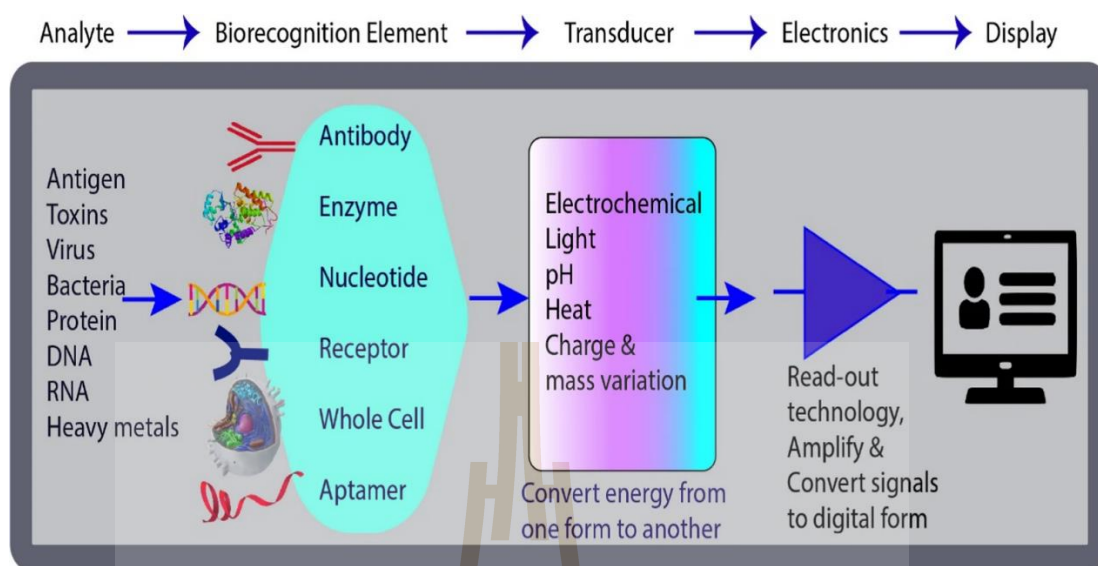


Figure 2.5 Schematic diagram of a component of biosensor, including a bioreceptor, transducer, electronic system, and display, alongside various types of bioreceptors and transducers utilized in biosensors. (Iqbal et al., 2022)

Currently, various technologies assert their suitability for biosensing applications, serving as proofs of concept or commercially viable products. The primary application domains of biosensor technology encompass medical, food, industrial, environmental measurement, and biodefense fields (Mehrotra, 2016). The extensive utilization of biosensor technology can be attributed to its substantial advantages over established analytical methods, including a minimal sample pretreatment, cost-effective instrumentation, rapid response times, and high sensitivity.

2.3 Types of Biosensors

Biosensors encompass a diverse and multidisciplinary field, typically categorized into four main groups. Firstly, based on bioreceptor components, they include enzyme, antibody-antigen, whole-cell, nucleic acid, biomimetic, aptamer, tissue, and phage-based biosensors. Secondly, classification by transducers and sensors divides them into electrochemical, optical, piezoelectric, thermal, and gravimetric types. Thirdly, biosensors may be classified based on fabrication technology. Lastly, they can be categorized based on sensing mechanisms, including optical, electrical,

electronic, thermal, magnetic, or mechanical approaches. This categorization is depicted in Figure 2.6.

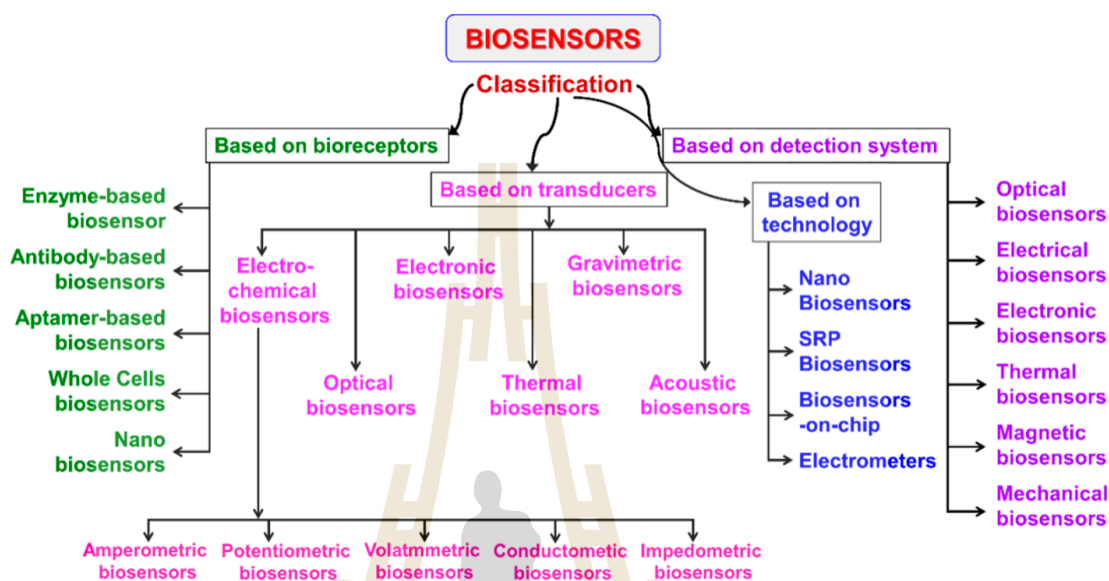


Figure 2.6 Classifications of biosensor. (Curulli, 2021)

2.3.1 Types of Acoustic Wave Biosensors

Acoustic biosensors offer promising solutions for the rapid, sensitive, and cost-effective detection of both gaseous and liquid analytes. These sensors typically utilize either bulk or SAW, as illustrated in Figure 2.7, to operate at different frequencies. Generally, they rely on the piezoelectric effect, where certain piezoelectric generate an electric potential when subjected to mechanical stress and vice versa, creating an internal mechanical stress in response to an applied electrical potential. The piezoelectric materials and their phenomenon are described in Section 2.4.

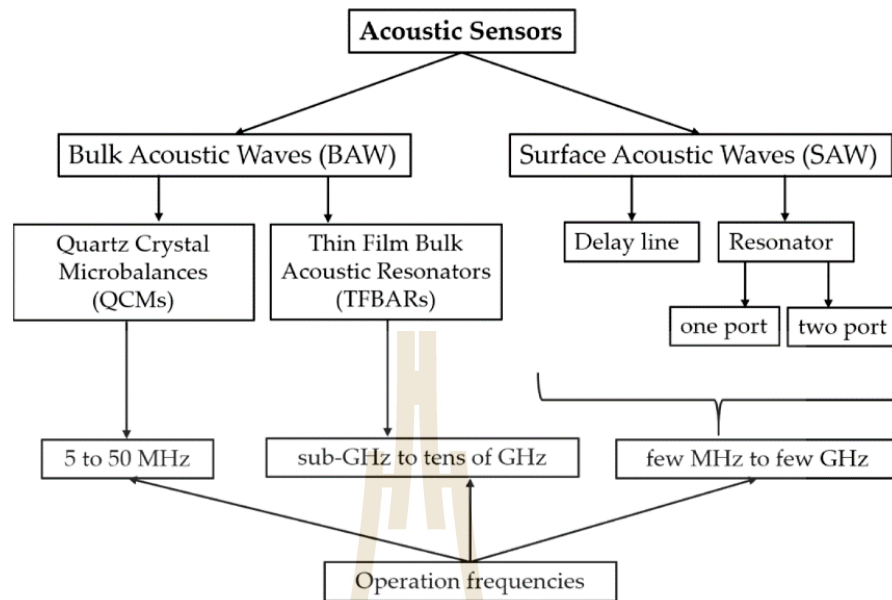


Figure 2.7 The various types of acoustic sensors and their corresponding operational frequencies. (Palla-Papavlu, Voicu, & Dinescu, 2021)

2.4 Piezoelectric Biosensors

Various types of transducers, including optical (light), electrochemical, and piezoelectric (mass variation), have been employed in the development of biosensors. The coupling of electrical and mechanical energy renders piezoelectric materials invaluable across a wide range of applications, including energy conversion, sensors and actuators, and telecommunication. Piezoelectric sensors are particularly suitable for assays involving changes in viscosity and surface elasticity, such as aggregations, coagulations, and precipitations, making them popular choices for biosensor detection.

In contrast to conventional methods that depend on labels such as enzymes, fluorophores, and radioactivity, the utilization of piezoelectric devices as biosensors has experienced a steady rise in interest. This is attributed to their appealing applications in mass-sensitive detection without the requirement for labeling at the sensor surface, along with their capability for real-time monitoring, consequently simplifying assay formats (Skládal, 2024). An advantage of piezoelectric devices over other techniques is illustrated in Table 2.2.

Table 2.2 Comparison of various detection techniques. (Narita et al., 2021)

Techniques	Advantage	Disadvantage
Piezoelectric	Label free Specific Highly sensitive Rapid	Rely on sample preparation Complex pretreatment steps Brittleness
PCR	Sensitive specific	Complex pretreatment steps Failing to distinguish between viable and nonviable cells Sophisticated instruments
Optical	Rapid Sensitive Specific	Complex pretreatment steps Sophisticated instruments

2.4.1 Piezoelectric Phenomena

In 1880, Pierre and Jacques Curie discovered piezoelectric materials through experiments that involved measuring surface charges on specially prepared crystals such as quartz, barium titanate, topaz, Rochelle salt (sodium potassium tartrate tetrahydrate ($\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$)), and tourmaline, when subjected to mechanical stress. The term "piezoelectric" originates from the Greek word "piezein," which translates to "to press" or "to squeeze." Therefore, "piezoelectricity" essentially signifies "pressure-induced electricity" (Behera, 2022; Sezer & Koç, 2021).

Piezoelectricity, or the piezoelectric effect, is a physical phenomenon that refers to the ability of piezoelectric materials to produce an electric charge when subjected to mechanical stress, known as the direct effect. Conversely, the effect also operates in the opposite situation, known as the converse effect, where a voltage applied to the surface of a piezoelectric material causes mechanical stress or oscillation. This phenomenon is depicted in Figures 2.8 and 2.9.

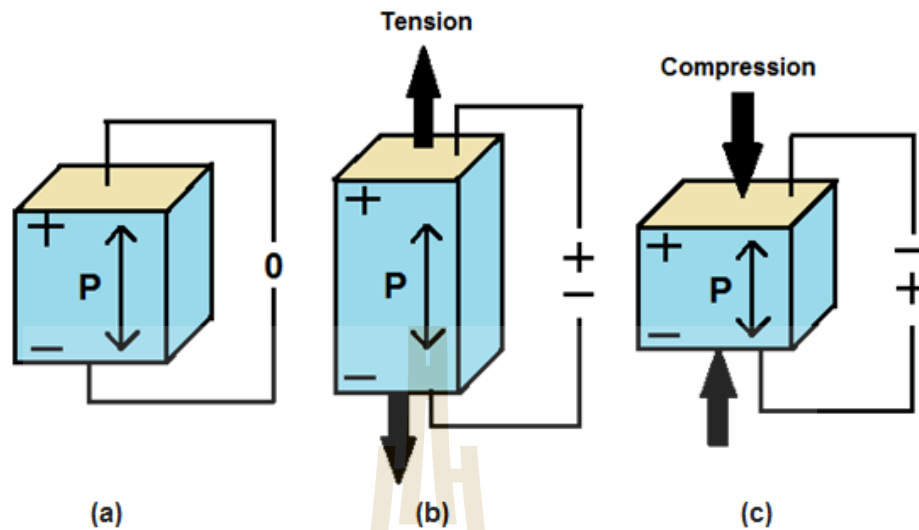


Figure 2.8 The direct piezoelectric effect with the following components: (a) A piezoelectric material without applying any force, (b) The application of tension to generate voltage, and (c) The application of compression to generate voltage. (Vatansever, Siores, & Shah, 2012)

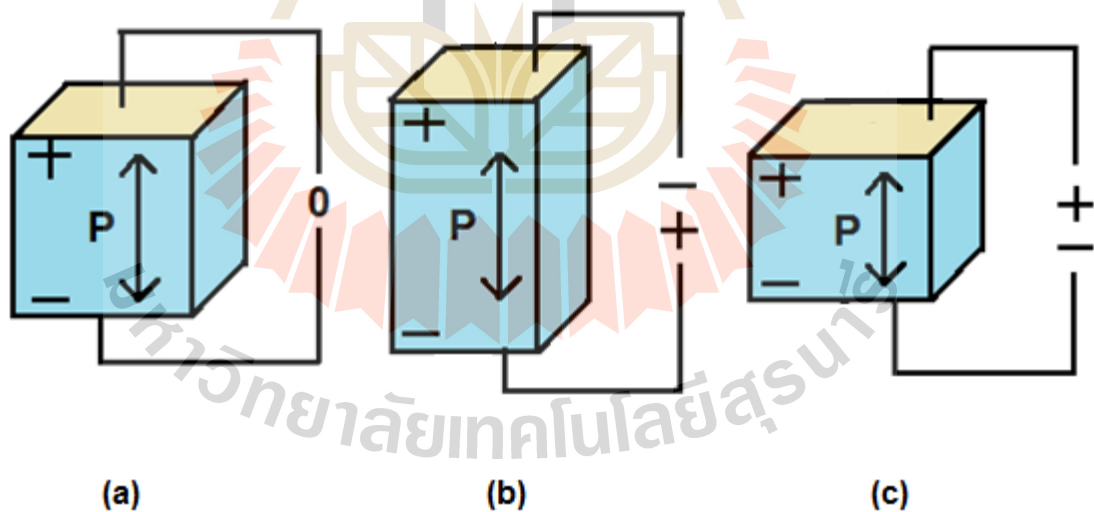


Figure 2.9 The converse or indirect piezoelectric effect as follows: (a) A piezoelectric material, (b) Expansion of the material caused by applying electrical current, and (c) Shrinkage of the material caused by applying opposite electrical current. (Vatansever et al., 2012)

The piezoelectric material is stimulated by an alternating voltage applied to its surface via electrodes. This alternating voltage induces mechanical oscillations in the piezoelectric material, and the frequency of these oscillations is measured using an oscillation circuit. In common biosensor applications, a simple oscillation is typically preferred. The piezoelectric platform is considered ideal for constructing biosensors. The characteristics of the oscillations can be varied depending on factors such as the material, electrical contacts, and material shape. These oscillations are commonly spread across the material, causing the generation of acoustic waves. Both SAWs propagating across the material's surface and bulk acoustic waves occurring within its depths can occur in the oscillating material.

2.4.1.1 Constitutive Properties of Piezoelectric Materials

The distinctive feature that distinguishes piezoelectric devices from others is their remarkable electromechanical interaction between the mechanical and electrical states in crystalline materials. Unlike purely mechanical or electrical devices, piezoelectric crystals can generate an electrical response when mechanically disturbed, and vice versa (Mandal & Banerjee, 2022).

The relation between stress, strain, electric field, and electric displacement field in a stress-charge form (Cheng, Lu, Yang, Gong, & Ke, 2023; Liang, Zhang, Xu, Tan, & Zhang, 2022) of a piezoelectric crystal is delineated by the piezoelectric linear constitutive equations, as described below:

$$T_{ij} = C_{ijkl}^E S_{kl} - e_{ikl}^T E_k \quad (2.1)$$

$$D_i = e_{ikl} S_{kl} + \epsilon_{ik}^S E_k \quad (2.2)$$

where T_{ij} is the second-order stress tensor, D_i are the electrical displacement vector (C/m²), S_{kl} is the strain tensor of the medium, E_k is the electric field vector (V/m). C_{ijkl}^E , e_{ikl} and ϵ_{ik}^S are the elasticity tensor (N/m²), the piezoelectric tensor (C/m²), the permittivity tensor (F/m), respectively.

According to the principle of the piezoelectric effect, piezoelectric materials can serve a valuable role in biosensor applications. In this context, these

materials function as oscillators based on the piezoelectric effect principle. Their interaction with analytes on their surface maximizes the advantages offered by the piezoelectric biosensor platform.

2.4.2 Principle of Piezoelectric Biosensor

Piezoelectric biosensors are analytical devices utilized for determining various analytes. These biosensors, employing the piezoelectric effect, rely on the propagation of high-frequency acoustic waves within the bulk or the surface of a medium. Changes in velocity and amplitude are induced by mass loading or medium viscosity. Detection mechanisms in these devices occur at one of the surfaces of the piezoelectric material, specifically on the top electrode, where variations resulting from the interaction between the analyte and immobilized biomolecules can be converted into frequency or phase changes (Di Pietrantonio, Cannatà, & Benetti, 2019). Figure 2.10 illustrates the principle of piezoelectric biosensors, specifically focusing on QCM, in the detection of antigen-antibody interaction. Here, an antibody serves as the biorecognition element, and the specificity of the antibody significantly influences its binding affinity for particular antigens, which serve as the analyte. The piezoelectric biosensor exhibits specificity when the employed antibody is specific, and the electrode, along with other sensitive parts of the piezoelectric material, remains insensitive to unspecific reactions with interfering compounds. Probe antibodies are immobilized on the upper electrode surface of the piezoelectric material, as depicted in Figure 2.10a. Both upper and lower electrodes drive the resonance. The target antigen subsequently binds with the antibodies, resulting in a mass change (Δm) on the electrode surface, which leads to a time-dependent frequency shift recorded, as illustrated in Figure 2.10b. This shift is represented by the frequency change (Δf) in the oscillation circuit (Narita et al., 2021; M. Pohanka, 2018).

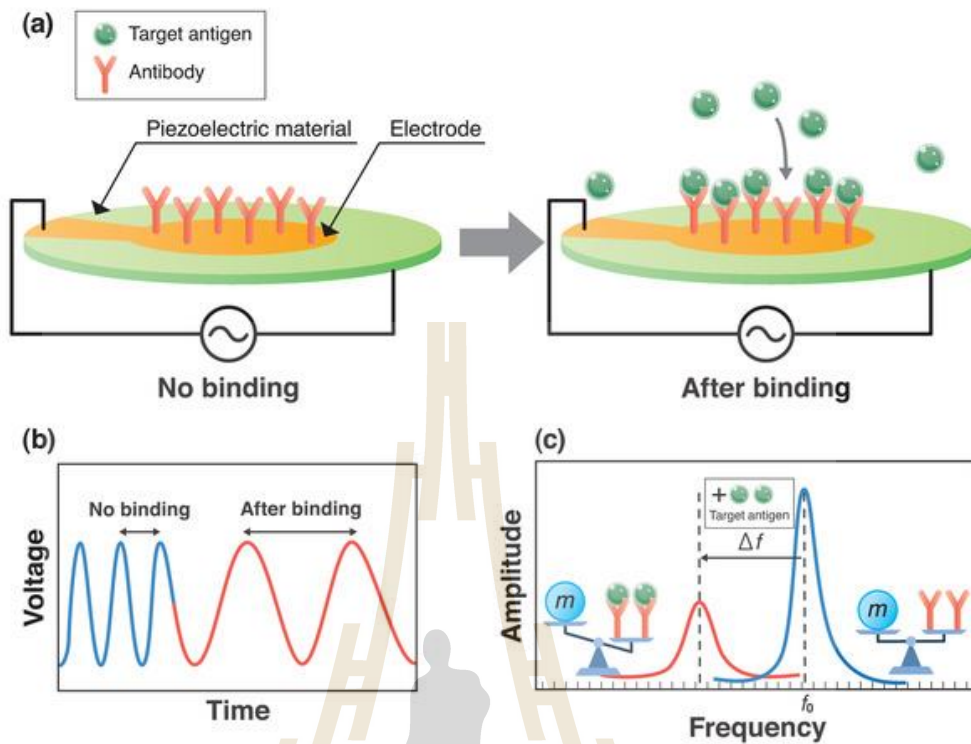


Figure 2.10 (a) The working principle of a piezoelectric biosensor designed for detecting antigen-antibody interactions, (b) The relationship between voltage and time and (c) Amplitude influences frequency during the detection process. (Narita et al., 2021)

Piezoelectric transducers have served as chemical sensors since Sauerbrey discovered the correlation between the mass of adsorbed films and resonant frequency. QCM and SAW piezoelectric devices are piezoelectric biosensors primarily detecting analytes through mass loading on the substrate's surface (Aslam, Zhang, Sreejith, Naghdi, & Ju, 2023; Di Pietrantonio et al., 2019). The mass loading effect, where the resonance frequency shift is proportional to adding mass and inversely proportional to the surface area of the working electrode, can be determined using Sauerbrey's equation (SAUERBREY, 1962), which is expressed as follows:

$$\Delta f = \frac{\Delta m f_0}{\rho A h} \quad (2.3)$$

where f_0 is the resonance frequency, Δf is the change in resonance frequency, Δm is the mass change, A is the active surface area, ρ is the effective density and h is the thickness of the device.

For the QCM, Sauerbrey's equation can be expressed as follows:

$$\Delta f = \frac{2 f_0^2}{\mu_p^{1/2} \rho_q^{1/2} A} \Delta m \quad (2.4)$$

where A is the active surface area of the QCM electrodes, ρ_q is the density of quartz (2.648 g cm^{-3}), and μ_q is the shear modulus of quartz (for AT-cut quartz, $2.947 \times 10^{11} \text{ g cm}^{-1} \text{ s}^{-2}$).

2.5 Piezoelectric Acoustic Biosensors

Acoustic components are based on acoustic waves generated from the piezoelectric effect, one of the most exciting material properties. Acoustic waves generated from the piezoelectric effect are broadly categorized into bulk acoustic waves (BAW) and SAW, as explained in section 2.3.1. The piezoelectric acoustic biosensors use acoustic waves generated by piezoelectric materials to extract information about the analytes. Commonly employed materials for bulk substrates include quartz, lithium tantalate (LiTaO_3), and lithium niobate (LiNbO_3). Additionally, deposited piezoelectric thin films such as lead zirconate titanate, aluminum nitride, and Zinc oxide are utilized, offering improved piezoelectric properties and facilitating the miniaturization of acoustic devices (Nair, Teo, & Li, 2022).

2.5.1 Acoustic Wave Mode

The specific wave mode depends on how mechanical waves propagate within piezoelectric materials. BAW and SAW waves each exhibit distinct modes that prove invaluable for biosensing applications. This diversity in operational modes underscores the pivotal role of acoustic biosensors in advancing compact, portable devices for various consumer-oriented sectors such as healthcare, food safety, environmental monitoring, and tailored medical applications (Nair et al., 2022). Figure 2.11 illustrates the schematic representation of different acoustic wave modes utilized

in biosensing, both BAW and SAW. An example of the propagation direction and particle displacement in different modes of BAW and SAW, as depicted in Figure 2.12.

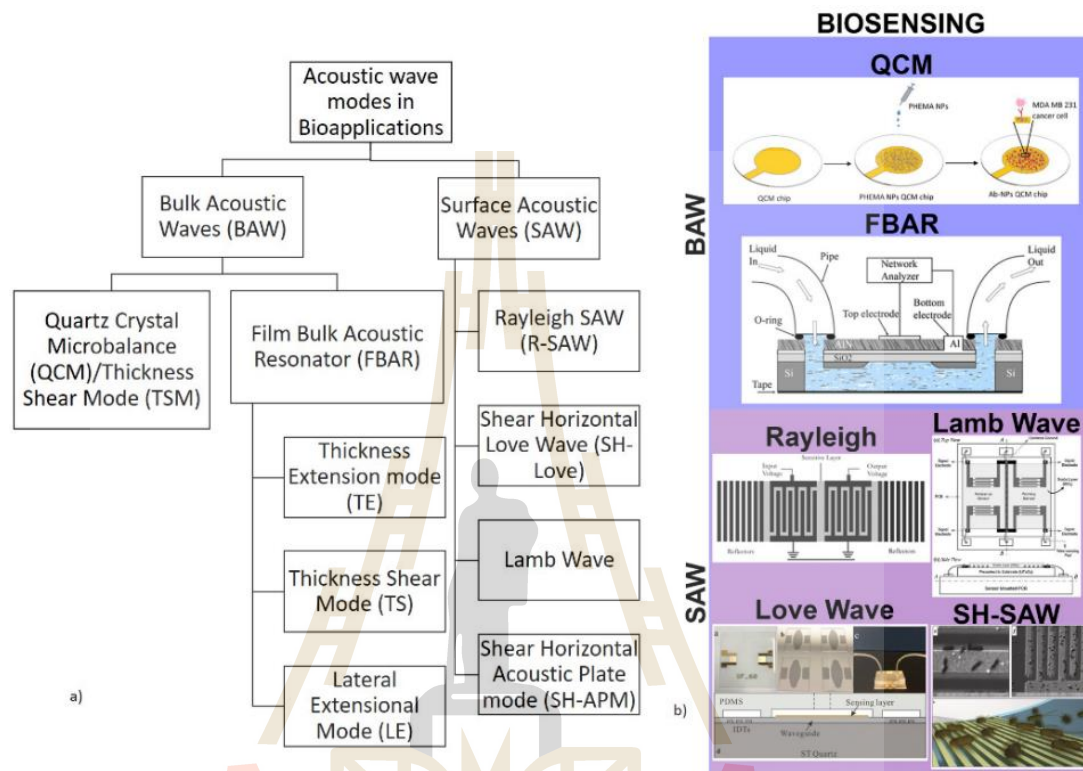


Figure 2.11 Classification of different wave modes into two categories: bulk acoustic waves and SAWs. (Nair et al., 2022)

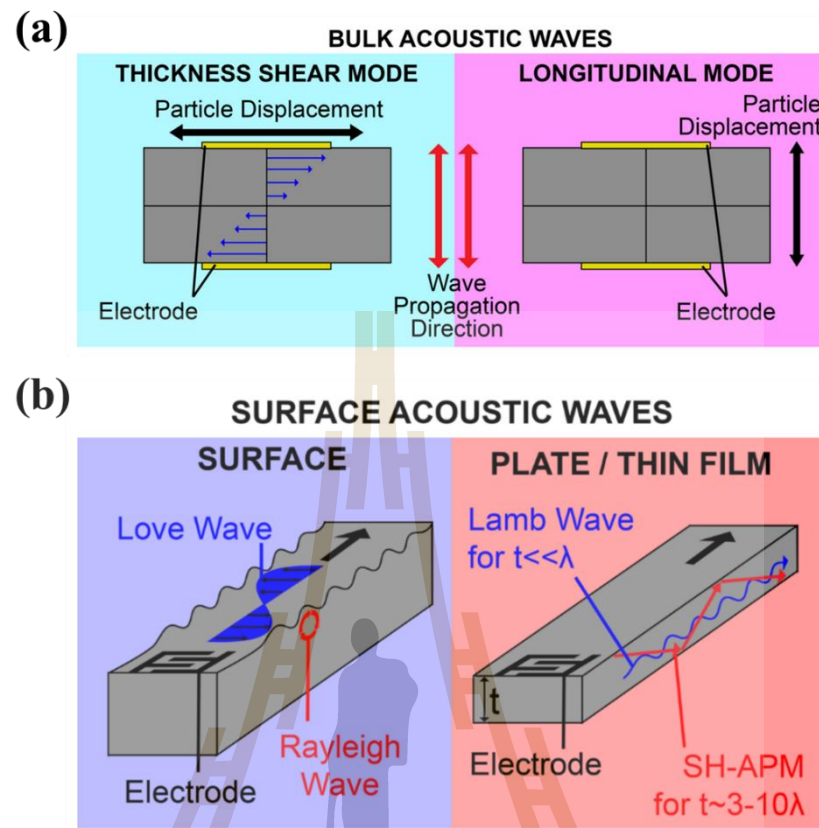


Figure 2.12 An example of wave propagation mode in BAW and SAW. (Nair et al., 2022)

2.5.2 Acoustic Wave Biosensor Principle

When the bioreceptor interacts with the analyte in the acoustic biosensor, it results in changes to the density, viscosity, elasticity, or electrical characteristics of the sensing element. These modifications influence the properties of the acoustic waves, including wave velocity/phase shift, amplitude, and frequency. The acoustic transducer then converts these changes into an electrical output. A simplified diagram, as seen in Figure 2.13, illustrating this working principle.

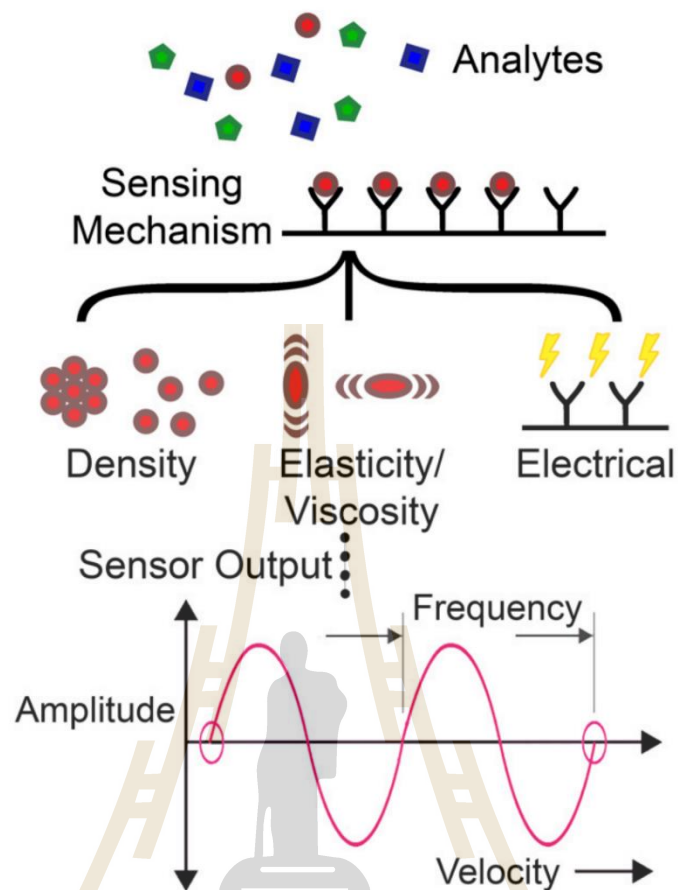


Figure 2.13 The basic working principle of an acoustic biosensor for detecting analytes, resulting in variations in the acoustic wave amplitude, phase/velocity, or frequency in the sensor output. (Nair et al., 2022)

Variations in the phase velocity of the acoustic wave can be ascribed to two main categories of factors, as delineated by Fu and co-worker (Fu et al., 2017):

(1) Intrinsic factors such as material properties: density, elasticity, phase transformation, viscosity, conductivity, permittivity, changes in carrier concentration and mobility.

(2) Extrinsic factors such as mass loading, temperature, deformation, pressure, strain, stress, humidity, pH values, ultraviolet (UV), and infrared (IR) sources, externally applied electric/magnetic fields and charge injection.

Acoustic phase velocity, v , is related to the resonant frequency by

$$f = \frac{v}{\lambda} \quad (2.5)$$

where λ represents the wavelength. A change in acoustic velocity leads to a change in resonant frequency.

The following relationship identifies the different sources that may contribute to the change in acoustic phase velocity (Δv) (Fu et al., 2017):

$$\frac{\Delta f}{f_0} = \frac{\Delta v}{v_{acoustic}} = \frac{1}{v} \left(\frac{\partial v}{\partial m} \Delta m + \frac{\partial v}{\partial \sigma} \Delta \sigma + \frac{\partial v}{\partial c} \Delta c + \frac{\partial v}{\partial T} \Delta T + \frac{\partial v}{\partial P} \Delta P + \frac{\partial v}{\partial \rho} \Delta \rho + \frac{\partial v}{\partial \eta} \Delta \eta + \frac{\partial v}{\partial \epsilon} \Delta \epsilon \dots \right) \quad (2.6)$$

The above equation assumes that any external perturbations listed below are small; Δm is the change in mass load, $\Delta \sigma$ the change in conductivity, Δc the change in mechanical constant, ΔT the change in temperature, ΔP the change in pressure, $\Delta \rho$ the change in density, $\Delta \eta$ the change in viscosity, and $\Delta \epsilon$ the change in dielectric constant.

The sensitivity of an acoustic wave sensor (S_r) to any external perturbation x is defined as (Fu et al., 2017):

$$S_r = \lim_{\Delta x \rightarrow 0} \frac{\Delta f}{f \Delta x} = \frac{df}{f dx} \quad (2.7)$$

where Δf is the frequency shift induced by Δx or the variation or perturbation of factors such as temperature, pressure, mass, density, viscosity or conductivity.

Most acoustic wave devices operate according to Sauerbrey's equations, as shown in Eq. (2.3). The equations demonstrate that mass sensitivity is directly proportional to mass per unit area and the square of the operating frequency. Therefore, enhancing the sensitivity of an acoustic wave device involves increasing the resonant frequency and minimizing both the base mass and surface areas of the device. While QCM functions as a gravimetric sensor, its sensitivity is constrained by factors such as substrate thickness and low operating frequency. Conversely, SAW

devices have been tailored for sensing applications due to their superior sensitivity stemming from higher operating frequencies and significantly reduced base mass. Figure 2.14 provides a schematic representation of sensitivity performance across various resonators about their typical operational frequency ranges.

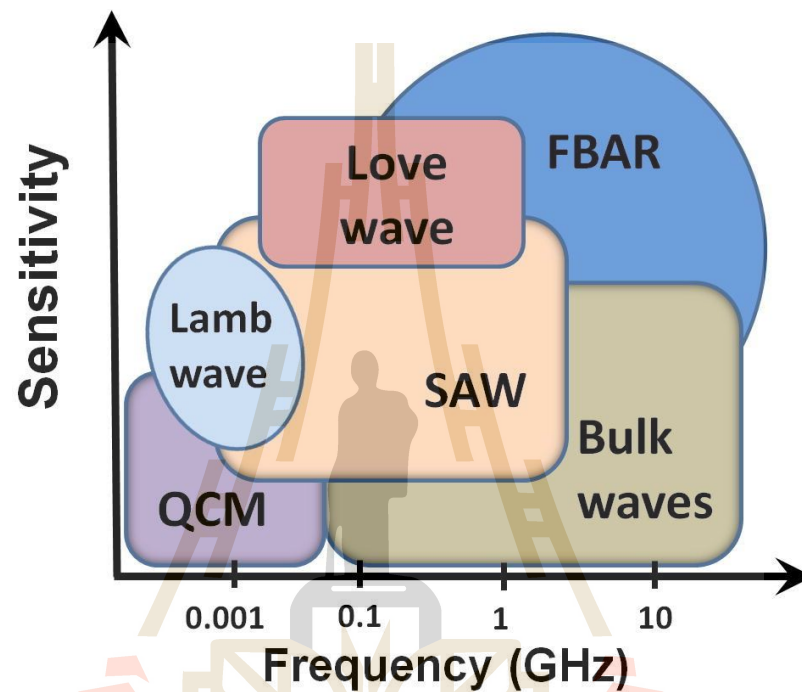


Figure 2.14 Comparison of frequency range and sensitivity of common acoustic wave devices. (Fu et al., 2017)

Table 2.3 outlines two acoustic wave devices based on piezoelectric materials for chemical and biochemical sensor applications, along with various details such as the type of acoustic wave, operating frequency, sensitivity, working medium, availability, manufacturing costs, commercial success, and general remarks.

Table 2.3 Overview of high frequency acoustic devices for chemical/biochemical sensor applications. (A. Mujahid, Afzal, & Dickert, 2019)

	QCM	SAW
Acoustic Wave Type	Bulk	Surface
Operating Frequency	5–200 MHz	100–1500 MHz
Sensitivity	Low	Intermediate
Working Medium	Liquids and Gases	Gase
Availability	Common and Easily Available	Common and Easily Available
Manufacturing Costs	Low	Moderate
Commercial Adaptability	Medium	High

2.6 Bulk Acoustic Wave

2.6.1 Quartz Crystal Microbalance

The QCM, sometimes referred to as the Quartz Crystal Nano Balance (QCN), belongs to the class of BAW devices. It comprises two essential components: a thin disc of QC and top and bottom electrodes known as the active area, as seen in Figure 2.15a. The QCM typically consists of a thin disk of AT-cut quartz (i.e., cut from a quartz mineral piece at a 35.25° orientation to its optical axis). The piezoelectric properties of quartz are significantly influenced by its cutting orientation, with commonly used commercial electrodes typically made from gold (Au). Enhancements in properties are achieved post-electrode deposition, either through the creation of Au-Ti electrode to improve cohesion energy or through specific applications utilizing SiO_2 -Au-Ti electrode. The QCM has its own natural frequency depending on the cutting angle and the thickness of the QC. The oscillation mode of QCM is called thickness-shear mode, which has the direction of displacement perpendicular to the quartz thickness with the maximum amplitude at the quartz plate surface, as seen in Figure 2.16 (Songkhla & Nakamoto, 2021).

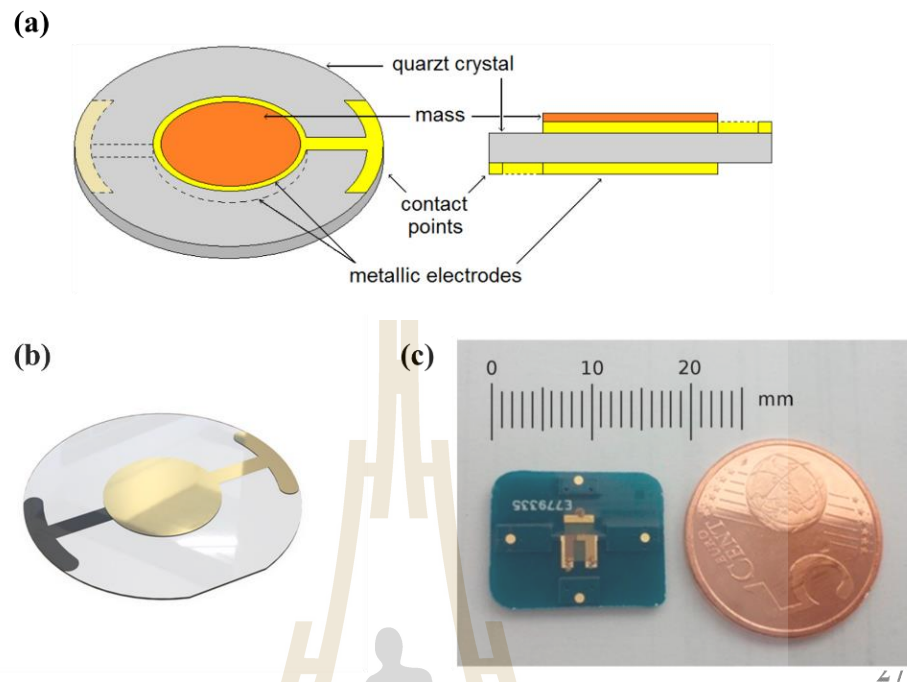


Figure 2.15 (a) QCM crystal's views (Karapınar, Gürkan, Öner, & Doğan, 2018), (b) 10 MHz QCM with 13.9 mm diameter and $\sim 160 \mu\text{m}$ thickness from NOVAETECH (<https://store.openqcm.com/openqcm/>), and (c) 150 MHz HFF-QCM integrated into customized PCBs measuring $17.5 \times 14 \text{ mm}^2$ with a thickness of 1.55 mm produced by Advanced Wave Sensors S. L. (Fernández et al., 2017)

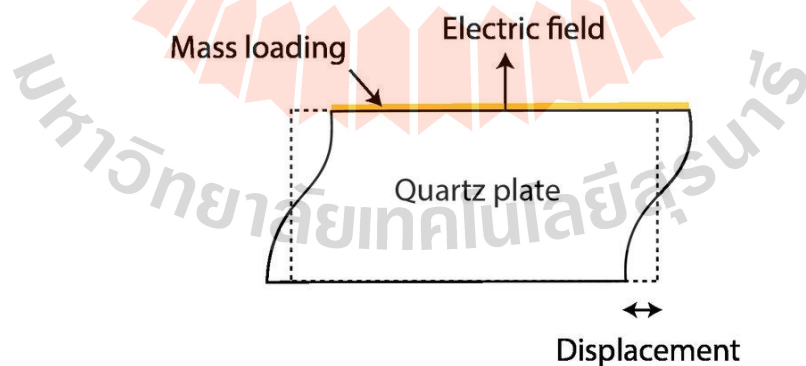


Figure 2.16 Thickness shear mode of QCM. (Songkhla & Nakamoto, 2021)

The QCM for biological detection exhibit stability in both liquid and dry conditions. Resonance frequencies for QCM typically range from 5, 10 (Figure 2.15b),

and 20 MHz to higher frequencies such as 150 MHz (Figure 2.15c) and 195 MHz (Mujahid, Afzal, & Dickert, 2019) for HFF-QCM, which necessitates integration with printed electronic boards (PCB) for handling. Mass variation per unit area is detected through changes in the frequency of the QC resonator. The resonance is affected by the addition or removal of a small mass, such as oxide growth/decay or film deposition on the surface of the acoustic resonator. QCM, among various piezoelectric sensor platforms, is a popular choice for biosensor construction due to its widespread availability and low cost (around \$20). It offers high-resolution mass sensing, measuring mass per unit area based on the piezoelectric effect. QCM is versatile and capable of operating in vacuum, gas, and liquid environments. Application of a voltage across these electrodes yields shear deformation of the crystal due to the piezoelectric effect. This device is known as a resonator because the crystal resonates as electromechanical standing waves are created. Maximum displacement is at the AT-cut crystal faces, resulting in a device intrinsically sensitive to surface interactions.

2.6.2 QCM Working Principle

The operation principle of the QCM relies on the Sauerbrey equation to estimate theoretical mass changes due to analyte absorption on the electrode. The sensitivity of a piezoelectric biosensor is influenced by factors such as oscillating frequency, active area size, and material thickness. To enhance sensitivity, materials should be thinner, have smaller active electrode areas, higher resonance frequencies, near-zero temperature coefficients of piezoelectric constant, and high-quality factors (Q). Figure 2.17 showed the variation of Q value on the various shape of electrode.

The Sauerbrey equation establishes a linear relationship between crystal resonant frequency and small mass increments, assuming tight coupling of the deposited mass to the crystal surface. This allows the QCM resonant frequency variation associated with mass increases at the surface to be predicted. The piezoelectric signal transduction facilitates label-free detection of various surface interactions, including protein adsorption, (bio)film deposition, antigen detection, biomolecule binding kinetics, cell adhesion, and DNA detection (Grainger & Castner, 2011).

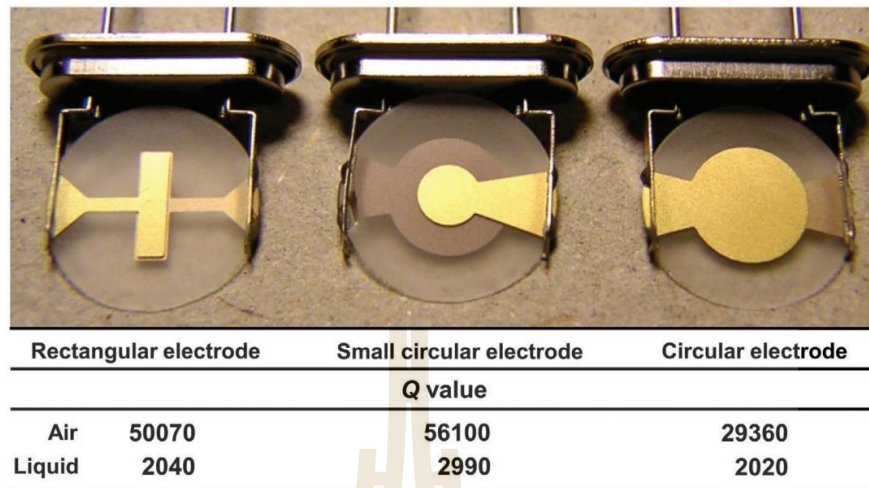


Figure 2.17 Photograph of QCs alongside measured Q values, with measurements conducted both in air and in phosphate-buffered saline. (Grainger & Castner, 2011)

2.7 Surface Acoustic Wave

In 1885, Lord Rayleigh discovered SAWs that propagate along the free surfaces of elastic solid materials, with their amplitudes typically decaying exponentially with depth into the substrate (Hadj-Larbi & Serhane, 2019; Wu, Chen, Nassar, & Huang, 2021). In 1965, White and Voltmer introduced the IDT, demonstrating its capability to generate traveling SAWs on quartz substrates with thin-film metal. By applying an AC to the SAW on a piezoelectric substrate with IDT. A standing SAW is produced as a result of the generation of a periodic strain field. Optimizing device performance requires careful consideration of IDT electrode construction, including parameters such as wavelength, aperture, number of fingers, and delay line. This device has garnered attention in telecommunications, superconducting qubits, quantum dots, and healthcare applications. With such a wide range of applications in research and commercial industries, over 1 billion SAW devices are manufactured every year. The SAW devices have signal frequencies ranging from 10 MHz to 1 GHz (Mazalan, Noor, Wahab, Yahud, & Zaman, 2021). Most SAW devices comprise a piezoelectric substrate, a substance and two IDTs electrodes (Mandal & Banerjee, 2022). Application of an RF voltage to an IDT port on a piezoelectric substrate excites SAWs and generates a potential wave due to the inverse piezoelectric effect. This effect involves the injection

of RF voltage at the first port, leading to elastic deformation. The transmittance of SAWs is measured at a second IDT port through the direct piezoelectric effect, which converts the elastic deformation back into a voltage signal (Puebla et al., 2020), as illustrated in Figure 2.18.

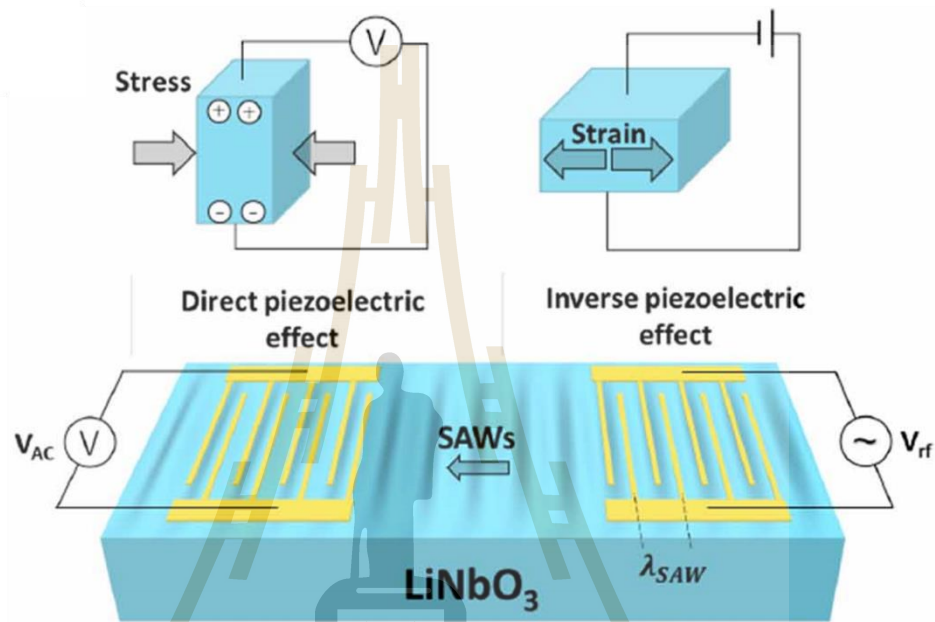


Figure 2.18 Schematic of a two-port IDT device on a piezoelectric substrate (Lithium Niobate) utilized for the generation of SAW. (Puebla et al., 2020)

SAW IDTs are commonly designed in two configurations: delay-line and resonator structures, as illustrated in Figure 2.19. In the setup of a standard SAW delay line, a piezoelectric substrate is used with input and output IDTs applied to it for the excitation and reception of acoustic waves. The gap between these IDT pairs forms the delay line. On the other hand, SAW resonators utilize grating reflectors with a period of $\lambda/2$ to bounce surface waves back towards the IDTs, inducing resonance. SAW resonator devices consist of one set of IDTs and two reflectors on each side of the crystal.

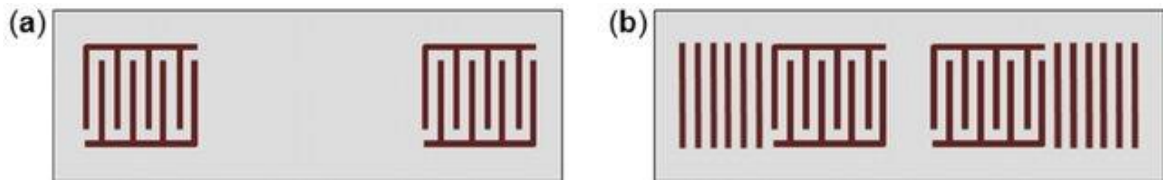


Figure 2.19 SAW sensor configurations (a) delay line and (b) resonator (two-port) (Hohmann et al., 2015).

2.7.1 SAW Simulation

SAW propagation can also be modeled more extensively using finite element method (FEM) analysis. Electrostatics, solid mechanics, and piezoelectric constitutive equations are solved at each finite element to determine SAW propagation throughout the computational domain. Figure 2.20 shows the results of the analysis of acoustic modes by eigenfrequencies of a SAW traveling along the free surface of 64°Y-X LiNbO₃ as calculated using COMSOL Multiphysics.

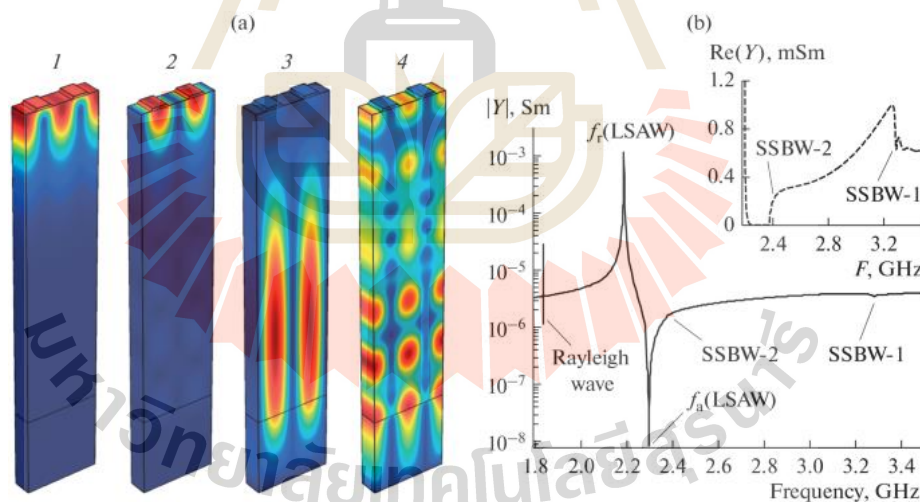


Figure 2.20 Results of the COMSOL analysis, showing: (a) Analysis of eigenfrequencies in the form of total mechanical displacement patterns for the Rayleigh wave (1), leaky SAWs (LSAWs) (2), and surface skimming bulk mode waves (SSBW) (3) and (4); and (b) Analysis in the frequency domain of the admittance characteristic. (Koigerov & Korlyakov, 2022)

The mathematical models for SAW sensors are derived from the piezoelectric constitutive equation, Newton's second law governing mechanical behavior, and Maxwell's equations governing electrical behavior (Cheng et al., 2023). The relationship between stress, strain, electric field, and electric displacement field in a stress-charge scenario within a piezoelectric crystal is expressed by the piezoelectric linear constitutive equations. The propagation of SAW in a piezoelectric material is governed by a comprehensive set of equations, encompassing the continuum equations of motion, Maxwell's equations (under the quasi-static assumption), strain-mechanical displacement relations, piezoelectric constitutive relations, and appropriate boundary conditions.

The mechanical behaviour of linear elastic materials is governed by the equation of motion, which is defined by Newton's second law (Liang et al., 2022):

$$\rho \frac{\partial^2 u_i}{\partial t^2} = \sum_j \frac{\partial T_{ij}}{\partial x_j} (i, j = 1, 2, 3) \quad (2.8)$$

where ρ is the mass density, u_i is the global displacement, and t is the time.

When an acoustic wave propagates through the substrate, its propagation velocity is approximately five orders of magnitude lower than that of electromagnetic wave. Therefore, the electromagnetic field coupled with SAW can be approximated as an electrostatic field, and E_k can be expressed as the gradient of a potential function ϕ (Cheng et al., 2023):

$$E_k = -\frac{\partial \phi}{\partial x_k} (k = 1, 2, 3) \quad (2.9)$$

additionally, the following relationship exists between strain and displacement piezoelectric materials are described as (Cheng et al., 2023):

$$S_{kl} = \frac{1}{2} \left(\frac{\partial u_k}{\partial x_l} + \frac{\partial u_l}{\partial x_k} \right) (k, l = 1, 2, 3) \quad (2.10)$$

and $S_{kl} = \frac{\partial u_l}{\partial x_k}$ is in the symmetry of the stain matrix.

By utilizing Equations (2.1), (2.8), (2.9), and (2.10), the wave equation can be formulated as:

$$T_{ij} = \sum_{k,l} C_{ijkl}^E \frac{\partial u_l}{\partial x_k} + \sum_k e_{ikl} \frac{\partial \phi}{\partial x_k} \quad (2.11)$$

$$\frac{\partial T_{ij}}{\partial x_j} = \sum_{k,j,l} C_{ijkl}^E \frac{\partial^2 u_l}{\partial x_j \partial x_k} + \sum_{k,j} e_{ikl}^T \frac{\partial^2 \phi}{\partial x_j \partial x_k} \quad (2.12)$$

$$\rho \frac{\partial^2 u_i}{\partial t^2} = \sum_{k,j,l} C_{ijkl}^E \frac{\partial^2 u_l}{\partial x_j \partial x_k} + \sum_{k,j} e_{ikl}^T \frac{\partial^2 \phi}{\partial x_j \partial x_k} \quad (2.13)$$

because of the insulating nature of the medium, this region exhibits no charges, and the electrical displacement is solenoidal:

$$\nabla \cdot D = \frac{\partial D_i}{\partial x_i} = 0 \quad (2.14)$$

$$D_i = \sum_{k,l} e_{ikl} \frac{\partial u_l}{\partial x_k} + \sum_k \varepsilon_{ik} \frac{\partial \phi}{\partial x_k} \quad (2.15)$$

$$\frac{\partial D_i}{\partial x_i} = \sum_{k,i,l} e_{ikl} \frac{\partial^2 u_l}{\partial x_i \partial x_k} + \sum_{k,i} \varepsilon_{ik} \frac{\partial^2 \phi}{\partial x_i \partial x_k} \quad (2.16)$$

$$0 = \sum_{k,i,l} e_{ikl} \frac{\partial^2 u_l}{\partial x_i \partial x_k} - \sum_{k,i} \varepsilon_{ik} \frac{\partial^2 \phi}{\partial x_i \partial x_k} \quad (2.17)$$

for $i, j, k, l = 1, 2, \text{ and } 3$.

Equations (13) and (17) can be employed to compute the wave velocity, displacement, and voltage at each node after establishing the boundary conditions and performing discretization using the FEM (Cheng et al., 2023).

The analysis and calculation algorithm in the COMSOL environment includes several steps, as seen in Figure 2.21, (Koigerov & Korlyakov, 2022):

- (1) Choice of 2-D/3-D model;
- (2) Drawing the geometry of the device;
- (3) Choice of material;
- (4) Input of Euler angles;
- (5) Setting the area of the perfectly matched layer;
- (6) Setting periodic and boundary conditions;
- (7) Mesh generation;
- (8) Selecting and configuring the Solver;
- (9) Start calculation;
- (10) Postprocessing: visualization and processing of numerical simulation results in the Results section.

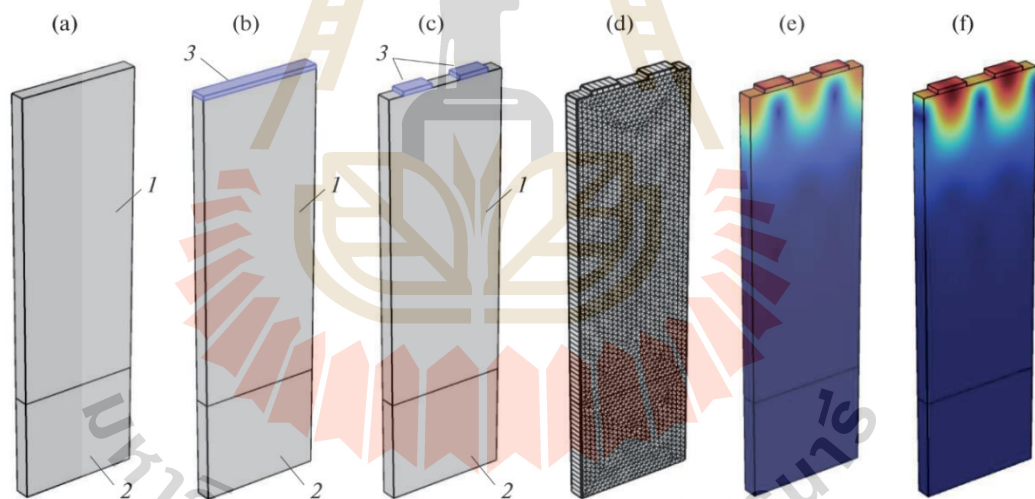


Figure 2.21 (a) Test cells for the analysis of SAW parameters on the free surface, (b) Identification of a metallized surface, (c) Identification of metal electrode, and definition of mesh; also, the results of numerical analysis for the (d) First and (e) Second eigenfrequencies. Geometry: 1. Piezoelectric material; 2. Perfectly matched layer; 3. Metal. (Koigerov & Korlyakov, 2022)

The examples of results simulated via COMSOL are shown in Figures 2.22 to 2.24.

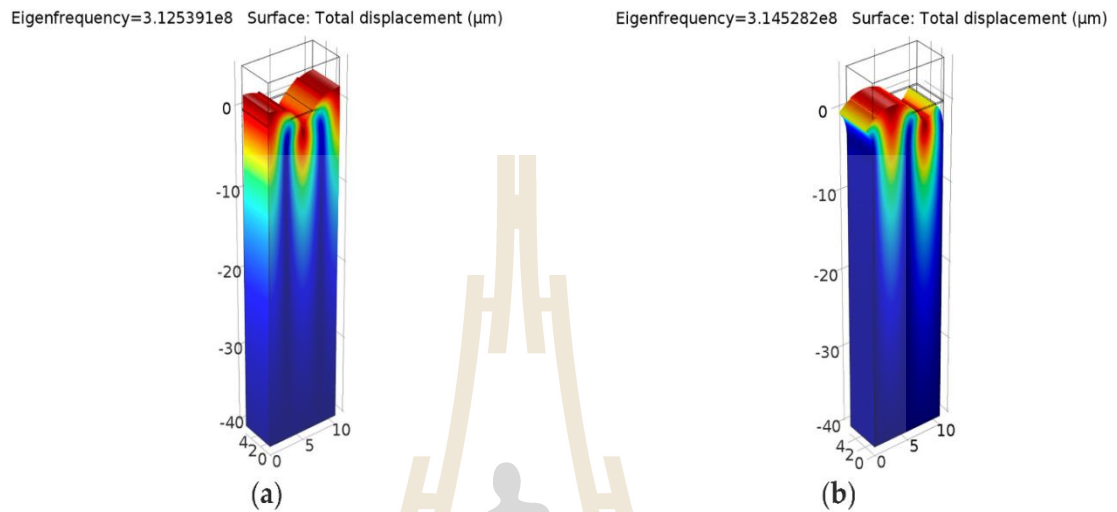


Figure 2.22 Displacement profiles of periodic shorted-grating on ST-X Quartz: (a) Eigenfrequency 312.539 (MHz); (b) Eigenfrequency 314.528 MHz. (Hao, Liu, Liu, Liang, & He, 2016)

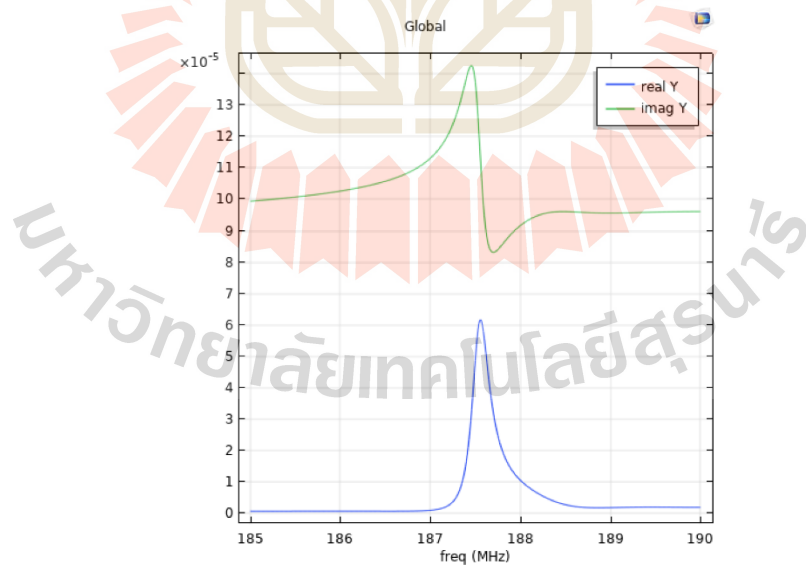


Figure 2.23 The real and imaginary part of the admittance Y vs. frequency curves showing the resonance peaks of Si/SiO₂/ZnO (Caliendo & Laidoudi, 2020).

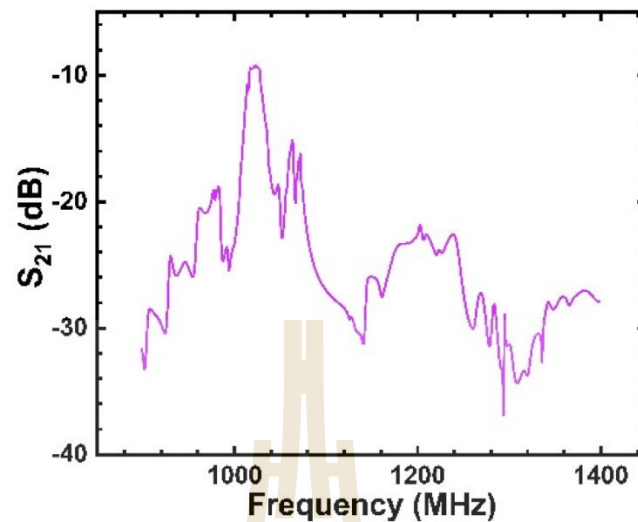


Figure 2.24 S_{21} characteristics of SAW filter of SiO₂/IDT/LiNbO₃/diamond/Si structure with Rayleigh mode. (Zhang & Wang, 2021)

2.7.2 Characterization of SAW

In addition to simulation results, the results of SAW experiments can be characterized using various techniques (Duquennoy, Smagin, Kadi, Ouaftouh, & Jenot, 2022; Kittmann et al., 2018; Kutepov et al., 2022; Luo, Fu, Wang, Wang, & Zhou, 2009; Smagin et al., 2022). For example, electrical measurements such as S-parameter analysis and impedance characteristics, as well as optical measurements including SAW attenuation, velocity measurements, and SAW propagation mapping. When the SAW interacts with an electrically loaded IDT, the total input wave energy divides into transmitting forward, reflecting, and absorbing components. Therefore, the IDT can be considered a scatterer of SAW. Understanding the scattering parameters (S-parameters) is crucial for the development of electroacoustic devices (Timoshenko, Kalinchuk, & Shirokov, 2018). S-parameters can be measured using a VNA, indicating how energy can propagate through an electrical network. S-parameters are commonly employed to describe an n-port network operating at high frequencies, such as RF and microwave frequencies. Other parameters, including impedance (Z), admittance (Y), hybrid (h), and ABCD parameters, are also utilized for describing an n-port network. The primary distinction between S-parameters and other parameter representations lies in the fact

that S-parameters describe normalized power waves when input and output ports are correctly terminated, while other parameters describe voltage and current with open or short ports. Furthermore, S-parameters can express additional electrical properties such as gain, insertion loss, return loss, voltage standing wave ratio, reflection coefficient, and amplifier stability (Huynh, Karlsson, & Gong, 2010).

An IDT delay line, depicted in Figure 2.19a, can be conceptualized as a two-port network (Figure 2.25).

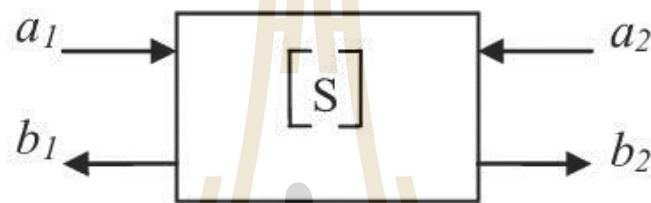


Figure 2.25 A two-port network can be described using S-parameter. (Huynh et al., 2010)

The general definition of the waves traveling into and out of an n-port (Caspers, 2012; Huynh et al., 2010) is as follows:

$$\begin{aligned} a_n &= \frac{1}{2\sqrt{Z_0}} (V_n + Z_0 I_n) \\ b_n &= \frac{1}{2\sqrt{Z_0}} (V_n - Z_0 I_n) \end{aligned} \quad (2.18)$$

where n represents a port number, the waves directed towards the n -port are denoted as $a = (a_1, a_2, \dots, a_n)$, while the waves traveling away from the n -port are represented as $b = (b_1, b_2, \dots, b_n)$. The wave a_1 entering the n -port at port 1 is derived from the voltage wave going into a matched load and Z_0 is the characteristic impedance at that specific port.

A two-port network can be described mathematically as:

$$S_{11} = \left. \frac{b_1}{a_1} \right|_{a_2=0} \quad S_{12} = \left. \frac{b_1}{a_1} \right|_{a_1=0} \quad S_{21} = \left. \frac{b_2}{a_1} \right|_{a_2=0} \quad S_{22} = \left. \frac{b_2}{a_2} \right|_{a_1=0} \quad (2.19)$$

and in matrix form:

$$\begin{Bmatrix} b_1 \\ b_2 \end{Bmatrix} = \begin{bmatrix} S_{11} & S_{12} \\ S_{21} & S_{22} \end{bmatrix} \begin{Bmatrix} a_1 \\ a_2 \end{Bmatrix} \quad (2.20)$$

where S_{11} represents the reflection at port 1, S_{12} denotes the reverse voltage gain, S_{21} signifies the forward voltage gain, and S_{22} indicates the reflection at port 2. When measuring S-parameters, it is crucial to ensure that no power wave is reflected at port 1 or 2, meaning $a_1 = 0$ or $a_2 = 0$. Otherwise, errors may be introduced into the results.

For an n-port network, it can be extended to the following expression:

$$\begin{Bmatrix} b_1 \\ b_2 \\ \vdots \\ b_n \end{Bmatrix} = \begin{bmatrix} S_{11} & S_{12} & \cdots & S_{1n} \\ S_{21} & S_{22} & \cdots & S_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ S_{n1} & S_{n2} & \cdots & S_{nn} \end{bmatrix} \begin{Bmatrix} a_1 \\ a_2 \\ \vdots \\ a_n \end{Bmatrix} \quad (2.21)$$

when IDTs are measured using the VNA, the resonance of the IDT can be observed as a peak in the frequency response of the electrical S_{21} (transmission) parameter and as a dip in the S_{11} (reflection) parameter. S_{21} measures the insertion loss, which represents the power transferred from Port 1 to Port 2, as illustrated in Figure 2.26a. Additionally, S_{11} represents the return loss of a device, indicating how much of the input power supplied to the device reflects back to the input port. Ideally, there should be no reflected power, and 100% of the power should be delivered to the device. The S_{11} measurement is also displayed, as seen in Figure 2.26b.

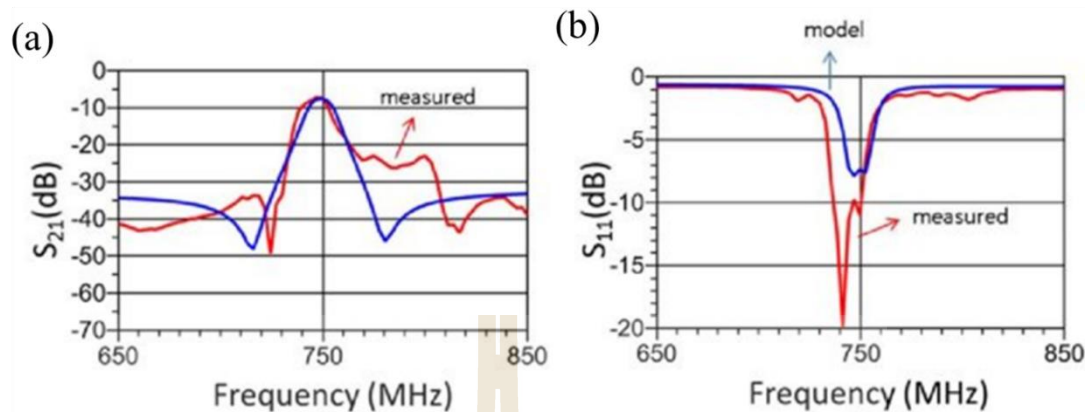


Figure 2.26 (a) The insertion loss (S_{21}) and (b) Return loss (S_{11}) for both the model's predictions and the measurement results. (Sis, 2014)

2.8 Acoustic Biosensor Devices and Nanotechnology-Enhanced Acoustic Biosensors for Biosensing Applications

M. Pohanka (Pohanka, 2021) and Y. Uludağ along with E. Tothill (Uludağ & Tothill, 2010), have employed a sandwich assay for PCa detection, which combines an antibody-modified QCM sensor with a nanoparticle amplification system, as shown in Figure 2.27a. Their experimental procedures for detecting PSA are quite similar. Initially, they coated the QCM electrode surface with a SAM. Subsequently, they immobilized anti-PSA antibodies on the QCM. Then, they introduced PSA over the PSA antibody on the QCM surface. After PSA binds to the QCM electrode, they introduced PSA antibody-modified AuNP onto the sensor surface, as shown in Figure 2.27b and 2.28. This approach incorporates the use of AuNPs in their assay design to increase the mass of bound antigen, which, in turn, enhances the sensitivity of their work.

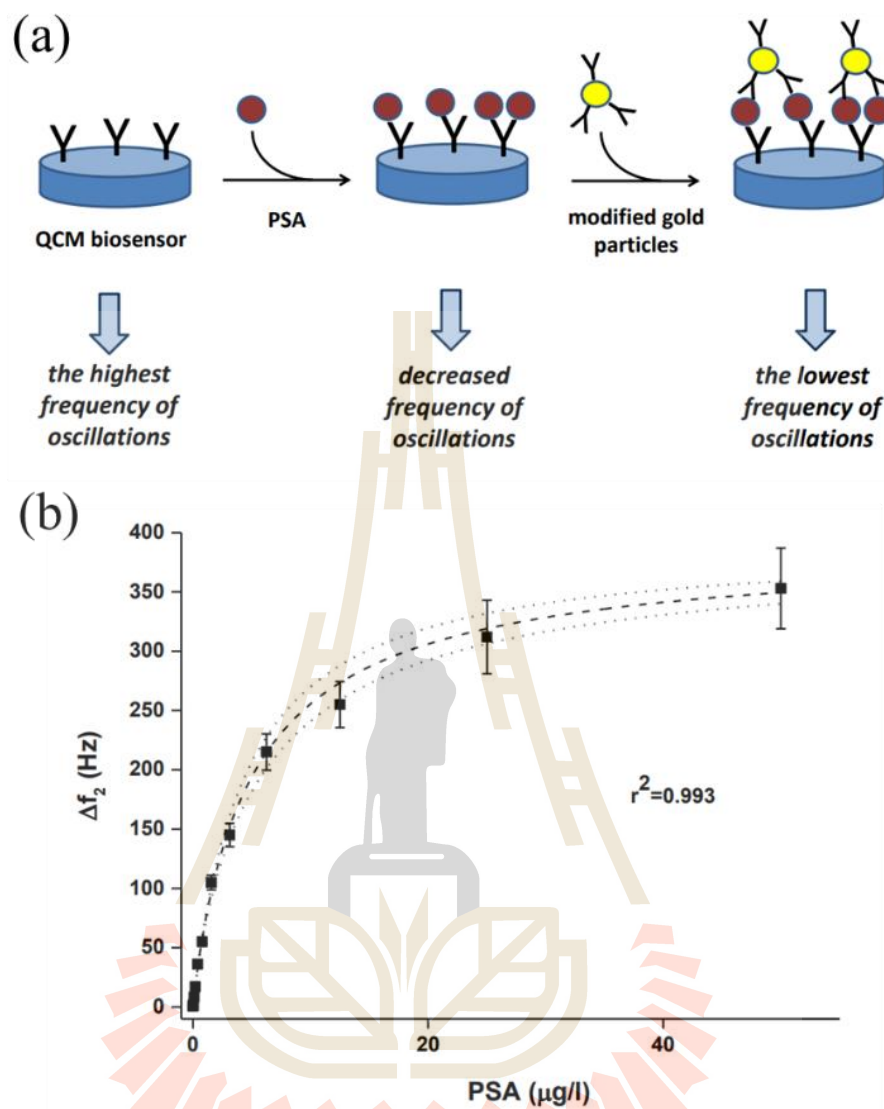


Figure 2.27 (a) The principle of the PSA assay using a QCM biosensor and (b) The results of PSA detection achieved through QCM biosensor technology. (Pohanka, 2021)

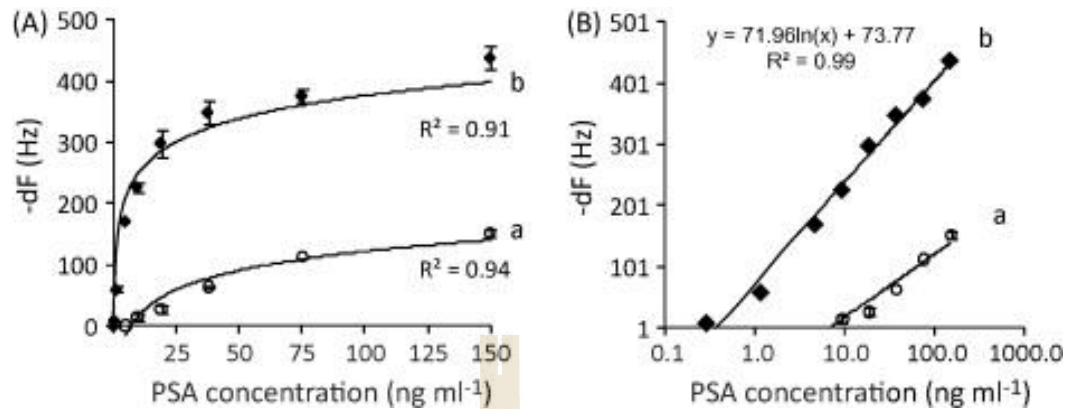


Figure 2.28 The injection of tPSA at varying concentrations (in 75% human serum) onto the tPSA capture antibody immobilized QCM sensor surface is followed by a sandwich assay, where (a) tPSA detection antibody and (b) Antibody-modified Au nanoparticles were injected. The resulting frequency changes due to the binding of nanoparticles are then recorded. (Uludağ & Tothill, 2010)

Li Su et al. (Su et al., 2013) focused on the detection of cancer biomarkers, specifically PSA and α -fetoprotein (AFP), using a piezoelectric ceramic biosensor. Unlike the commonly used QCM, which has higher costs (\$10 USD), They opted for a more cost-effective option, the PZT ceramic resonator, which costs only \$0.5 USD. The PZT ceramic resonator served as the transducer in their biosensor setup. The commercial PZT ceramic utilized had the chemical formula $\text{Pb}[\text{Zr}_x\text{Ti}_{1-x}]\text{O}_3$, with x ranging from 0 to 1. The 40MHz frequency of the PZT ceramic resonator was connected to an impedance analyzer. The study demonstrated the frequency changes of the PSA (2 ng mL^{-1}) to PSA antigen-antibody immobilization resonator in both the detection channel (with PSA antibody) and the control channel (without PSA antibody) at 4 minutes and 1 hour intervals, as shown in Figure 2.29a. Additionally, their findings indicated that the incubation time significantly influences the piezoelectric biosensor, with the frequency of the PSA channel decreasing upon the addition of PSA antigen, and the frequency of the AFP channel decreasing upon the addition of AFP antigen, as shown in Figure 2.29b. This highlights the sensitivity of the biosensor to detect these biomarkers, making it highly desirable for biosensor applications.

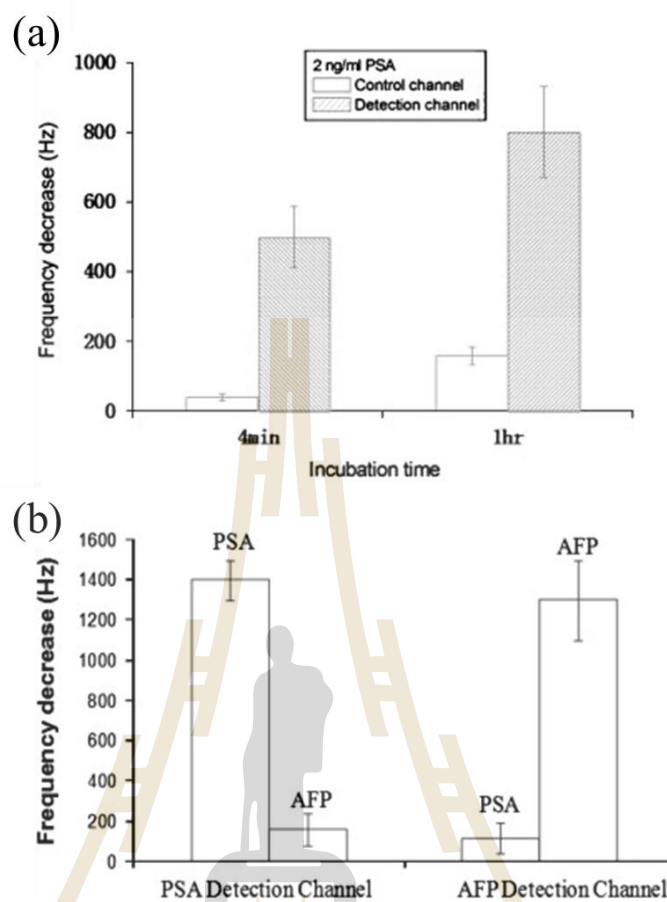


Figure 2.29 (a) The frequency change in the detection channel (with PSA antibody), and the control channel (without PSA antibody), where frequency increases with longer incubation times and (b) The relationship between frequency decrease and PSA antigen in the PSA detection channel, with no observed change for the AFP biomarker. (Su et al., 2013)

Catarina Lino et al (Catarina Lino et al., 2023) pioneered the creation of an innovative DNA-based biosensor utilizing QCM technology. This biosensor is designed for the early and dependable detection of non-small cell lung cancer (NSCLC)-associated mutations, notably EGFR deletions, as shown in Figure 2.30, in liquid biopsy specimens. It functions by binding NSCLC-specific probes with the DNA extracted from the samples, demonstrating high selectivity and considerable potential for clinical implementation. Its utility spans from aiding in treatment decisions to facilitating

patient monitoring, marking a significant advancement in the realm of liquid biopsy analysis for NSCLC diagnosis.

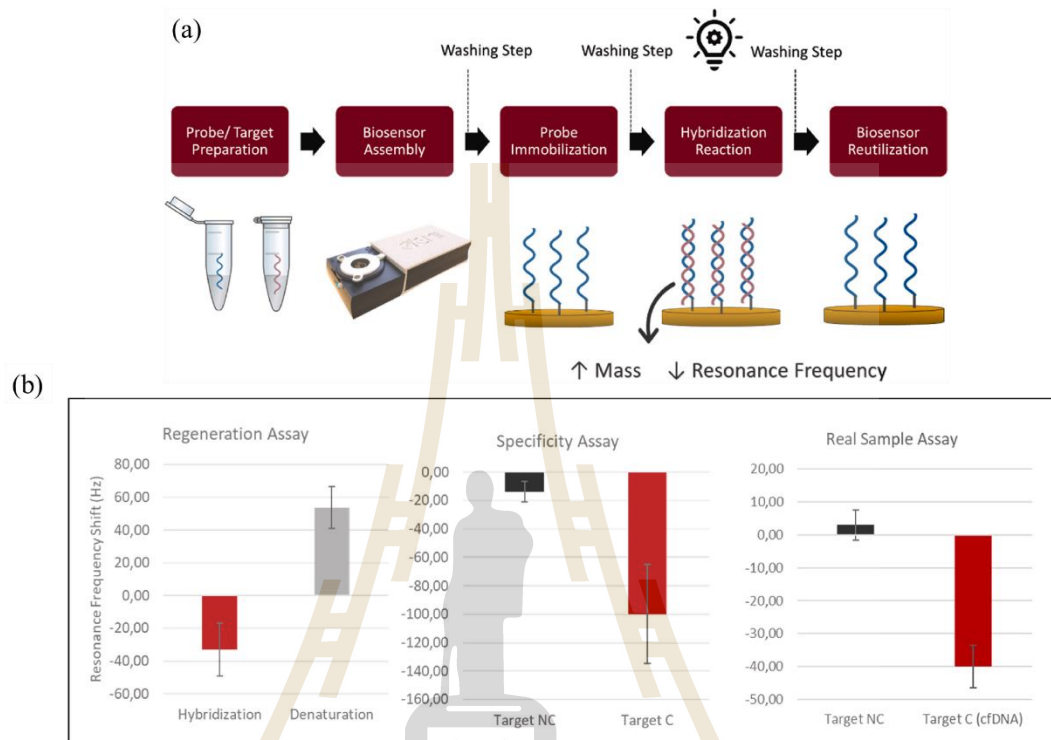


Figure 2.30 (a) The experimental setup for detecting hybridization EGFR reactions using a QCM system and (b) Resonance frequency shifts of denaturation (grey) and hybridization reactions with complementary target DNA (red) and Resonance frequency shifts from the detection of Non-complementary Target EGFR Del (NC) (black) and Complementary Target EGFR (C) (red), and Results from three replicates of the assay performed with real DNA samples. (Catarina Lino et al., 2023)

Hyeoun Ji Park et al. (Park & Lee, 2018) focused on utilizing QCM as a piezoelectric biosensor (Figure 2.31a) for blood analysis, specifically targeting the detection of hemoglobin A1c (HbA1c) (Figure 2.31b). The QCM measures mass variation per unit area by monitoring frequency changes of a QC resonator, enabling quantitative measurement of HbA1c (Figure 2.31c). Their findings indicated that the AuNP-based QCM biosensor can effectively detect HbA1c in whole blood samples. The

enlargement of AuNPs enhanced the sensitivity of the QCM sensor, achieving a remarkable detection limit of 0.147% for HbA1c with a coefficient of variation of less than 10%.

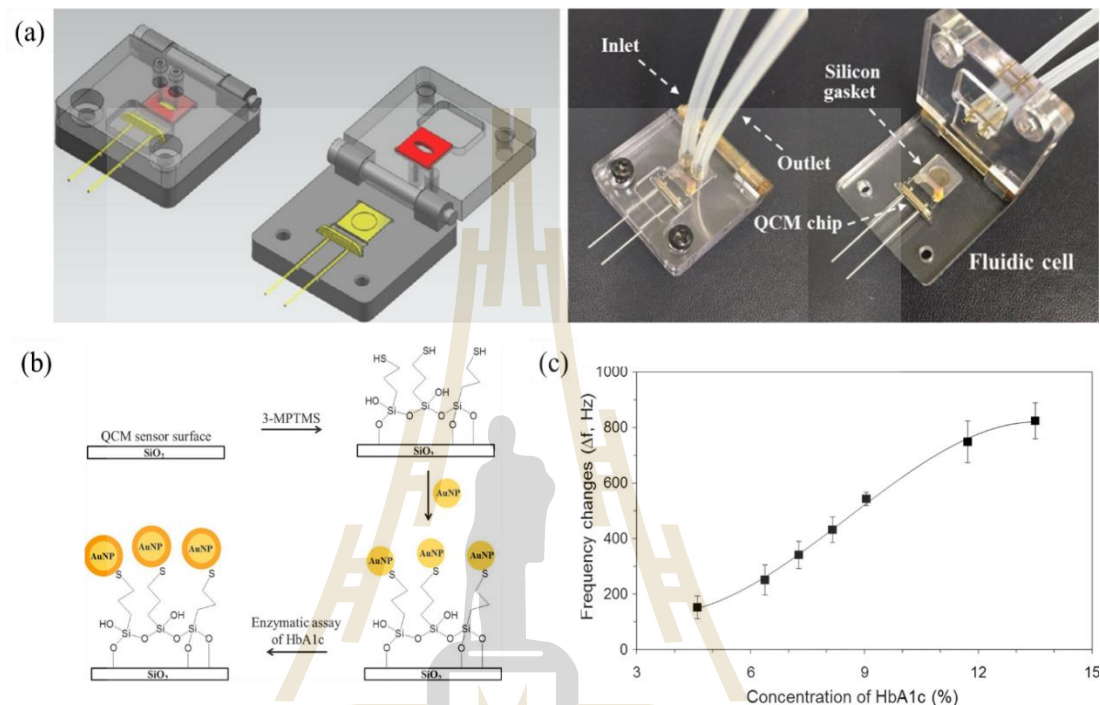


Figure 2.31 (a) Custom-made fluidic modules: schematic description (left) and practical photograph (right), (b) The procedure for HbA1c detection combined with the size enlargement of AuNPs on the QCM sensor surface, and (c) The changes in resonance frequency (Δf) versus concentrations of HbA1c with respect to hemoglobin. (Park & Lee, 2018)

Miroslav Pohanka et al. (Miroslav Pohanka, 2018) conducted a study on the determination of Tumor Necrosis Factor Alpha (TNF α) using a Piezoelectric biosensor (Figure 2.32a). They assessed the accuracy of TNF α detection by comparing the piezoelectric biosensor with Enzyme-Linked Immuno-Sorbent Assay (ELISA) through statistical analysis. The constructed QCM Immunosensors were tested with standard solutions of human TNF α (Figure 2.32b). The LOD was calculated to be 1.62 pg/ml and 3.23 pg/ml, with coefficient of determination (r^2) values of 0.977 and 0.991,

respectively. Furthermore, the immunosensor was subjected to a 90-day test (Figure 2.32c), displaying minimal changes in frequency over time, indicating its stability and long-term reliability.

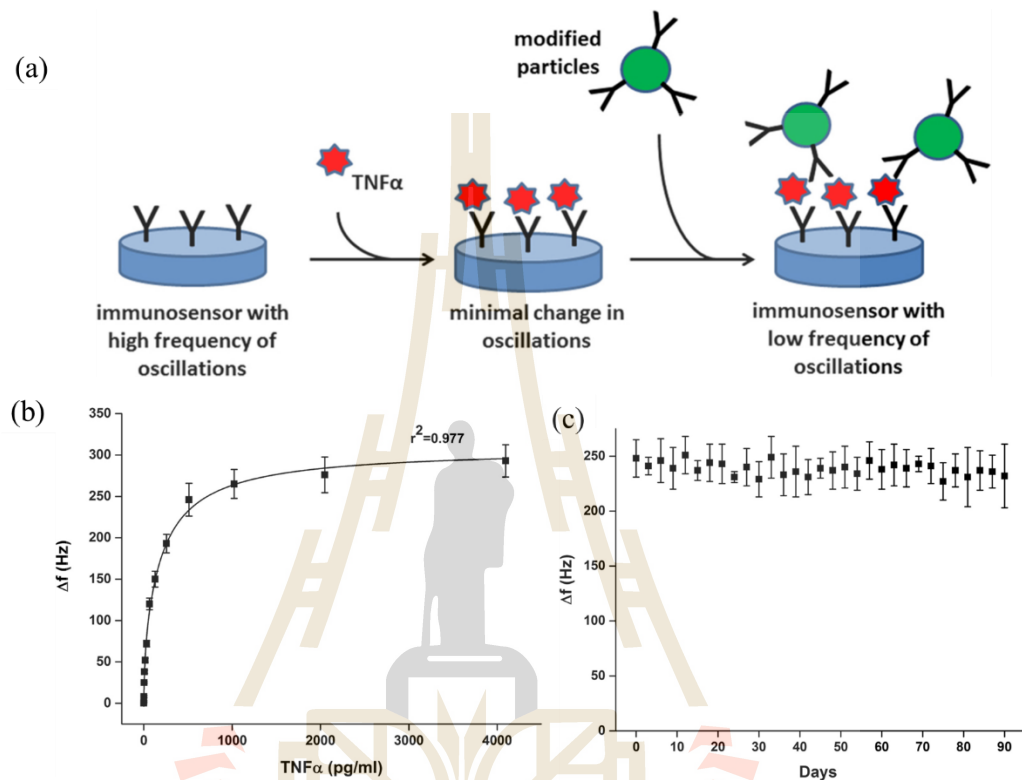


Figure 2.32 (a) The principle of the constructed immunosensor-based assay, (b) Detection of human TNF α using a QCM-based piezoelectric immunosensor, and (c) The long-term stability of the immunosensors. (Miroslav Pohanka, 2018)

Hao-Chun Chiang et al. (2019) developed AuNPs of varying sizes using the electrochemical deposition technique to enhance biosensor sensitivity. They examined the relationship between different AuNP sizes and their interaction with various components such as aqueous electrolyte solution, Bovine Serum Albumin (BSA), Glucose Oxidase (GOx), and casein protein. Their study involved AuNps produced with different electrodeposition parameters. The results suggested that the sizes of the proteins Casein > GOx > BSA could influence the absorption of AuNp, with BSA

exhibiting easier absorption on smaller AuNps, as seen in Figure 2.33 (Chiang, Wang, Zhang, & Levon, 2019).

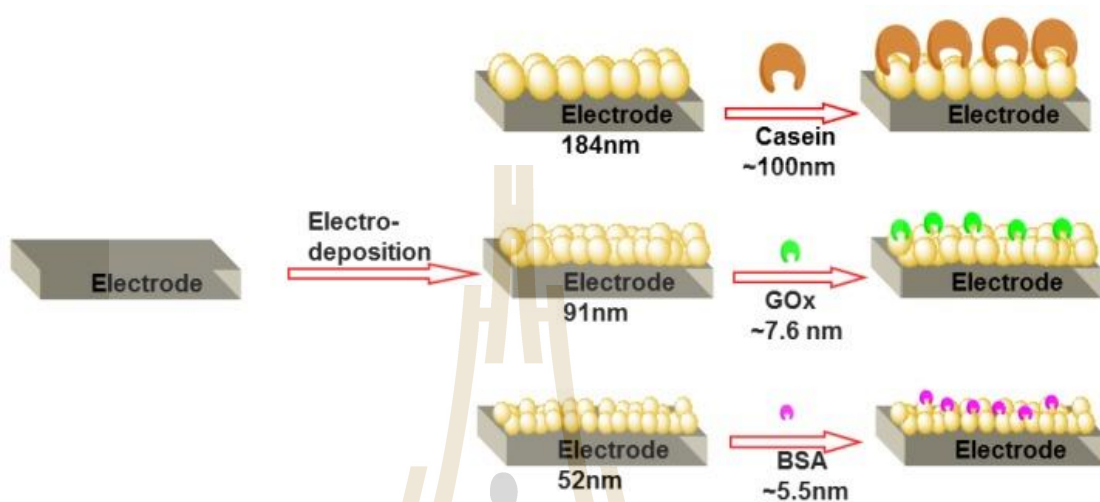


Figure 2.33 The adsorption of proteins onto surfaces featuring varying sizes of AuNPs. (Chiang et al., 2019)

Naoto Asai et al. (Asai, Terasawa, Shimizu, Shingubara, & Ito, 2018) utilized electroplating to generate an Au dendrite nanostructure, maximizing the surface area on a QCM. Their investigation revealed that the frequency shift induced by the antigen–antibody interaction on the Au-modified surface of the QCM was significantly larger than that observed on a flat QCM surface, with a shift of 110 Hz. This result occurred during CV when a potential of -0.95 V was applied for 2 minutes, as depicted in Figure 2.34.

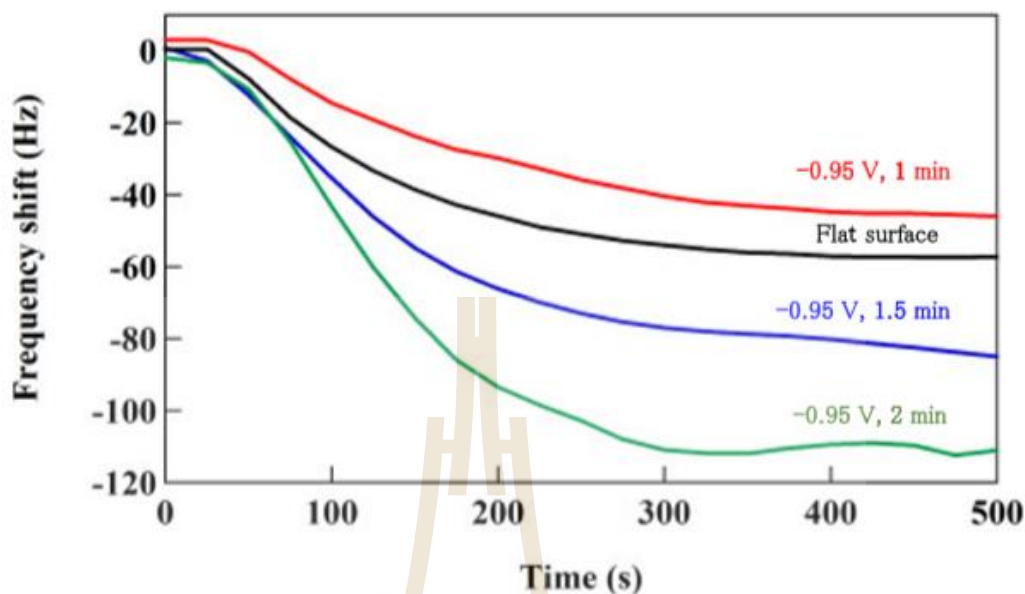


Figure 2.34 The frequency shift of both the dendrite structured and flat surface QCM chips during the detection of the antigen-antibody interaction. (Asai et al., 2018)

2.9 Chapter Summary

In conclusion, this chapter presents the comprehensive overview of the current trends and challenges in cancer detection, with a particular focus on the significance of cancer biomarkers such as PSA. It presents the principles and applications of biosensors, especially acoustic wave biosensors, highlighting their various types and underlying mechanisms, including piezoelectric phenomena. The discussion extends to specific devices like QCM and SAW, elucidating their working principles and characterization methods. Moreover, it explores the integration of nanotechnology with acoustic biosensors for biosensing applications. This chapter underscores the importance of acoustic biosensors in advancing cancer detection and offers valuable insights for further research and development in this field. The experimental results obtained by QCM and SAW will be presented in the next chapters.

2.10 References

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CHAPTER III

THE EFFECT OF SURFACE MODIFIED QUARTZ CRYSTAL MICROBALNCE ON DETECTION SENSITIVITY FOR PROSTATE SPECIFIC ANTIGEN

3.1 Introduction

Over the past decade, significant advancements have been made in cancer detection through biosensors, as detailed in Chapter 2. Various types of biosensors are capable of detecting cancer. The exceptional properties of the QCM piezoelectric sensor prompted its selection as a biosensor for exploring the potential in PCa detection.

Three strategies for improving QCM sensitivity are identified: (1) reducing the QC thickness, (2) decreasing the active electrode area of quartz, and (3) nano structuring through surface modification methods. In recent research, multiple studies conducted by various researchers (N. Asai et al., 2019; Naoto Asai, Terasawa, Shimizu, Shingubara, & Ito, 2018; Chiang, Wang, Zhang, & Levon, 2019; Gagner, Lopez, Dordick, & Siegel, 2011; Guo et al., 2018) consistently highlight the positive impact of nano structuring on QCM electrodes. These investigations consistently demonstrate an enhancement in the sensitivity of QCM electrodes through the implementation of nano structuring. These collective findings underscore the significance of adopting nano structuring as a strategic approach for improving QCM electrode sensitivity.

Due to the outstanding properties of the QCM piezoelectric sensor, as discussed in Chapter 1, the QCM will be taken as a biosensor to explore the potential for the detection of PCa. PSA and its corresponding antibody are employed as established biomarkers for PCa. The impact of surface modification on the QCM electrode, including enhancements in surface area and roughness, is detailed in this chapter. To investigate the surface morphology, roughness, and crystal structure, various characterization techniques such as SEM, XRD, and AFM are utilized.

Subsequently, the sensitivity of the QCM is then compared with that of the pristine sample through the detection of the PSA biomarker.

This chapter is divided into several sections. Section 3.2 outlines the experimental procedures employed in this study. Section 3.3 provides the results and discussion of surface characterization, including the investigation of the performance of the surface-modified QCM through the detection of the PSA biomarker. This encompasses sections 3.3.1 to 3.3.3, which focus on the phase, microstructure, surface roughness and surface area, and section 3.3.4 delves into the resonance frequency shift observed during PSA detection on the developed QCM. Finally, section 3.4 concludes the chapter.

3.2 Experimental Procedure

3.2.1 Materials and reagents

The QCM kit and standard AT-cut QCs were purchased from Novaetech S.r.l. (Italy) to conduct the proposed experiment. The QCs specifications are 10 MHz resonance frequency, 13.9 mm disc (QC) diameter, and 6 mm electrode diameter. Before the electroplating, QCs were cleaned by using piranha solution (70% of H_2SO_4 , 30% of H_2O_2 , and DI-water in 3:1:1 ratio) for 5 minutes, followed by deionized (DI) water several times and finally dried with nitrogen (N_2) gas blow. The chemical solutions: Sulfuric acid (H_2SO_4 , Anapure, New Zealand), Hydrogen peroxide (H_2O_2 -30%, Qrec, Nong Khaem 10160, Bangkok), were purchased from the referenced companies. In order to modify the surface area (by electroplating method), a Gold (Au) electrolyte (IntelliChem, Bangkok, Thailand) was used.

3.2.2 Direct-Current Electrodeposition

During the electroplating, both QC (as a cathode) and platinum grating mesh (as an anode) were immersed in the electrolyte solution for 30 and 60 minutes and applied a 20 mA/cm^2 current density. The sketch diagram of the whole electroplating process is shown in Figure 3.1. Electroplating of QCs performed at Micromachining Laboratory, Synchrotron Light Research Institute (SLRI), Thailand.

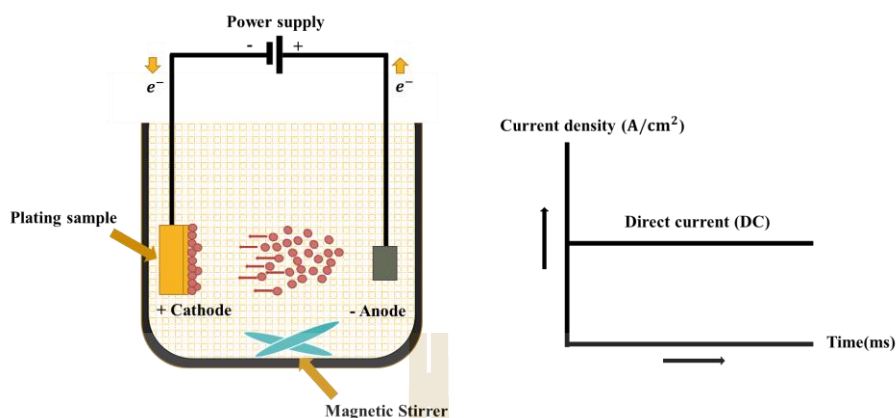


Figure 3.1 Schematic representation of the electroplating method. (Sonklin, Munthala, Suksaweang, Janphuang, & Pojprapai, 2022)

3.2.3 Antigen-antibody coated nanostructure-modified QCM

Soon after the electroplating, a linker group solution (10 μl of protein-A with 1 mg/ml concentration) was added to the electrode surface and incubated for 24 hrs. After Protein-A incubation, 2 μl of PSA antibody (0.5 mg/ml) was added to the QCs and then incubated for 1 hr. A blocker solution (20 μl of 3% BSA) was added to the QCs to block the non-specific bindings and incubated for 2 hr. Finally, 2 mg mL⁻¹ of PSA (in 1X diluted PBS) was added to the QCs and incubated for 1 hr at ambient temperature to allow antibody-antigen interaction. Polyclonal PSA (Saint Louis, MO 63103, USA), protein A (Saint Louis, MO 63103, USA), PSA antibody (Assaypro, St. Charles, MO, US), Bovine Serum Albumin (Billerica Ma, USA), and Phosphate-buffered saline (Calbiochem, La Jolla, CA) were purchased from Sigma Aldrich. After every incubation process, QCs were washed in DI water carefully to remove the excess biological parts. The electroplating process, mobilization, and immobilization of antibody-antigens are represented in a schematic diagram as shown in Figure 3.2. Figure 3.2(a) describes a pristine QC with an unmodified electrode surface; Figure 3.2(b) presents the surface modified QC by the electroplating method; Figure 3.2(c) illustrates the mobilization of the PSA antibody onto the modified electrode surface; and Figure 3.2(d) shows the immobilization of the PSA.

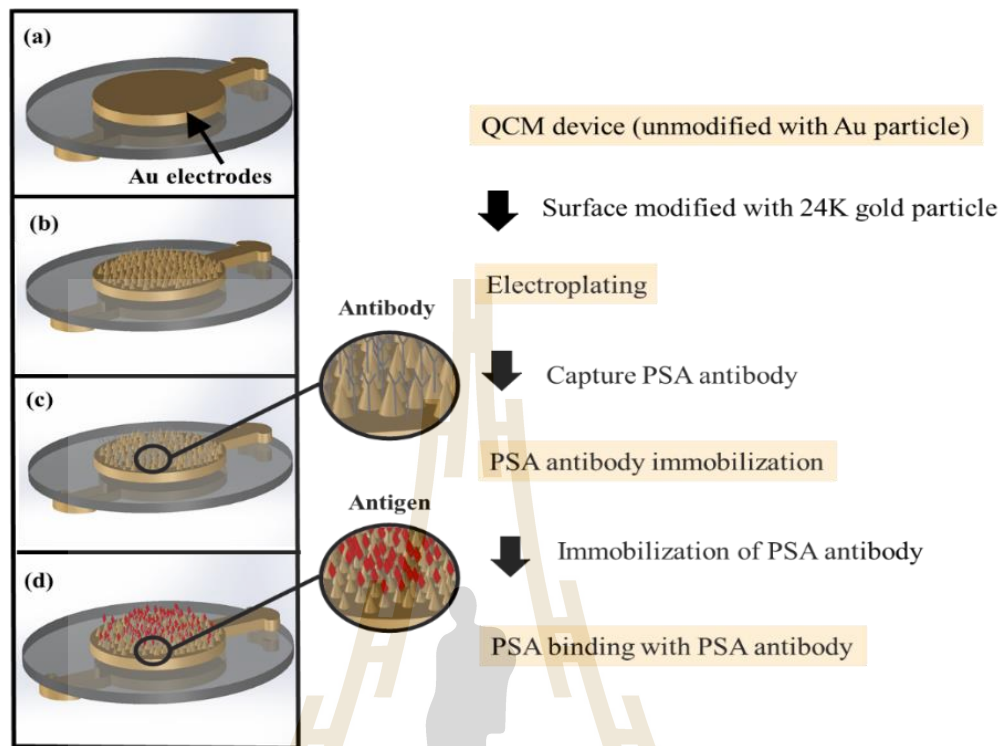


Figure 3.2 The surface modification and PSA antibody-PSA interaction on QCs: (a) Pristine QCs device, (b) Deposition of gold through electroplating to develop gold on QCs electrode surface, (c) PSA antibody immobilization, and finally (d) PSA binding with PSA antibody. (Sonklin et al., 2022)

3.2.4 Characterizations

SEM (JEOL JSM-6010LV), AFM (Park Systems AFM XE-120), and XRD (Bruker D8 ADVANCE) techniques were employed to characterize the modified and unmodified QCs. The resonance frequency of both modified, unmodified QC was recorded using the OpenQCM analyzer and analyzed using openQCM software.

3.3 Results and Discussion

3.3.1 SEM Results

The surface morphology, the surface roughness of unmodified and modified QCs was characterized using SEM and AFM techniques, as shown in Figure 3.3. Unmodified QC electrode surface showed more uniform nanostructure, smoother,

relatively smaller grain sizes than that of surface-modified, as shown in Figure 3.3(a-c). During the electroplating, the Au ions in the electrolyte get deposited on the QC electrode surface, and consequently, the nanostructure formed on the surface. With increasing electroplating time from 30 to 60 minutes, the nanostructure formation increases.

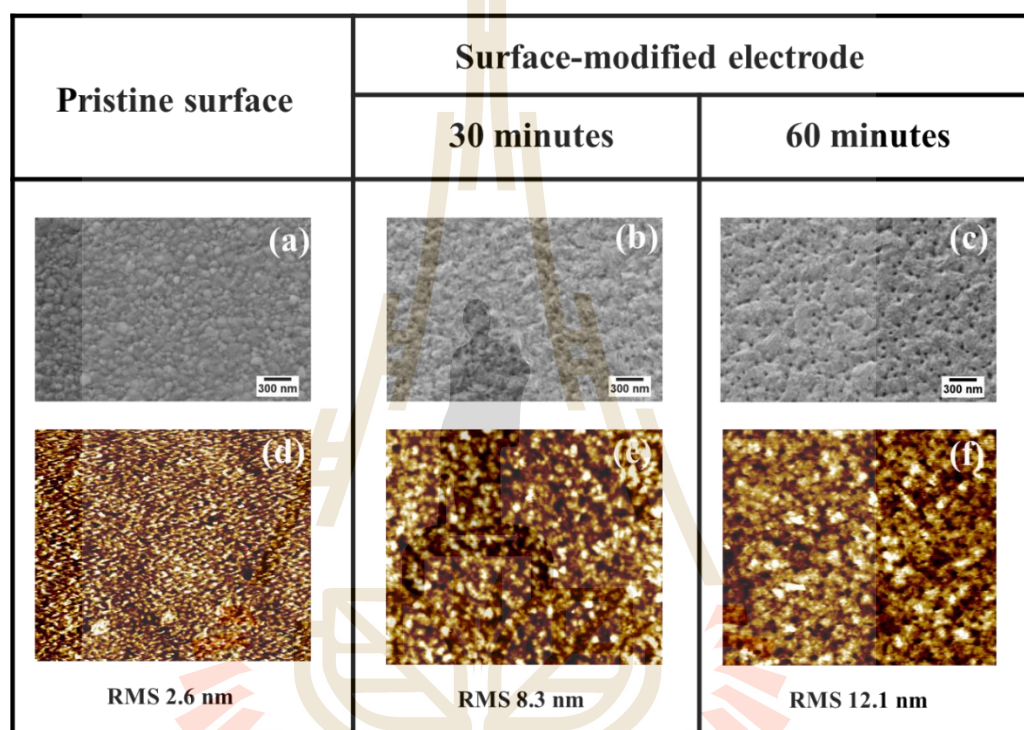


Figure 3.3 Surface morphology of electroplated QCs (SEM and AFM images): (a and d) Pristine QCs, (b and e) 30-minute electroplated QCs, and (c and f) 60-minute electroplated QCs. (Sonklin et al., 2022)

3.3.2 AFM Results

The measured RMS values of unmodified QC, 30-minute electroplated QC, and 60-minute electroplated QC are 2.6, 8.3, and 12.1 nm, respectively. It is seen that the roughness increased with electroplating time, as shown in Figure 3.3d to 3.3f. The surface area of nanostructured QCs was calculated. The estimated surface area values of unmodified QC, 30- and 60-minute electroplated QCs are 25.07, 25.58, and

26.53 nm², respectively, with respect to the increase of electroplating time. The measured RMS roughness and surface area values are also depicted in Table 3.1. The dimensions of the IgG antibody, as determined by cryo-AFM, are roughly 12 nm in length, 12 nm in width, and 3 nm in height (Yeow, Tabor, & Garnier, 2017). Its width closely resembles the nanostructure observed in the 60-minute electroplated sample, as indicated by the RMS value.

Table 3.1 The summary of RMS and surface area values estimated from the AFM topographical images. (Sonklin et al., 2022)

Sample and condition	RMS (nm)	Surface area (nm ²)
Pristine	2.6	25.1
30-minute	8.3	25.6
60-minute	12.1	26.5

3.3.3 XRD Results

The XRD spectra of unmodified and modified QCs electrode surfaces are shown in Figure 3.4. The XRD scan range was from 35° to 85° of 2 θ , and the resolution was 0.02°. The diffraction pattern peaks at 38.2° and 81.8° of 2 θ correspond to the (111) and (222) crystal planes. These XRD diffraction patterns are matched with JCPDS file 00-004-0784 for Gold (Au). The peak at 54.9° corresponds to (222) plan and is assigned to the hexagonal phase of SiO₂ (Quartz) and is well-matched with JCPDS file 00-02-0458. Due to electroplating, a significant enhancement in the (111), (200), (220), (311), and (222) diffraction peak which belongs to Au and the intensity of peaks increased with electroplating time. The preferential growth (peak at 38.2° of (111) plane) was observed after the electroplating process.

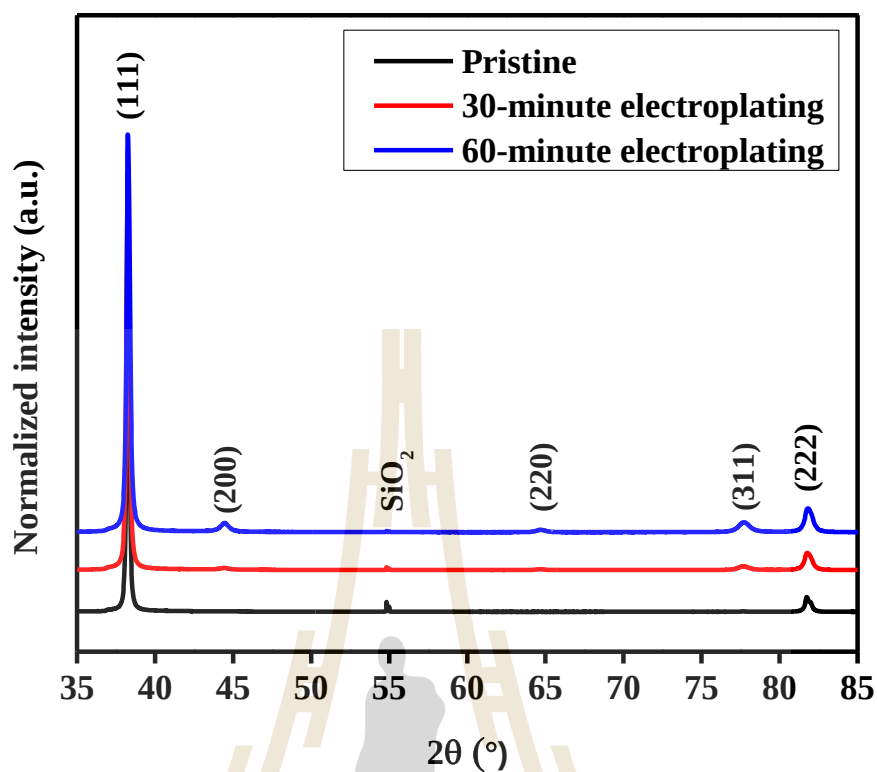


Figure 3.4 XRD patterns of pristine and electroplated QCs. (Sonklin et al., 2022)

3.3.4 Resonance Frequency Shift of PSA Detection

The resonance frequencies, corresponding shifts of 30- and 60-minute electroplated QCs are mentioned in Figure 3.5 and shown in Table 3.2. Owing to the increase of the electroplating time (30 and 60 minutes), the resonance frequency shift increased, and it is in agreement with the Sauerbrey equation. A -255 kHz of frequency shift was observed for the 30-minute electroplated QC, and -592 kHz of frequency shift was observed for 60-minute electroplated QC, compared to the resonance frequency of the pristine QC.

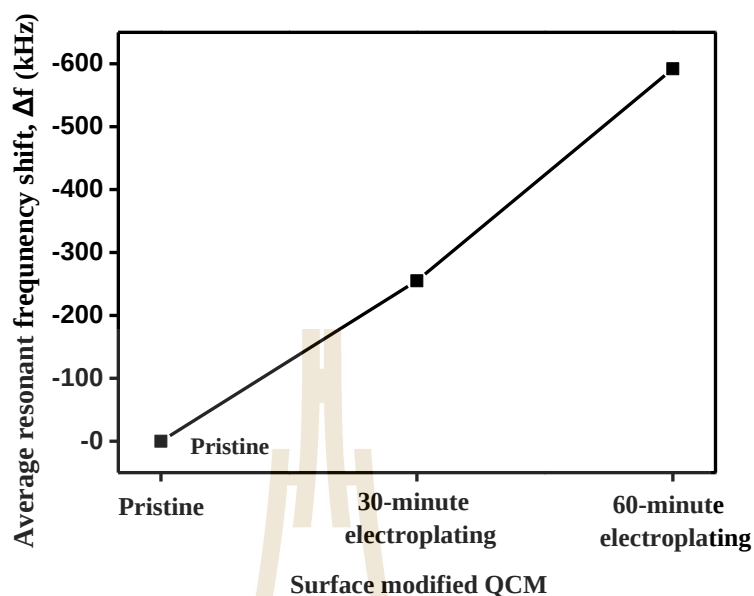


Figure 3.5 Resonance frequency shifts of electroplated QCs at different electroplating durations. (Sonklin et al., 2022)

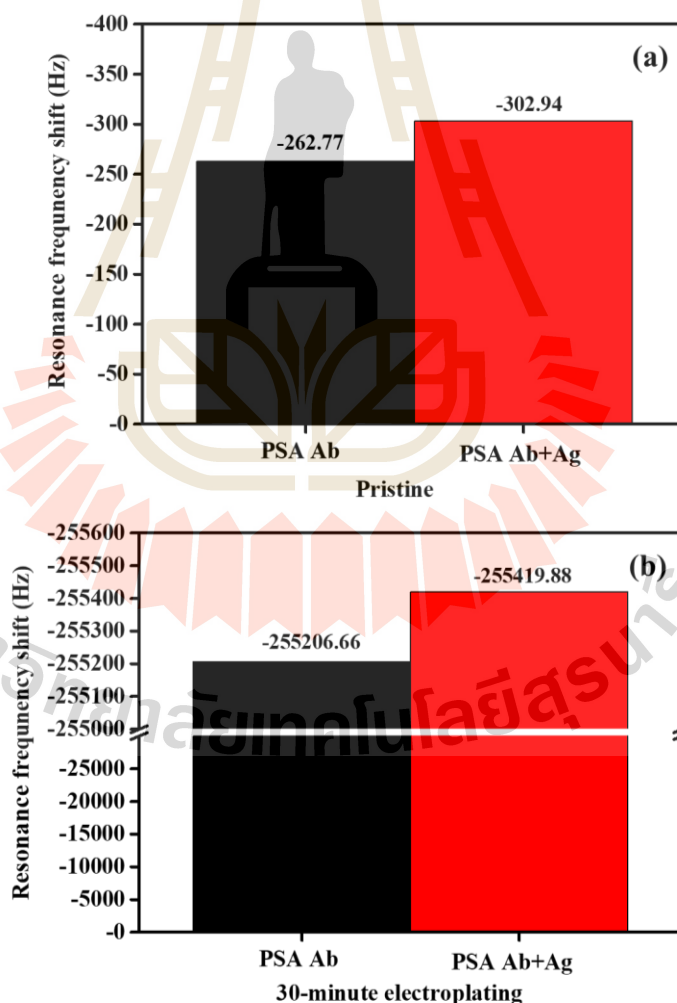
Table 3.2 The resonance frequency shifts at different stages of the experiment. (Sonklin et al., 2022)

Sample and condition	1)frequency shift after surface modification (kHz)*	2)Resonance frequency after adding PSA Antibody (Hz)	3)Resonance frequency after adding PSA (Hz)	4)frequency shift due to Antigen-Antibody interaction (Hz)**
Pristine	0	10008940.1	10008901.0	-39
30-minute	-255	9760151.3	9759937.5	-213
60-minute	-592	9515700.4	9515217.3	-483

*The resonance frequency of the surface modified samples compared to that of pristine. ** The resonance frequency of 3) minus 2).

Further, PSA antibody was added to the protein-A incubated pristine QCs and surface modified QCs (of both 30-and 60-minute) and incubated for 1 hr. The corresponding frequencies are -262 Hz, -255206 and -491415 Hz with respect to the

pristine resonance frequency. Then, 2.5 mg/ml of PSA has added to the antibody incubated QCs and incubated for 1 hr. The corresponding frequencies are -302, -255419 and -491897 Hz with respect to the pristine resonance frequency. All the corresponding frequency shifts are shown in Figure 3.6. The estimated frequency shifts from antibody to antigens are 39 Hz (in case of pristine QCs), 213 Hz (in case of 30-minute QCs), 483 Hz (in case of 60-minute QCs), and the same values are mentioned in Figure 3.7. These obtained results suggest that the sensitivity of 30- and 60-minute modified QCs is 3 times and 5 times greater than the pristine QCs. The large surface area with the nanostructured surface might be facilitated the high binding ability between antibody-antibody.



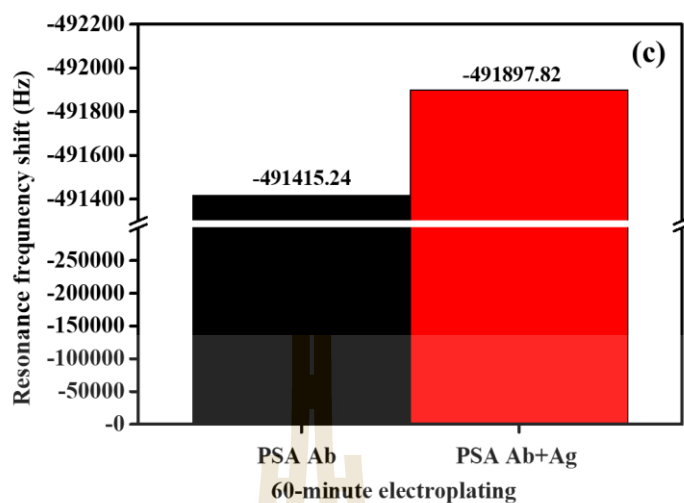


Figure 3.6 The resonance frequency shifts with respect to PSA antibody and PSA. (Sonklin et al., 2022)

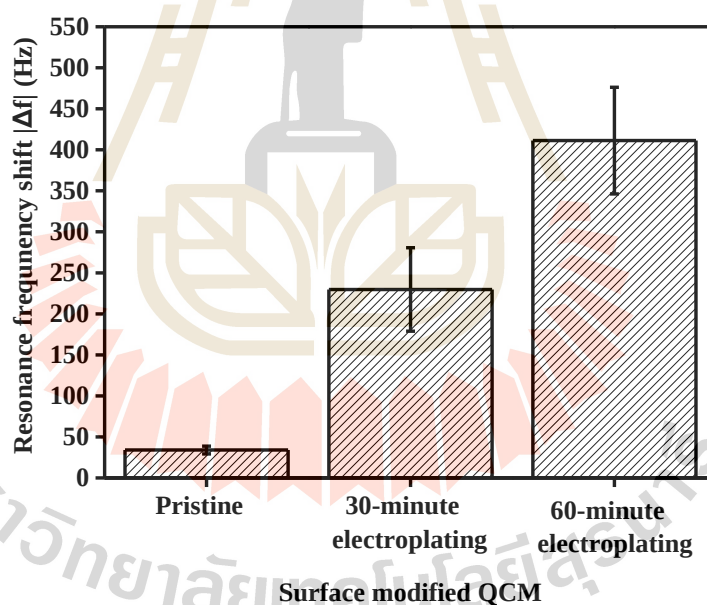


Figure 3.7 The average of resonance frequencies shifts caused by Antigen and antibody binding on the pristine, 30-minute electroplated, and 60-minute electroplated. The test was all at a concentration of 2 mg/ml of PSA. (Sonklin et al., 2022)

3.4 Chapter Summary

In this chapter, the surface-modified QCs were successfully prepared by using the electroplating method. The surface roughness and the surface area values are increased with the electroplating time. Characterization techniques, SEM, AFM, and XRD, were utilized to confirm the surface morphology, roughness, and crystal structure. The sensitivity of QC of 30-, 60-minute QCs is 3 and 5 times that of pristine QCs. These positive results suggest that the surface modification of QCM electrode can be a promising candidate for PSA biomarker detection.

3.5 References

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CHAPTER IV

GOLD NANOSPIKES-MODIFIED QUARTZ CRYSTAL MICROBALNCE BIOSENSOR FOR PROSTATE SPECIFIC ANTIGEN DETECTION

4.1 Introduction

The application of a direct current (DC) electroplating technique is employed to generate gold (Au) nanostructures on the active area of the QCM, serving as a signal amplification strategy. Anti-PSA is utilized as the recognition element for detecting PSA, as detailed in Chapter 3. Our previous research demonstrated the feasibility of employing Au nanostructures on a QCM as an alternative biosensing platform for PSA detection. However, there remains a necessity to explore PSA detection at different concentrations on a functionalized QCM electrode and improve the detection process. Additionally, certain specific issues require attention. The surface modification using Au microparticles in the electrolyte lacked significant distinctiveness from the pristine surface. Excessive Au deposition resulted in a notable frequency shift that exceeded the measurement range. Addressing these challenges is essential to enhance the overall effectiveness of this biosensing platform.

This chapter reports a new approach, PCD, to enhance a surface area for the detection of PSA biomarker by creating a distinctive structure with minimal nanoparticle mass loading on the QCM electrode surface. Furthermore, this research broadens to explore the detection of the PSA biomarker at varying concentrations. In contrast to our previous method, this study employs L-cysteine and EDC/NHS as linkers instead of protein A. This choice results in increased physical strength due to the adhesive properties of L-cysteine, forming covalent bonds, and the use of EDC/NHS, which enhances the efficiency of the cross-linking reaction.

The chapter is structured into several sections, covering the fabrication of gold nanostructures on the QCM electrode (Section 4.2.2), characterization of these nanostructures on the QCM electrode surface (Section 4.2.3), and the electrode surface treatments and immobilization steps of PSA, as well as a procedure of QCM

measurements (Sections 4.2.4 and 4.2.5). The investigation on the impact of various parameters related to the chemical treatment on the QCM electrode surface through wettability characterization and the identification of functional groups to confirm the surface's suitability for biomolecule immobilization, is presented in Section 4.3 of the results and discussion. This section further includes a thorough examination of the resonance frequency response after surface modification on the QCM electrode with different % duty cycles, XRD spectra of Au-pulsed QCM with different duty cycles, surface characterization of the Au-QCM electrodes, electroactive surface area, XPS analysis, FT-IR bands detecting various chemical treatments on the QCM electrode surface, and the wettability of the various chemical treatments on the QCM electrode. The performance of the QCM biosensor for PSA detection is also discussed in this section. Finally, Section 4.4 is the chapter summary.

4.2 Experimental procedure

4.2.1 Materials and reagents

All chemicals used in this work were of analytical grade and were used as received, without further purification. A QCMs with a 13.9 mm diameter and a 160 μm thickness, operating at 10 MHz, were obtained from Novaetech (Pompei, Italy). The crystal's electrodes have a 200 nm thick gold (Au) layer deposited on a 10 nm titanium (Ti) adhesive layer. Piranha solution was made by combining a 70:30 v/v solution of 96% sulfuric acid (H_2SO_4 , Anapure, New Zealand) and 30% hydrogen peroxide (H_2O_2 , Qrec, New Zealand). 99.9% ethanol was purchased from Merck (Darmstadt, Germany). Human PSA antibody was purchased from Assaypro (St. Charles, MO, US) and PSA antigen was purchased from Sigma Aldrich (Saint Louis, US). Phosphate-buffered saline (PBS, pH 6.4) was obtained from Calbiochem (La Jolla, CA, US). A surfactant, Tween 20, was from Chem-Supply (Gillman, SA, Australia) and Au electrolyte was supplied by IntelliChem (Bangkok, Thailand). 1-Ethyl-3-(3-dimethylamino propyl) carbodiimide (EDC), N-Hydroxy succinimide (NHS), and L-cysteine were provided by NPChemicals (Bangkok, Thailand). Potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$] and potassium ferrocyanide [$\text{K}_4\text{Fe}(\text{CN})_6$] were obtained from Fisher Scientific (Gujarat, India). In all experiments,

autoclaved deionized (DI) water was used. The QCMs were electrodeposited in the Micromachining Laboratory of the Synchrotron Light Research Institute (SLRI), Thailand.

4.2.2 Fabrication of gold nanostructures on QCM electrode

PCD was utilized for the formation of a nanostructured thin film on the QCM electrode surface. The experimental setup is depicted in Figure 4.1(a). Au nanostructures were prepared by immersing a pristine QCM in an Au electrolyte solution. The QCM functioned as a cathode and a platinum grating mesh functioned as an anode. A magnetic stirrer was utilized, rotating a stir bar to facilitate the dispersion of the Au electrolyte solution. An electrical current was applied between the electrodes, resulting movement of electrons from anode to cathode. The Au ions are formed on the QCM electrode. PCD involves three variable parameters: pulse ON-time (t_{on}), pulse OFF-time (t_{off}), and peak current (I_p). The ON-time corresponds to when a positive current is applied, and the OFF-time occurs when no current is applied, with both contributing to the total time period with an average current (I_a) (Nassar, Meissner, Wadhwa, Korvink, & Mager, 2019). The t_{on} and t_{off} are described in terms of a duty cycle that can be expressed as follows (Chandrasekar & Pushpavanam, 2008):

$$\text{Duty cycle (\%)} = \left[\frac{t_{on}}{t_{on} + t_{off}} \right] \times 100 \quad (4.1)$$

The diffusion layer, grain size, and nucleation rate can be controlled by modulating the duty cycle. During the t_{on} , a negatively charged layer is formed around the cathode, which is discharged during the t_{off} . The periodic ON and OFF times not only provide an easier path for ion transfer to the cathode but also allow for relaxation and rearrangement of the deposited atoms, resulting in a more uniform ion distribution on the surface of the cathode. As a result, the deposition uniformity is improved, and the porosity of the deposited surface is reduced (Sreekanth, Bulusu, Suhash, & Shrikant, 2017). In this study, the electrical current was maintained at 0.5 mA for 30 minutes with a duty cycle of 20% and 80%.

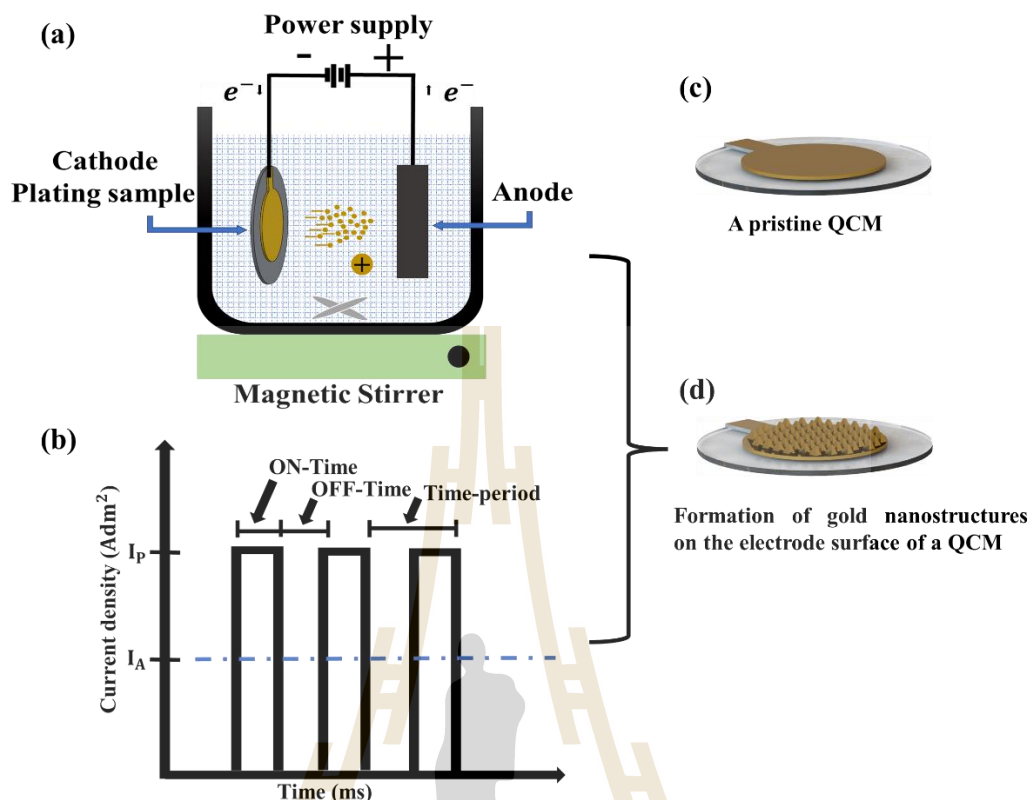


Figure 4.1 (a) working principle of a pulse current deposition technique used to deposit AuNS on QCM electrode surfaces, (b) a typical pulse current waveform, (c) the as-received QCM, and (d) the nanostructure-modified QCM electrode surface. (Sonklin et al., 2024)

4.2.3 Characterization of gold nanostructures on QCM electrode surface

The morphology of Au structure coated QCM electrode was observed by field emission scanning electron microscopy (FESEM, JEOL JSM-6010LV) at an acceleration voltage of 20 kV and a working distance of 5.5 mm revealed the structures at magnifications of 30,000x and 100,000x. The topographical image was obtained using atomic force microscopy (AFM, Parker, USA) in C-AFM mode with a scan rate of 0.3 Hz over the area of $3 \times 3 \mu\text{m}^2$. X-ray diffraction (XRD) patterns were obtained utilizing a Bruker D8 Advance X-Ray diffractometer with Cu-K α 1 radiation ($\lambda = 0.15406 \text{ nm}$), operating at 40 kV and 40 mA. Scans were conducted from 35° to 80° in 0.02° increments to investigate the crystal structure and analyze crystallite size. Cyclic

voltammetry measurements were conducted using an electrochemical analyzer (CHI660D, CH Instruments Inc., United States) with a conventional three-electrode system (Ag/AgCl reference electrode, platinum wire counter electrode, and QCM as the working electrode) to investigate an effective surface area. X-ray photoelectron spectroscopy (XPS) was performed using ESCALAB250Xi X-ray photoelectron spectrometer (Thermo Scientific, UK) with a monochromated Al K alpha excitation source operating at 120 W (13.8 kV x 8.7 mA), maintaining a base pressure better than 2×10^{-9} mbar.

4.2.4 Electrode surface treatments and immobilization steps of PSA

Gold nanostructure-coated QCM electrodes were cleaned with piranha solution via a shaker at 20 revolutions Per Minute (rpm) for 30 minutes. Subsequently, the QCMs were cleaned in DI-water, followed by ethanol and dried under ambient temperature. The cleaned QCMs were then placed in a custom-made moisture chamber to minimize the evaporation of the reagents on the QCM electrodes. L-cysteine was used as the linker due to its ability to form Gold-Sulfur (Au-S) covalent bonds. The electrodes were further incubated with 30 μ L of 1 M L-cysteine for 2 hours at room temperature. The electrodes were further activated using EDC/NHS which enhances the effectiveness of cross-linking reaction. To activate carboxyl group and increase the effective antibody immobilization, 50 μ L of 0.1 M EDC/NHS was added to the electrode surface for 2 hours. Then, 10 μ L of anti-PSA antibody at a concentration of 0.408 mg mL⁻¹ in PBS with a pH of 7.4, 50% Glycerol (v/v), and 0.02% (w/v) Sodium Azide (NaN₃) was added and incubated on the electrode overnight at 20 °C. To prevent non-specific binding, the surface was treated with 2.5% bovine serum albumin (BSA) in 1X PBS and 0.05% Tween for 1 hour. To investigate the response of the QCM signal, various concentrations of PSA (0.1, 1, 10, 100, and 1000 ng mL⁻¹) were utilized in this work. 30 μ L of PSA was bound with anti-PSA antibody for 1 hour at room temperature. All experimental steps were performed using the drop-casting method. During each incubation step, the QCM was extensively rinsed with autoclaved DI water to eliminate any unbound molecules from the electrode surface. Subsequently, the drying process was carried out at room temperature before recording the frequency response. The schematic representation of the immobilization step is provided in Figure 4.2.

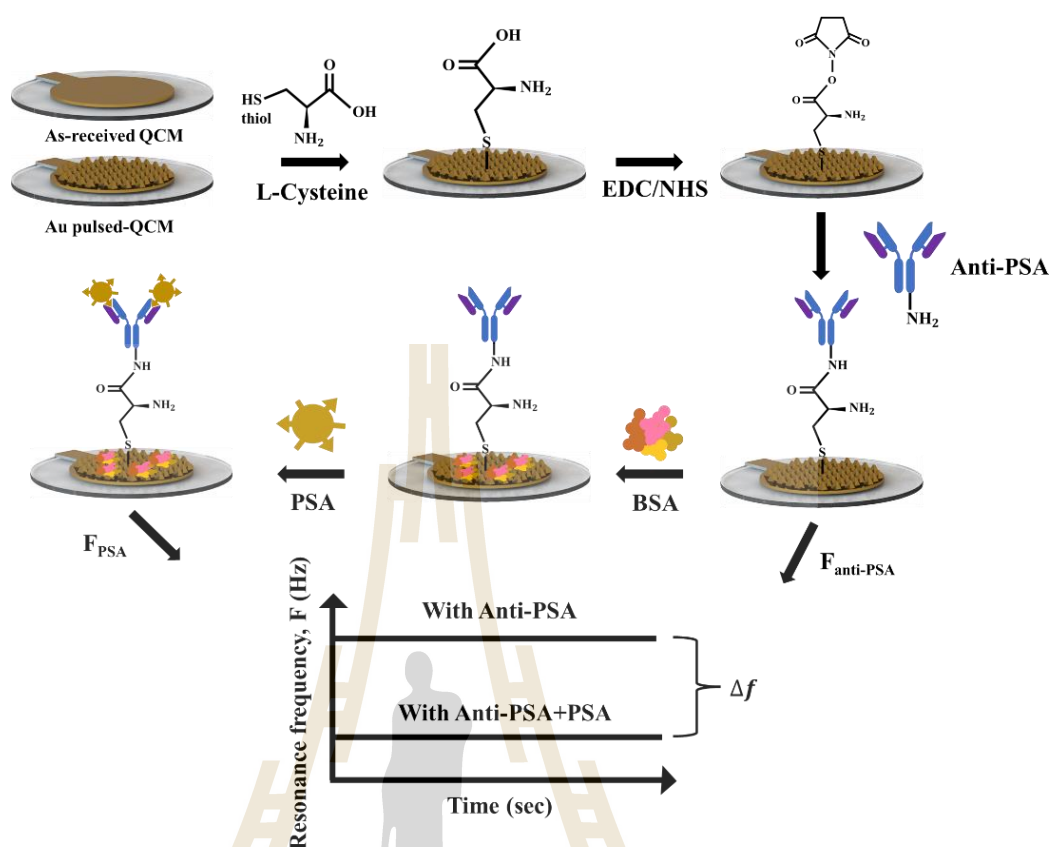


Figure 4.2 Schematic illustration of the QCM biosensor construction for the PSA detection procedure. (Sonklin et al., 2024)

4.2.5 QCM measurements

The frequency response of the QCM before and after immobilization was evaluated using a portable QCM instrument (Surazense, Thailand). The electronic module, designed and developed at Suranaree University of Technology, Thailand, incorporated an open-cover fluidic cell purchased from Novaetech (Pompei, Italy). Data acquisition was performed with the Surazense software. The measurements were conducted under dry conditions at room temperature to mitigate the changes in the viscosity of the assay media on the QCM. The frequency was recorded before and after immobilizing PSA on the Au nanostructure-coated QCM. For each measurement, the average QCM frequency was recorded over 3 minutes.

4.2.6 Characterization of chemical treatments on QCM electrode

The wettability resulting from the chemical treatment of the QCM surface were determined through contact angle (CA) measurements. These measurements were conducted using a custom-made water drop test station equipped with Dino-Lite digital microscopes (Netherlands). The contact angle of water on the QCM electrode surface with various chemical treatments was recorded using Dino Capture 2.0 software. The CA was measured by placing a 10 μ L water droplet from a syringe needle onto the various chemically treated QCM surfaces along the Y-axis. Fourier-Transform Infrared (FTIR) spectroscopy (Bruker, Tensor 27) was employed to analyze the QCM electrodes and identify the functional groups involved in the chemical treatment of the QCM surfaces.

4.3 Results and discussion

4.3.1 Resonance frequency response after surface modification on the QCM electrode with different %duty cycles

The presence of Au structures on the QCM electrode after electrodeposition was confirmed by measuring the frequency shift (Δf) before and after the deposition ($F_{\text{after deposition}} - F_{\text{before deposition}}$), as shown in Figure 4.3. The results demonstrated that the Δf value for the pulse-deposited Au with a 20% duty cycle was approximately $-38,560 \pm 836$ Hz, while for the 80% duty cycle, it was $-50,863 \pm 9,480$ Hz. It is indicated that the mass loading of the Au structures on QCM under the 80% was higher compared to the 20% duty cycle. This result is consistent with the theory of pulse electrodeposition, where a higher duty cycle, indicating a longer duration of the ON-time, typically leads to a greater mass deposition, as described by Eq. (2).

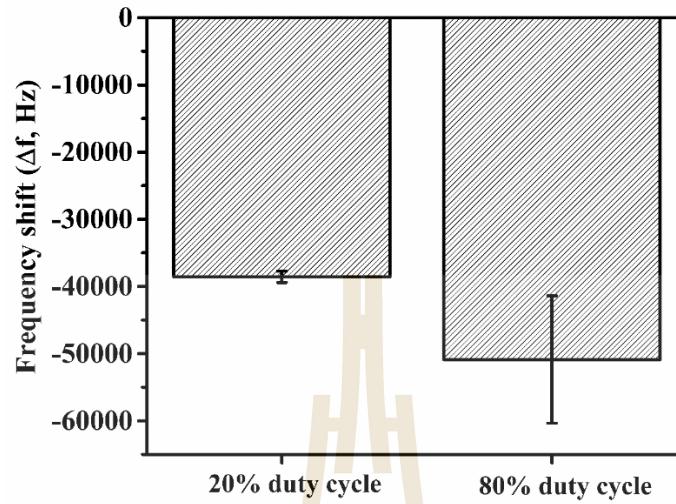


Figure 4.3 Resonance frequency shift resulting from the development of AuNS structures on the QCM electrode. (Sonklin et al., 2024)

4.3.2 XRD spectra of Au-pulsed QCM with different duty cycles

XRD spectra of the QCM electrode with/without the formation of Au structure via electrodeposition was obtained to investigate grain shape and growth direction. The XRD diffraction patterns are matched with JCPDS file 00-004-0784 for Gold (Au) and JCPDS file 01-088-2321 for Titanium (Ti). The XRD spectra have been assigned to the (111), (200), (220), and (311) peaks of Au/Ti, with the dominant peak corresponding to (111) as shown in Figure 4.4a. It is observed that the (111) intensity increases as the portion of duty cycle increases, indicating the Au/Ti of pulse-deposited Au on QCM electrode grew preferentially in the (111) orientation. The predominant peak spectra can be fitted using the Lorentz spectral function. The correlation coefficient (R^2) was greater than 0.99, and the (111) peak was deconvoluted into peaks near 38.27° and 38.36° , corresponding to Au (111) and Ti (111), as depicted in Figure 4.4b. The (111) peak intensity, crystallite size, and Full Width at Half Maximum (FWHM) of the Au (111) were investigated. According to our previous experiment (Sonklin, Munthala, Leuasongnoen, Janphuang, & Pojprapai, 2022), the Debye-Scherrer equation for calculating the crystallite size (D) is applied:

$$D = \frac{K\lambda}{\beta \cos \theta} \quad (4.2)$$

where K is the shape factor (taken as 0.94), λ is the incident X-ray wavelength ($\lambda=1.5406 \text{ \AA}$), β is the FWHM (in radians) of the (002), and θ is the diffraction angle.

Compared to the pristine QCM, the extracted (111) Au peak revealed a significant increase in intensities when applying 20% duty cycle and 80% duty cycle of Au deposition on the QCM electrode, as shown in Figure 4.4c. The FWHM decreases when the electrode was controlled at 20% duty cycle, followed by a gradual decline in FWHM with 80% duty cycle. The calculated crystallite size is inversely proportional to FWHM. The crystallite size is about 695, 807, 811 nm of pristine QCM, 20% duty cycle and 80% duty cycle Au modified-QCM, respectively, as depicted in Figure 4.4d. From this result, it can be observed that an increase in the percentage of duty cycle leads to the agglomeration of Au particles on the QCM electrode, resulting in the increase of frequency shift, the crystallite size and the X-ray intensities of Au, particularly in the (111) plane.

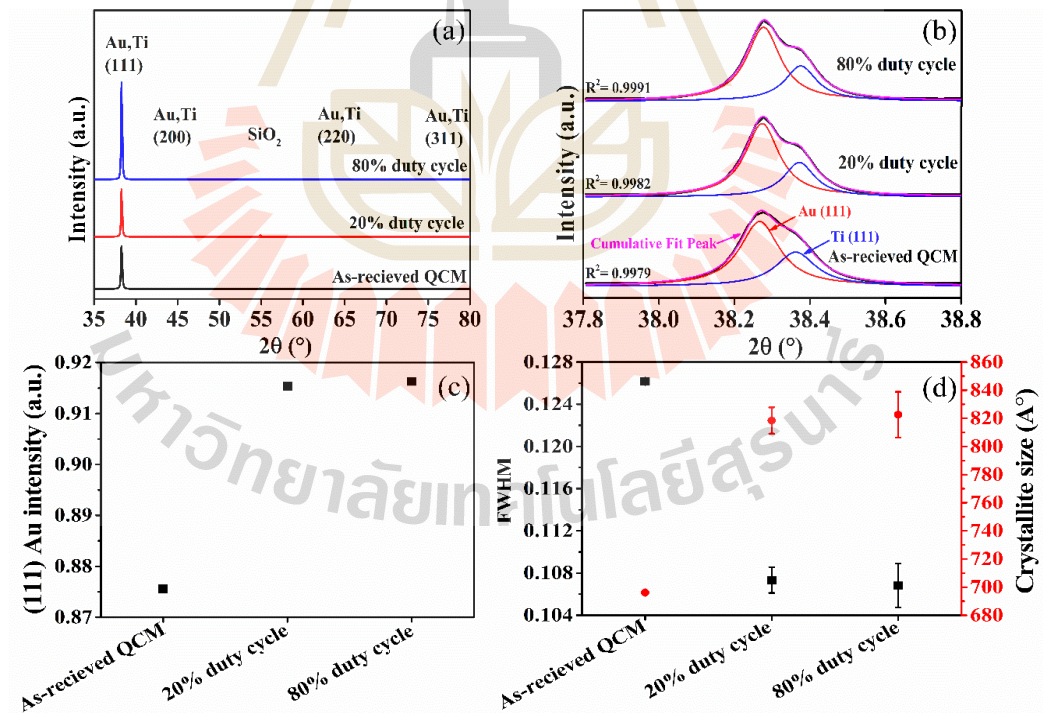


Figure 4.4 (a) XRD patterns, (b) XRD peak intensities, (c) fitting result of (111) Au peaks, (d) FWHM and crystallite size values of QCM pristine and different percentages of duty cycle. (Sonklin et al., 2024)

4.3.3 Surface characterization of the Au-QCM electrodes

FESEM micrographs were used to analyze the surface morphology. Figure 4.5a to 4.5c displays FESEM images of the as-received QCM, 20% duty cycle Au-modified QCM, and 80% duty cycle Au-modified QCM. As displayed in Figure 4.5a, the as-received QCM exhibited a relatively smooth and flattened surface. After inducing the electrodeposition, the formation of gold nanospikes (AuNS) was observed on the QCM electrode surface. As depicted in Figure 4.5b, the 20% duty cycle showed a well-formed nanospike structures with small grains. The 80% duty cycle demonstrated bigger nanospike structures of Au, as shown in Figure 4.5c. The results indicate that during the 80% duty cycle of deposition, a higher aggregation of Au nanoparticles occurs on the electrode. This suggests that the shape of the nanostructure is dependent on the adjusted percentage of the duty cycle. AFM measurements were carried out on the QCM's working electrode surface to explore the surface's 2D and 3D profile, as shown in Figure 4.5d to 4.5i. The analysis of 2D AFM images using XEI software yielded numerical data concerning roughness parameters and surface area, as detailed in Table 4.1. An average (R_a) and Root Mean Square (RMS) roughness values exhibited a concurrent increase with the elevation in the % duty cycle. The initial observation from the AFM image of the As-received QCM, as illustrated in Figure 4.5 (d,g), showed the distinctive grainy structure typical of gold. This surface exhibited a relatively flat profile with an RMS roughness measuring 3.1 nm and a surface area of $9.0951 \mu\text{m}^2$. Following the deposition of AuNS at 20% (Figure 4.5 (e,h)) and 80% duty cycle (Figure 4.5 (f,i)), a noticeable and significant transformation took place on the surface, resulting in observable alterations in grain shape. This alteration led to a surge in the RMS roughness values, reaching 9.5 nm and 10.5 nm, while the surface area increased to $9.5833 \mu\text{m}^2$ (5% increase compared to the pristine state) and $9.282 \mu\text{m}^2$ (2% increase compared to the pristine state), respectively.

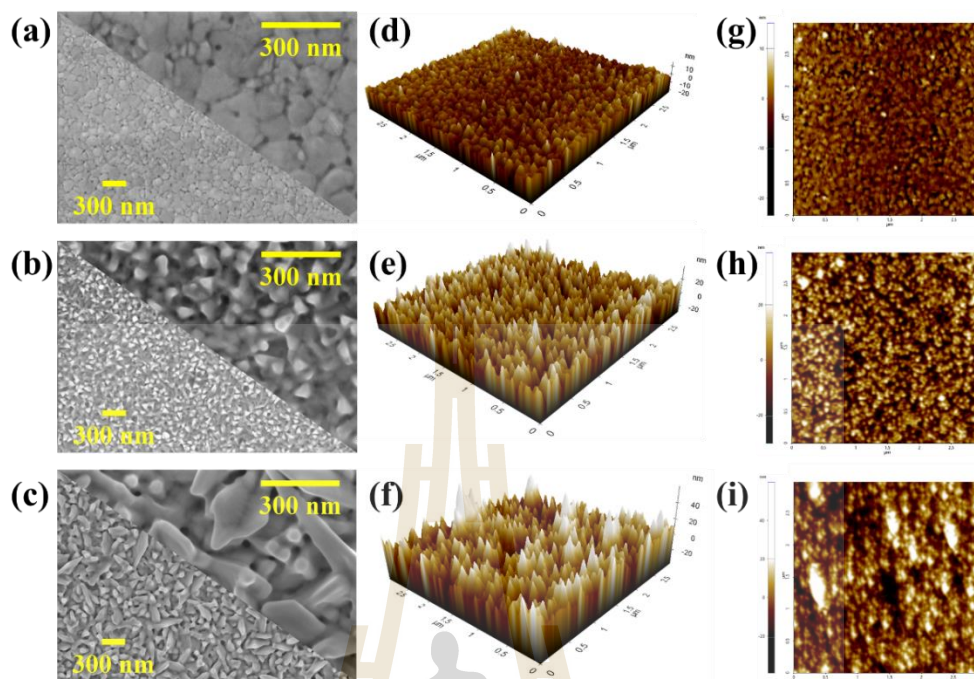


Figure 4.5 SEM and AFM images of the QCM working electrode surface: (a,d,g) As-received QCM, (b,c,h) AuNS-modified QCM with 20% duty cycle, and (c,f,i) AuNS-modified QCM with 80% duty cycle. (Sonklin et al., 2024)

Table 4.1 Surface area, roughness average, root-mean-square roughness values from AFM and effective surface area values from CV for the as-received, 20% duty cycle pulse-deposited Au, and 80% duty cycle pulse-deposited Au on the QCM. (Sonklin et al., 2024)

Sample	Effective surface area (cm ²) from CV	Surface area (μm ²) from AFM	Roughness average(nm)	Root-mean-squared roughness (nm)
As-received QCM	0.167	9.0951	3.1	4.2
AuNS-modified QCM (20 % duty cycle)	0.234	9.5833	7.7	9.5
AuNS-modified QCM (80 % duty cycle)	0.169	9.282	8.1	10.5

4.3.4 Electroactive surface area

The cyclic voltammetry (CV) experiments were performed to examine the impact of PCD technique on the enhancement of the QCM electrode's surface area. The CV was conducted in a three-electrode configuration, with a gold electrode as the working electrode, an Ag/AgCl electrode as the reference electrode, and a platinum wire as the counter electrode. The electrolyte chosen for this study was in PBS (0.1 M, pH 6.4), containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$. Figure 4.6a shows CV results of 10 mV s^{-1} for the as-received QCM and the AuNS-modified QCM electrodes. The result reveals an increase in peak current when comparing the pulse conditions to the as-received QCM electrode. This increase suggests a reduction in electron-transfer barriers attributed to the presence of nanoparticles. However, at 80% duty cycle, a decrease in voltammetric peak currents is observed, indicating lower conductivity compared to the 20% duty cycle. This decrease in peak current at 80% duty cycle can be attributed to the larger size of the AuNS formed during the QCM deposition process. The electroactive surface area (A) can be expressed by Randles-Sevcik equation (Bharti, Agnihotri, & Prabhakar, 2019):

$$A = \frac{I_{pa}}{2.69 \times 10^5 n^{3/2} C_0 D_r^{1/2} \nu^{1/2}} \quad (4.3)$$

where A is the effective surface area (cm^2), I_{pa} is anodic peak current (A), n is number of electrons participating in the redox reaction (taken as 1), C_0 is concentration of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (mol cm^{-3}), D_r is diffusion coefficient of $7.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, and ν is the scan rate (V s^{-1}). The CV was measured within a potential range of -0.1 to 0.5 V at different scan rates. The I_{pa} was found to be proportional to the square root of the sweep rate, and the slope was utilized to calculate the effective surface area, as shown in Figure 4.6b. Under 20% duty cycle, the highest I_{pa} was observed. According to Randles-Sevcik equation, the estimated effective surface areas are displayed in Table 4.1. The surface area of the as-received QCM and the AuNS-modified QCM electrodes with 20% and 80% duty cycle were calculated to be 0.167 cm^2 , 0.234 cm^2 , and 0.169 cm^2 , respectively. Interestingly, a 20% duty cycle results in

the highest surface area, whereas 80% duty cycle results in the lower surface area. Figure 4.7 presents a schematic diagram depicting a random Au particle formed around the QCM electrode during pulse current deposition with 20 % and 80% duty cycle. A low duty cycle results in a short On-time, limiting the movement of Au ions and leading to less Au deposition on the electrode surface, as illustrated in Figure 4.7a. On the other hand, a higher duty cycle with a longer On-time promotes higher ion mobility, causing Au particles to merge and aggregate on the electrode, as shown in Figure 4.7b. During the OFF-time period, stress relaxation, atom rearrangement, and texture formation were occurred. A longer OFF-time, indicating lower duty cycle, provide sufficient time for stress relaxation, rearrangement of the deposited atoms. The surface area results obtained from AFM and CV offered significant insights into the correlation between the geometric surface area, determined by topographical features, and the electrochemically active surface area. These findings substantiated a consistent trend within the QCM samples, affirming similar correlations concerning the % duty cycle. The PCD technique for AuNS formation leads to an increased surface area and a distinctive surface morphology. These results are consistent with the work done by Kamnerdkhag et al. (Kamnerdkhag, Free, Shah, & Rodchanarowan, 2017), which demonstrates that an increase in the duty cycle promotes larger crystallite growth, leading to a decrease in surface area. During pulse deposition, the growth of larger crystallites is correlated with a decrease in the surface area. It can be concluded that improving the Au formation process on the QCM maintains a similar crystal structure while creating exceptional nanostructures on the QCM electrode surfaces. Furthermore, this improvement demonstrates minimal mass loading after electrodeposition, as evidenced by frequency shifts of 15% and 20% at 20% and 80% duty cycles, respectively, compared to the frequency shifts observed in the previous work (Sonklin, Munthala, Suksaweang, Janphuang, & Pojprapai, 2022).

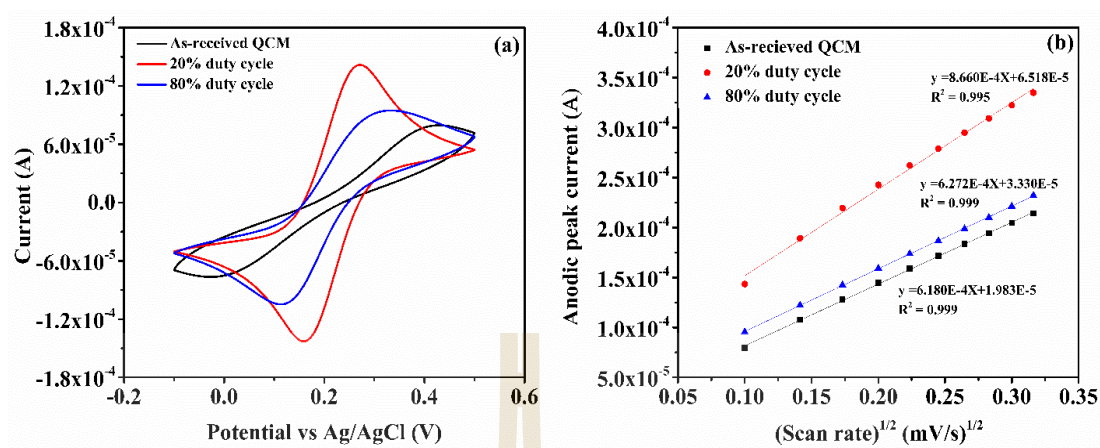


Figure 4.6 Electrochemical results of (a) CV plot within a scan range from -0.1 V to 0.5 V at a scan rate of 10 mV s^{-1} and (b) the relationship between anodic peak current and the square root of the scan rate at different scan rates of as-received QCM, 20% duty cycle, and 80% duty cycle of AuNS-modified QCM. (Sonklin et al., 2024)

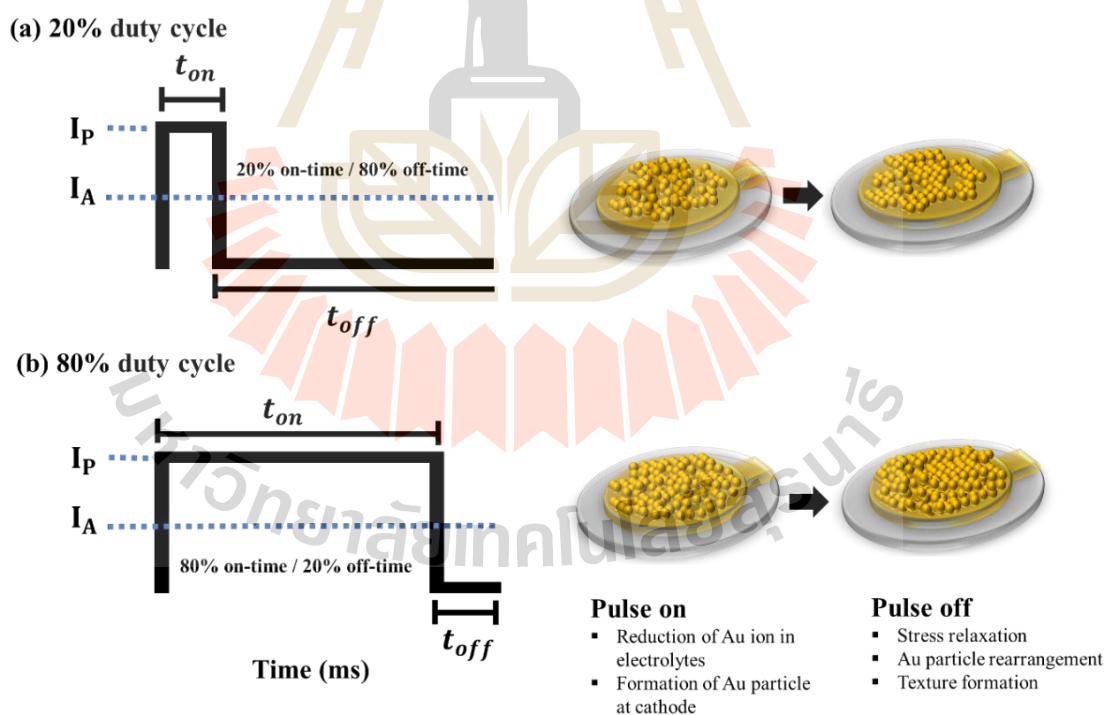


Figure 4.7 Schematic representation of Au deposition mechanism on a QCM during electrodeposition with various the percentage of duty cycle. (Sonklin et al., 2024)

4.3.5 XPS analysis

XPS analyses were performed to acquire precise information regarding the elemental composition of AuNS and to reveal the chemical state of gold, as illustrated in Figure 4.8. While preceding AFM and electrochemical analyses provided information solely about the surface structure, XPS is an inherently surface-sensitive technique that probes to a depth of approximately ~ 10 nm. Figure 4.8a displays a wide-ranging survey scan of the XPS spectrum of the pristine and AuNS-modified QCM. This spectrum showed prominent peaks of Au 4f, C 1s, O 1s, and Na 1s elements in increasing binding energy (BE) order. It primarily confirms the presence of carbon (C), oxygen (O), and gold (Au) atoms in all the samples. The presence of Na 1s observed in the AuNS-modified QCM suggested that it may originate from residual sodium cyanide solution used in Au electrolyte during the PCD process. A high-resolution narrow scan of the predominant peak Au 4f is displayed in Figure 4.8b. The measured spectrum is in a solid black line and the fitted spectrum is in a dashed line. The XPS spectrum within the Au 4f region reveals distinct doublets at approximately ~ 83.96 eV and 87.65 eV, exhibiting a characteristic energy separation (ΔE) of approximately 3.69 eV. Deconvolution of these doublet peaks indicates the presence of bands centered at ~ 83.96 eV and 87.65 eV, corresponding to 4f $7/2$ and 4f $5/2$ Au₀, respectively. These results primarily denote the presence of neutral Au₀ atoms on the surface of the As-received QCM and AuNS-modified QCM, remaining entrapped within the QCM electrode. Table 2 presents the Au atomic percentages, FWHM, binding energies, peak area, and peak position for each sample condition. The oxidation state significantly influences the binding energy and peak position. Atoms with higher positive oxidation states exhibit peaks at higher binding energies due to interactions between photoemitted electrons and the ion core (Hepperle et al., 2022). Despite this, there is no significant change observed in the peak position among the three conditions, suggesting that the oxidation states of the gold nanoparticles remained consistent throughout the process of modifying Au on the working electrode. The oxygen (O1s) and carbon (C1s) peaks observed on the gold QCM surface are attributed to contaminants originating from atmospheric exposure, the cleaning procedure, or the manipulation of the substrates.

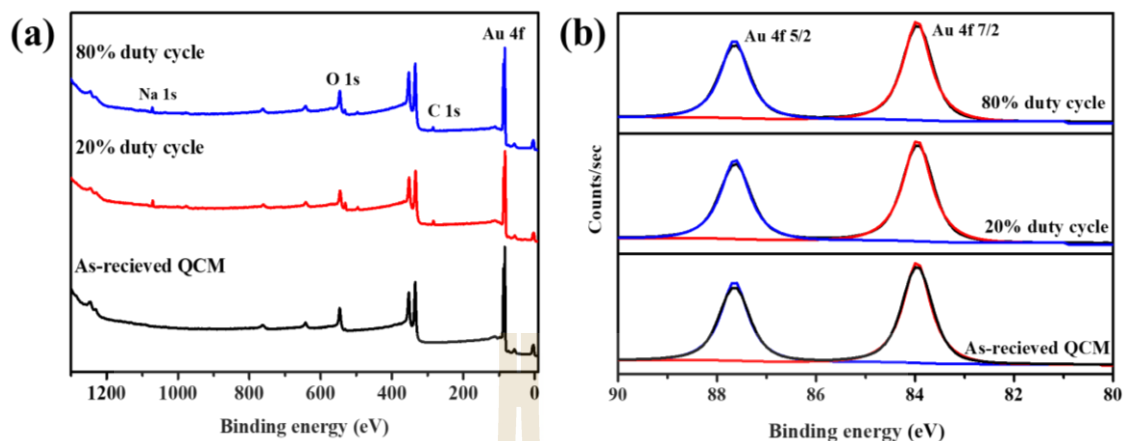


Figure 4.8 (a) XPS survey spectra and (b) narrow region XPS spectra for Au 4f. (Sonklin et al., 2024)

Table 4.2 XPS Au 4f binding energies, FWHM, peak area, and peak position for as-received QCM, 20% duty cycle, and 80% duty cycle of AuNS-modified QCM. (Sonklin et al., 2024)

Sample	Position(eV)	FWHM(eV)	Peak area (counts)	Atomic %
As-received QCM	83.96	0.66	99931.88	90.56
20% duty cycle	83.96	0.66	83913.89	41.68
80% duty cycle	83.96	0.69	95548.39	50.22

4.3.6 FT-IR bands detected the various chemical treatment on QCM electrode surface

Figure 4.9 shows the molecular vibration characteristics of the QCM surface treated with various chemicals, as measured by FTIR spectra in the wavelength range of 500-4000 cm^{-1} . The QCM was used separately for each chemical treatment and cleaning process. The initial observation, the transmittance bands were observed near 1180 cm^{-1} , which correspond to C-O bonds, were associated with organic residues and carbonates. The absence of these bands after the cleaning process suggests that the piranha solution was effective in eliminating organic contamination (Schmidt, 2022) from the Au surface. After adding L-cysteine, the bands were observed in the range between 1681-1380 cm^{-1} which are correlated with Amide I (C=O, C-N), (N-H), Amide II

(C-N, N-H), and Amide III (C=O), (C-N), (N-H), (O=C=N). Additionally, the decreased band was observed at wavenumber 2341-2550 cm^{-1} , indicating S-H stretching vibration of the thiol group. The reduction of bands of thiol group can be attributed to the splitting of the thiol group in the L-cysteine molecule, which is the prerequisite for forming Au-S bonding. According to Li et al. (Li, Liao, Ding, & Zeng, 2017), a bifunctional ligand of L-cysteine can provide a thiol group to form a chemical bond on nanoparticle surfaces. The bands of the amide group tend to be clearly visible after adding EDC/NHS, indicating their strong bond of amide group and carboxyl acid groups on the surface. The main functional EDC/NHS containing amines and carboxyl acid groups (Skopinska-Wisniewska, Tuszyńska, & Olewnik-Kruszkowska, 2021) thus allows the appearance transmittance bands in this range. The chemical-treated Au surface demonstrates the influence of chemical activity on chemical bonding and molecule vibrations. This result confirms the successful chemical treatment of the QCM surface, making the electrode ready for antigen-antibody immobilization.

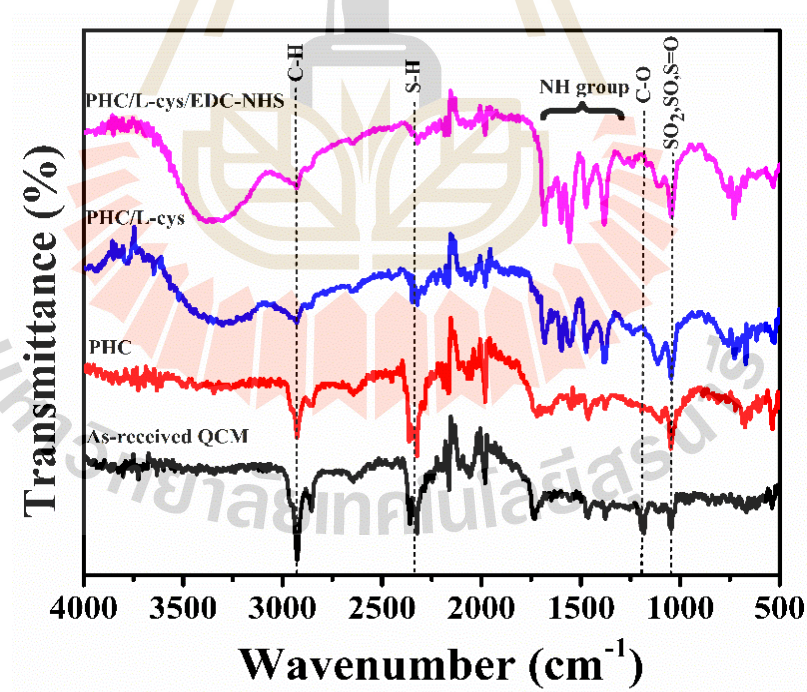


Figure 4.9 FT-IR spectra for stepwise chemical treatment on the Au-QCM surface. Piranha solution: PHC. (Sonklin et al., 2024)

4.3.7 Wettability of the various chemical treatment on QCM electrode

Before biomolecule immobilization, contact angle (CA) reflecting the wettability of the electrode surface after chemical treatment were measured. The wettability of a surface depends on the affinity between the material's surface and the liquid, which can be classified into two main classes: hydrophobic and hydrophilic. While, extreme of wettability are referred to its superhydrophobic and superhydrophilic nature. According to wettability theory, it is based on CA measurement, which is strongly influenced by liquid surface tension (Martinez-Urrutia, Fernandez de Arroiabe, Ramirez, Martinez-Agirre, & Mounir Bou-Ali, 2018). The relationship between CA and the surface tension is described by Young's equation (Hebbar, Isloor, & Ismail, 2017), which can be expressed as follows:

$$\cos \theta = \frac{\gamma_{SG} - \gamma_{SL}}{\gamma_{LG}} \quad (4.4)$$

where γ_{SG} , γ_{SL} and γ_{LG} is solid-vapor, solid-liquid, and liquid-vapor interfacial tension, respectively, and θ represent the CA. Therefore, the CA of a droplet of liquid on a solid surface is determined by the equilibrium of these three interfacial tensions.

For a hydrophobic surface, the CA > 90°, whereas for a hydrophilic surface, the CA < 90°; for a superhydrophobic surface, the CA > 150°, whereas for a superhydrophilic surface, the CA < 50° (Wang, Su, Shen, Ruths, & Sun, 2017). The wettability of a surface is a critical factor in influencing the biosensor. The bioreceptor surfaces need to be hydrophilic in order to facilitate easy interaction with the analyte biomolecule (Jandas et al., 2020). The result is presented in Figure 4.10. The captured snapshots indicate a reduction in droplet size, which is consistent with a decrease in the CA over the chemical-treated surface. The CA measured was 69° for the untreated Au-QCM surface. After the cleaning process with piranha solution, the CA decreased to 55°, and the CA value consecutively decreased from 44° of the QCM surface treated with L-cysteine to 36° of the QCM surface treated with EDC/NHS. The QCM surfaces were cleaned with the piranha solution, exhibited hydrophilic character that might be an effect of a thin native layer on the surface and plenty of hydroxyl (-OH) (Yang,

Zhong, Diallo, Wang, & Yue, 2014). After treating the Au surface with L-cysteine, there was a transfer from hydrophilic to super hydrophilic character, resulting in the reduction of the CA by 11°. Interestingly, a significant decrease in the CA value of the Au surface of the QCM was observed when the QCM was cleaned, L-cysteine and EDC/NHS were added. This suggested that the chemical treatment helps to increase the hydrophilic nature and. This ensures the potential effective attachment of the analyte biomolecule on the Au-QCM surface.

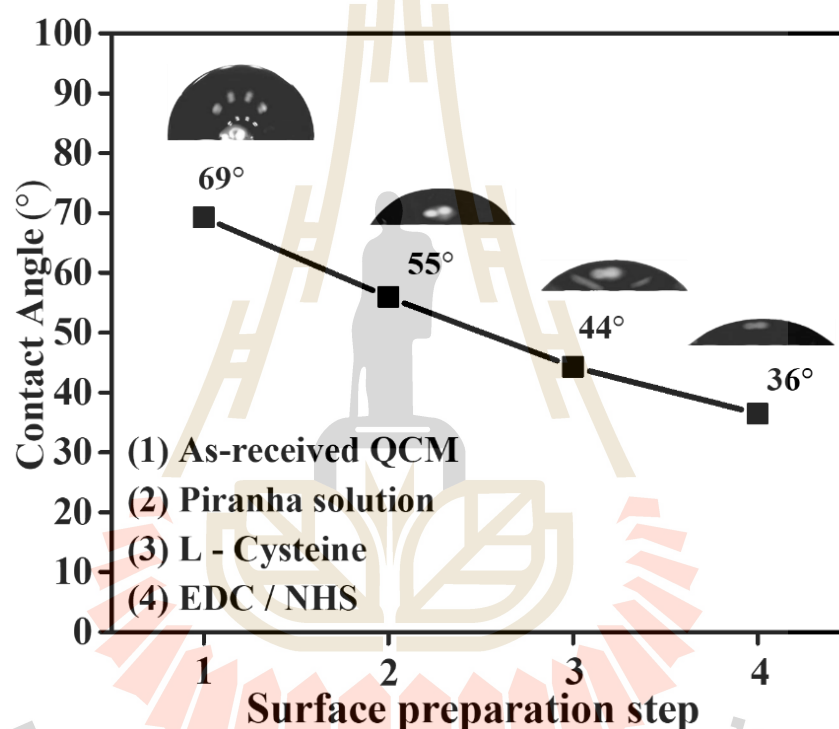


Figure 4.10 Contact angle of liquid on the Au-QCM surface that was cleaned with cleaning solutions, treated by L-cysteine, and EDC/NHS. (Sonklin et al., 2024)

4.3.8 Performance of QCM biosensor for PSA detection

To examine the sensitivity and quantitative detection range of the QCM, various concentrations of PSA were applied to the pristine QCM and the AuNS-modified QCM with 20% and 80% duty cycles. The frequency shift as a function of the logarithm of PSA concentration is shown in Figure 4.11. The frequency shift observed on the QCM with AuNS structures were higher compared to the pristine QCM when PSA was

loaded. This difference in frequency shift indicated that the AuNS structures amplifies the signal and improves the detection of the PSA biomarker. The enhanced detection can be attributed to the facilitated attachment of biomolecules onto the nanospikes structures, resulting in a stronger interaction between the biomarker and the AuNS-modified QCM electrode surface.

The initial observation of the relationship between the resonance frequency shift and the various duty cycles of the nanospike structures formation, it was observed that the shift in frequency during the antibody-antigen interaction, at the same concentration, was not directly correlated to the increase of percentage of duty cycle of the Au pulsed-QCM. However, the surface morphology from FESEM and the surface area obtained from CV results revealed a significant correlation with the resonance shift. The highest resonance shift was observed in the 20% duty cycle, which exhibited the highest surface area and the smallest grain size. This observation suggested that the attachment of antibody and antigen on the electrode surface was enhanced by the increased surface area created by the nanospikes on the QCM electrode surface. The presence of nanospike structures with a larger surface area could offer additional sites for biomolecule attachment, facilitating more effective interactions between biomolecules and the electrode surface. The limit of detection (LOD) for the highest frequency shift was calculated using the following equation. (Zhao et al., 2022):

$$LOD = \frac{3S}{K} \quad (4.5)$$

where S is the standard deviation and K is the slope of the calibration curve. The limit of detection (LOD) depicted in Table 3. It was found to be 24 pg mL^{-1} with a linear range of detection range $0.1\text{-}100 \text{ ng mL}^{-1}$ at 20% duty cycle of pulse-deposited Au. These conclusions support the idea that the immobilization surface plays a significant role in determining the frequency shift during the antibody-antigen interaction. A larger surface area provides more binding sites for biomolecules, resulting in stronger interactions between the antibody and antigen and leading to an enhanced resonance shift. In addition, we compared the sensitivity of our method

with previously work that utilize QCM with various surface modifications for signal amplification. The results of this comparison are presented in Table 4.3.

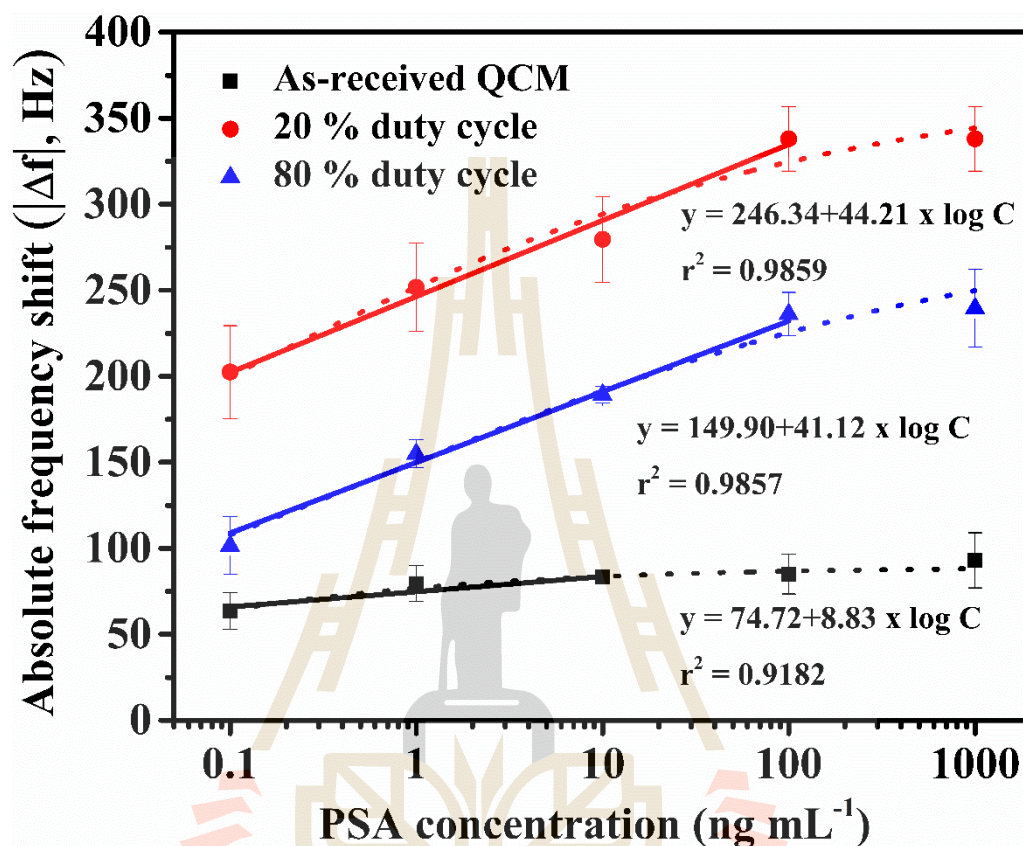


Figure 4.11 Resonance frequency response with PSA at various concentration. (Sonklin et al., 2024)

Table 4.3 A comparison of QCM biosensors with different signal amplification approaches reported for PCa detection. Gold nanoparticle: AuNP Gold nanospikes: AuNS. (Sonklin et al., 2024)

Signal amplification	Biomarker	Linear range (ng mL ⁻¹)	Detection limit (ng mL ⁻¹)	Response time	Ref
Anti-PSA antibody - AuNP- QCM	PSA	-	0.054	1 hour (including drying)	(Pohanka, 2021)
Anti-PSA antibody - AuNP- QCM	PSA and PSA- alpha 1- antichymotrypsin complex (in 75% serum)	0.29–150	0.29	8 min reaction time	(Uludağ & Tothill, 2010)
	tPSA in the whole (100%) serum	0.39–200	0.39		
Bare QCM	PSA	0.1-10	0.17	1 hour	This work
AuNS (20% duty cycle) - modified surface QCM	PSA	0.1-100	0.024	1 hour	This work
AuNS (80% duty cycle) - modified surface QCM	PSA	0.1-100	0.045	1 hour	This work

4.4 Conclusion

In summary, the incorporation of gold nanospikes (AuNS) into the QCM biosensor shows promise for point-of-care PSA diagnosis. This approach is convenient for biomedical research and can extend its use for analyzing other cancer-related

biomolecules. This developed biosensor enhances recognition element immobilization, amplifies signal transduction, and improves detection limits. It holds significant implications for early cancer detection and screening, addressing the growing demand for improved healthcare systems. The results confirm the successful deposition of gold nanostructures, increasing the effective surface area. The most notable resonance frequency shift in PSA detection was observed with a 20% duty cycle. Interestingly, an increase in the duty cycle did not necessarily correlate with an increase in the PSA detection signal measured via resonance frequency shift. It is plausible that the 20% duty cycle provides a larger surface area compared to an 80% duty cycle. Furthermore, the attachment of antibodies and antigens on the electrode surface was enhanced by the increased surface area facilitated by the nanospikes on the QCM electrode surface. The various chemical treatments provided in this work have improved surface properties suitable for biomolecule immobilization. The observed frequency shift demonstrates the AuNS-modified QCM biosensor's ability to detect PSA at various concentrations, with a remarkable limit of detection (LOD) of 24 pg mL^{-1} and a linear range of $0.1\text{-}100 \text{ ng mL}^{-1}$, displaying remarkable sensitivity. These approaches set the stage for the development of customized AuNS-QCM biosensors for specific biosensing applications in future research. Further investigations into the size of the AuNS structure and fine-tuning the relationship between the Au structure on the developed QCM electrode surface and the size of biomolecule immobilization are essential to enhance QCM efficiency for biomarker detection.

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CHAPTER V

THE EFFECT OF ANGLE-DEPENDENT GRAIN AND COLUMNAR GROWTH IN ZnO FILM DEPOSITED BY USING RF MAGNETRON SPUTTERING METHOD

5.1 Introduction

Chapter 3 and 4 highlight the potential use of the piezoelectric QCM sensor in biomarker detection. The resonance frequency and sensitivity of the QCM are influenced by factors such as crystal quality, electrode type, and electrode shape. To achieve greater sensitivity, the QCM requires a higher resonance frequency. However, achieving a high resonance frequency necessitates the use of a thin quartz substrate, rendering the QCM brittle, fragile, and challenging to handle. To address these issues, this chapter focuses on the fabrication of a new piezoelectric biosensor, the SAW. The SAWs are typically constructed from bulk piezoelectric materials like quartz, lithium tantalate, and lithium niobate, these materials also render them brittle and fragile. Recent advancements in piezoelectric thin film deposition have opened up new possibilities for thin film-based SAW devices (Y. Q. Fu et al., 2017; Länge, Rapp, & Rapp, 2008; Tigli, Bivona, Berg, & Zaghloul, 2010), offering integration potential with existing microelectronics.

ZnO is a wurtzite piezoelectric semiconductor that is widely investigated for such devices due to its high electromechanical coupling coefficient and its variety of choices for thin film fabrication (Borysiewicz, 2019; Triboulet & Perrière, 2003; Zhong Lin, 2004). This material can be grown on various substrates, including Silicon which is low-price and compatible with CMOS technology. The key issue in ZnO film deposition for making biosensor is to optimize the growth conditions to achieve a good quality of the film. The growth conditions, including parameters such as the deposition angle, growth temperature, laser energy density, deposition pressure, and temperature of the substrate,

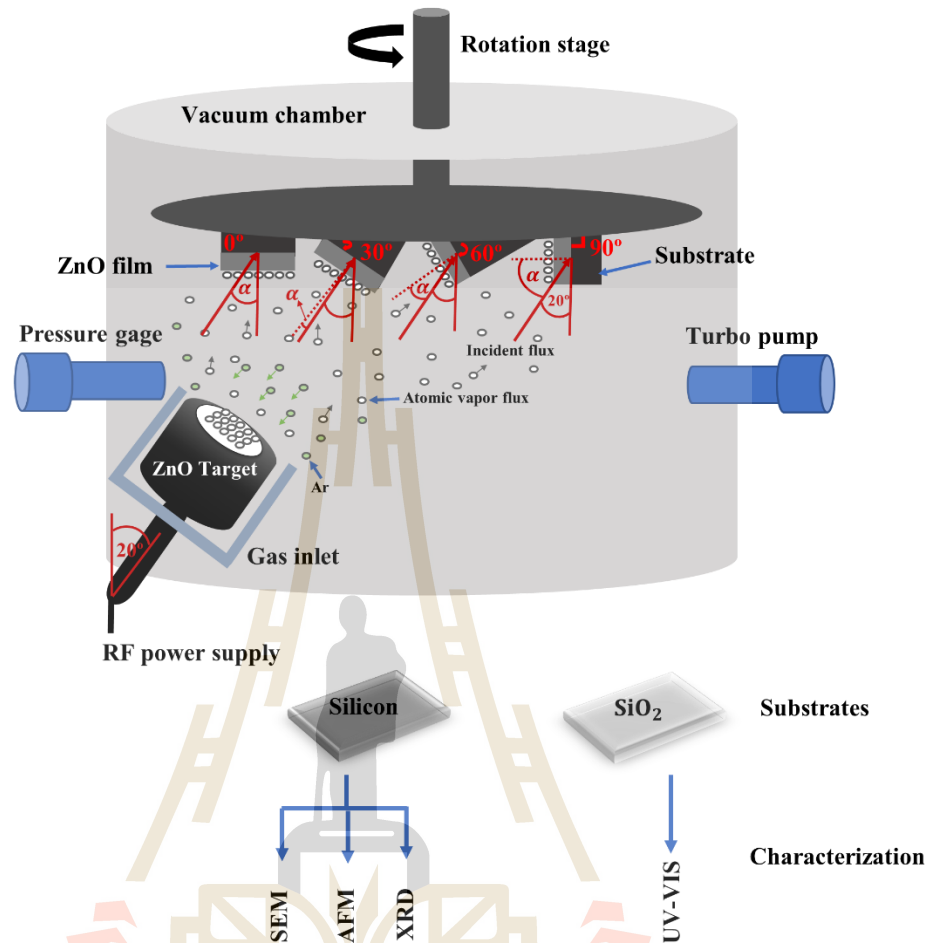
must be optimized to produce high-quality films with excellent properties and desired film orientations.

This chapter presents an attempt to develop uniformly inclined growth of piezoelectric ZnO films on Si substrates using the GLAD method. The ZnO films are fabricated by using RF magnetron sputtering method at different glancing angles from 0° to 90° with respect to the substrate holder. We report that the specific role of oblique angle deposition influences growth direction, phase formation, formation of nanostructure, and surface morphology of deposited films. Film deposition and comprehensive film characterization, such as surface roughness, and crystallography are performed with various techniques.

5.2 Experimental Procedure

ZnO thin films were deposited on commercially purchased boron-doped P-type Si (100) with a 1 - 10 ohm-cm resistivity and 1×1 cm² commercially purchased quartz substrates by using RF-magnetron sputtering method, facilitated at beamline 6a (BL-6a), Synchrotron Light Research Institute (SLRI), Thailand. A 99.99% pure, 3-inch diameter, and 3 mm thick commercially purchased ZnO sputter target was used to deposit the ZnO films. The substrates were thoroughly cleaned by using the Radio Corporation of America (RCA) process before the prior depositions (Kern, 1990). The pre-cleaned Si substrates and quartz substrates were clamped to the custom-made holder at different tilt angles (0° , 30° , 60° , and 90°) with respect to the substrate holder and the custom-made holder was mounted to the substrate holder in the sputtering chamber. The schematic representation of the experimental setup is illustrated in Figure 5.1a, and sputter deposition conditions are mentioned in Table 5.1. The optical properties were measured using UV-Vis (Agilent Cary300). SEM (JEOL JSM-6010LV) was performed to reveal the surface morphology of ZnO films. The surface topology was investigated by using AFM (Park Systems AFM XE-120 in contact mode with a scan area 5×5 μm^2). XRD (Bruker D8 ADVANCE) technique was performed to study the crystallite size and crystalline structure.

a)



b)

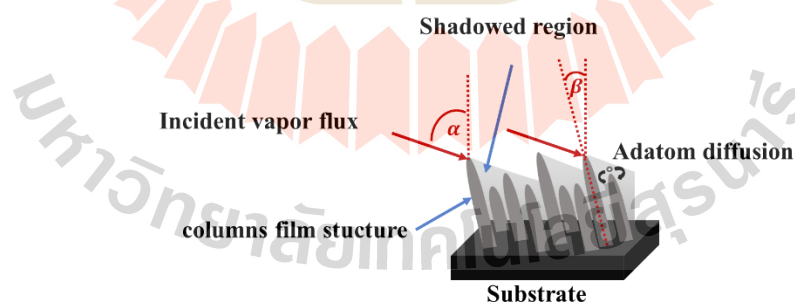


Figure 5.1 Schematic representation of (a) The substrate tilt angle-dependent ZnO films deposited by RF sputtering method and (b) Shadowing effect, the angle deposition (α), and the growth column angle (β). (Sonklin, Munthala, Leuasoongnoen, Janphuang, & Pojprapai, 2022)

Table 5.1 RF sputter deposition conditions. (Sonklin et al., 2022)

Sputtering parameter	Conditions
Target material	ZnO (99.999%)
Target diameter(cm)	3
Sputtering gas	Ar
Base pressure (mTorr)	8.67×10^{-7}
Working pressure (mTorr)	1.54×10^{-2}
RF Sputtering power (Watt)	200
Substrate-to-target distance (cm)	6
Substrate materials	100 P-type Silicon and glass (SiO ₂) slide
Deposition time (hours)	3

5.3 Results and Discussion

Figure 5.2 shows the XRD patterns of glancing angle deposited ZnO films on Si substrates. The scan range of each XRD spectra was measured from 20 to 60° of 2θ . The intensity of diffracted peaks typically depends on the film thickness and crystalline quality of films. The XRD peak positions at 34.4°, 36.1°, and 47.5° of 2θ correspond to the (002), (101), and (102) directions of hexagonal wurtzite ZnO structure. The above peaks are well matching with the ICDD file (PDF# 03-0888). The peak intensities and FWHM are measured to estimate the crystallite size and the same values are plotted in Figure 5.3 in terms of deposition angle. The Debye-Scherrer equation is used to calculate the crystallite size (D), as shown in Eq. 4.3.

The calculated crystal sizes are 21, 19, 22, and 24 nm, respectively, for 0°, 30°, 60°, and 90° of substrate tilt angles deposited film. It seems to be that both intensity and FWHM values are high for 30° tilt angle deposited films compared to other tilt angle depositions. Both values slightly decreased from 0° to 30° and further increased as the deposition angle increased to 90°. As seen above, the crystal size is less for 30° deposition. This could be due to the directional grain growth effect and selectively less for 30° tilt angle. The hexagonal lattice constant (c) and interplanar spacing (d) are calculated by the following equations (Hammad, Abdel-wahab, Vattamkandathil, & Ansari, 2018),

$$n\lambda = 2d_{hkl} \sin \theta \quad (5.1)$$

$$\frac{1}{d_{hkl}^2} = \frac{4}{3} \left(\frac{h^2 + hk + k^2}{a^2} \right) + \frac{l^2}{c^2} \quad (5.2)$$

Where h, k, l are the miller index, a, c are the lattice parameters, d_{hkl} is the interplanar spacing, θ is the incident angle, and λ is the incident X-ray wavelength (1.5406 Å). In this case, (hkl) values are considered from (002) peak since it is the dominant peak in XRD spectra. The estimated hexagonal lattice constant values are 5.226, 5.218, 5.204, and 5.209 Å, respectively for 0°, 30°, 60°, and 90° deposited films. Minor changes are observed in the estimated hexagonal lattice constant and its value decreases with an increase of tilt angle. Also, (002) peak position is shifted for all tilt angle depositions and this might be due to the stress/strain formation in the films (Bhattacharya, Gupta, Kahol, & Ghosh, 2010).

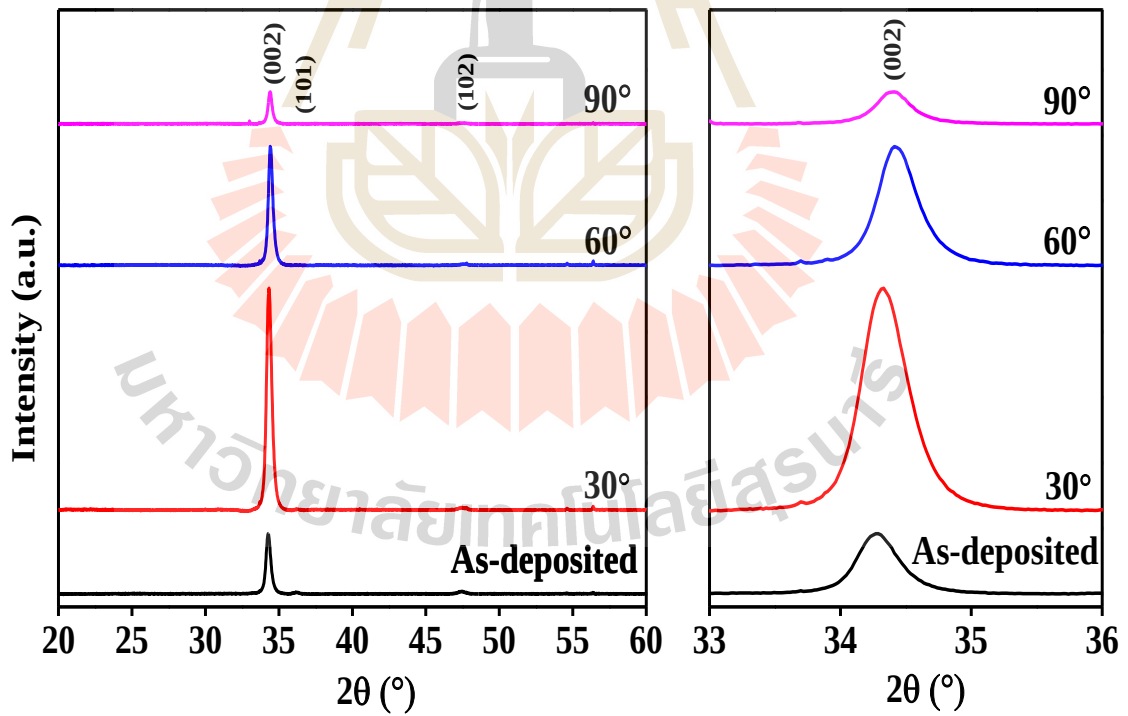


Figure 5.2 XRD patterns of ZnO films deposited at different substrate tilt angles. (Sonklin et al., 2022)

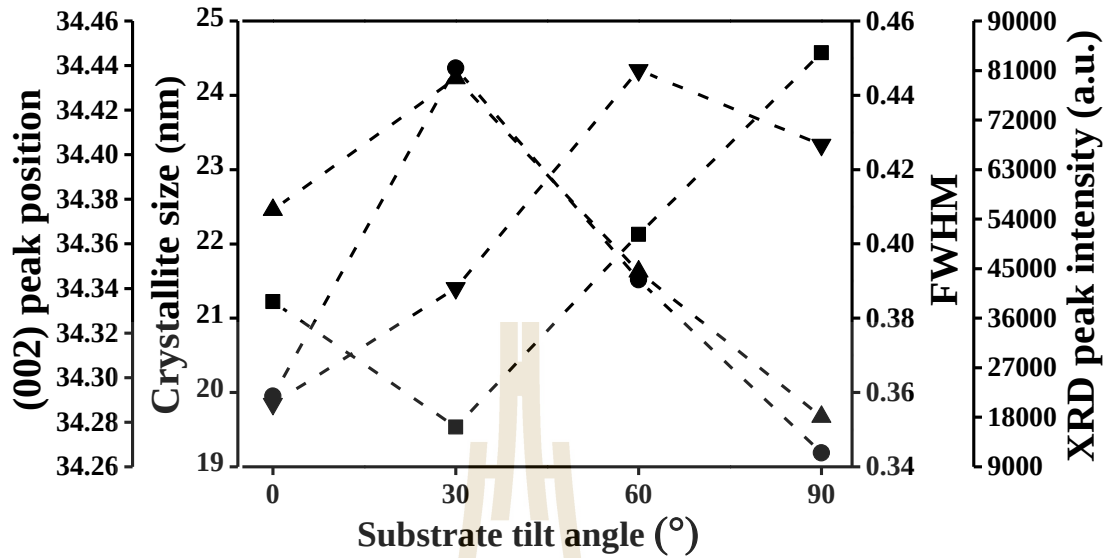


Figure 5.3 XRD peak intensities, peak positions, FWHM, and crystallite size values of ZnO films deposited at different substrate tilt angles. (Sonklin et al., 2022)

The strain (ε) present in the ZnO films can be calculated by the following equation (Hammad et al., 2018),

$$\varepsilon(\%) = \frac{c - c_0}{c_0} \times 100 \quad (5.3)$$

where c is the calculated lattice parameter of the film and c_0 is the lattice parameter of ZnO in bulk ($5.206 \text{ \AA}$). The estimated strain values are 0.39, 0.24, -0.03, and 0.06, respectively, for 0° , 30° , 60° , and 90° deposited ZnO films. The above-estimated values are also mentioned in Table 5.2. The lattice stain is related to film stress along the c -axis. A negative value corresponds to the tensile stress, while a positive value denotes the film's compressive stress. A decreasing lattice stain related to film stress implies that a greater number of defects are formed in the ZnO films with an increasing substrate tilt angle (H. F. Pang et al., 2012).

Table 5.2 Extracted ZnO films lattice parameters, strain, film thickness, and RMS roughness values. (Sonklin et al., 2022)

Tilted angle (°)	C-axis length (Å)	Strain (%)	Thickness (nm)	RMS (nm)
0	5.226	0.39	819	5.40 $\pm$ 0.26
30	5.218	0.24	731	4.38 $\pm$ 0.21
60	5.204	-0.03	427	4.73 $\pm$ 0.21
90	5.209	0.06	393	5.85 $\pm$ 0.07

The surface morphology, grain size, grain shape, film thickness and growth direction of ZnO films are analyzed using FE-SEM images is displayed in Figure 5.4. An initial observation shows that the film grains are flake-like structures, and these structures are observed for all the deposited substrate angles. The flake-like structures are generally observable at high sputtering powers and at higher deposition rates. Due to high powers, highly energized Ar ions can provide translational energy to the adatoms, providing high nucleation rates and irregular grain growth (Asgary et al., 2021; Le, Sohn, Jae Won, & Good-Sun, 2010; Toma et al., 2021). It is found that the flake-like grains have sharp edges; grain distribution is irregular, and the average grain size is 156 nm in length and 73 nm in width for 0° tilt angle deposition, as shown in Figure 5.4a. In the case of 30° tilt angle deposition, grains merged together and formed a dense surface, and the average grain size is 124 nm in length and 75 nm in width, as shown in Figure 5.4b. Similarly, when the substrate is 60° with the substrate holder, the average grain size varies from 55 to 96 nm and apparently, high grain density compared to other depositions is observed, as shown in Figure 5.4c. Interestingly, the grain size dropped sharply and sharp edges are not visible in the case of 60° angle deposition. In the case of 90° deposited films (i.e., the angle between the substrate-to-substrate holder is 90°) the grains are nearly the same as 0° deposited film, and the observed grain size varied from 145 nm in length and 80 nm width, as shown in Figure 5.4d. It is clearly noticeable that the porosity is high for 0° tilt angle deposition, less for 30° tilt angle deposition, and moderate for other depositions. The above behavior of film surfaces depends on nucleation rates and diffusion of adatoms.

A cross-sectional view of angle-dependent deposited ZnO films is analyzed to see growth direction and film thickness, and the corresponding images are shown in Figure 5.4e to 5.4h. The estimated film thicknesses are approximately 819, 731, 427, and 393 nm, for 0°, 30°, 60°, and 90° deposited films, respectively. The slight difference in the distance from the target to inclined substrates might induce the difference in incoming flux directions of sputtered atoms on the substrates, resulting in film thickness variation. Film thickness is drastically decreased with an increase in deposition angle. At an initial stage, evaporated atoms reach the substrate and condense on the substrate surface to spontaneously form nucleation. The nucleation rate and diffusion length of adatoms provide a necessary situation to create valleys and hillocks. The newly formed valleys and hillocks obstacle for the incoming flux to behind regions and form a tilt columnar nanostructure on the substrate. The valleys are shadowed by neighboring hillocks and which are caused by receiving insufficient vapor flux (Yildiz et al., 2015). Moreover, the movement of atom vapor during the substrate tilt-angle along the deposition process is insufficient to reach the substrate surface, leading to a decrease in the film thickness (Ravankhah & Savaloni, 2017).

At the shadow regions, incident vapor atoms arriving to the substrates with deposition angle (α) are prevented by growing peak regions where nanostructure growth did not occur. On the contrary, the peak regions receive enough adatoms flux, resulting in a tilt column nanostructure with different growth angle (β). A schematic representation of the so-called shadowing effect is explained in Figure 5.1b. Meanwhile, the surface diffusion and the film surface oppose nanostructure formation by providing smooth surfaces that might decrease the columnar branching (Ai & Zhao, 2018). In this experiment, the incoming flux direction of deposition angle is approximately 30° with respect to the substrate holder. In the case of 0° tilt angle deposition, the substrate normal is 60° inclined with the incoming flux direction. In this case, as observed from the FE-SEM (Figure 5.4e) image, the columnar growth direction is about 13° only. When it comes to the 30° tilt angle deposition, the substrate normal is parallel to the flux direction and the columnar growth direction is almost 90° (as observed in Figure 5.4f). Similarly, in the case of 60° tilt angle deposition, substrate normal is 60° inclined with the incoming flux direction and the columnar growth direction is about 13°, as shown in Figure 5.4g. Similarly, in the case of 90° tilt angle deposition, the substrate normal is 60° inclined with the incoming flux direction and

the columnar growth direction is approximately 18° , as shown in Figure 5.4h. The shift of normal substrate-tilt angles provides the columnar inclination. Compared to 0° tilt angle (Figure 5.4e) with 90° tilt angle depositions (Figure 5.4h), the total rotation of the columnar growth is 31° .

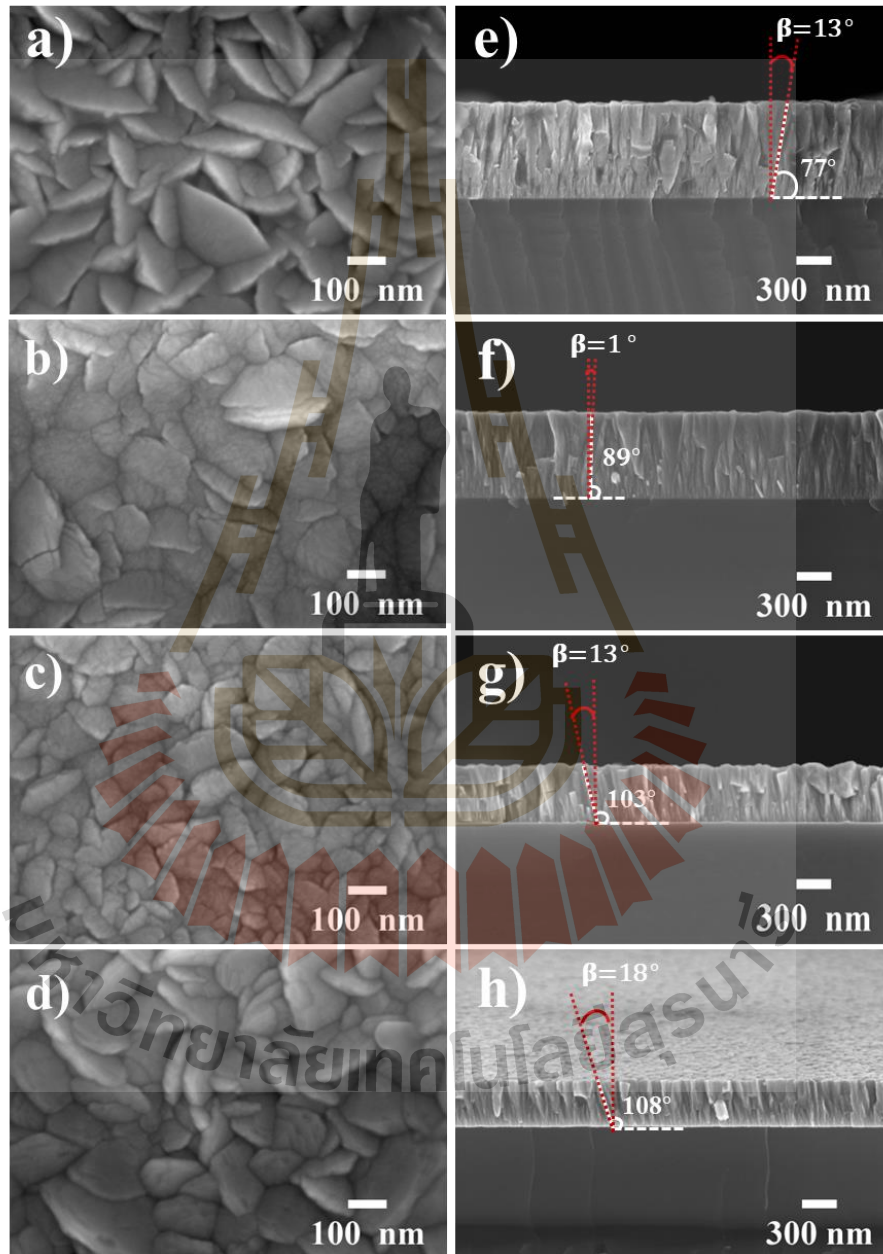


Figure 5.4 The FE-SEM images of ZnO films: (a, b, c, d) Surface images belong to 0° , 30° , 60° , and 90° deposited films. (e, f, g, h) Cross-sectional images belong to 0° , 30° , 60° , and 90° deposited films. (Sonklin et al., 2022)

The surface roughness of ZnO thin films was analyzed by using a contact mode AFM, and the measurement area was $5 \times 5 \mu\text{m}^2$ (as shown in Figure 5.5). The measured RMS values are 5.40, 4.38, 4.73, and 5.85 nm, respectively, for 0° , 30° , 60° , and 90° deposited ZnO films. The minimum RMS value was observed for 30° , and the maximum RMS value was observed for 90° deposited film. The decrease and increase of RMS values might be due to growth direction-induced surface roughness. The growing film surface depends on the substrate tilt angle and flux direction, influencing the c-axis orientation of sputter-deposited ZnO thin films.

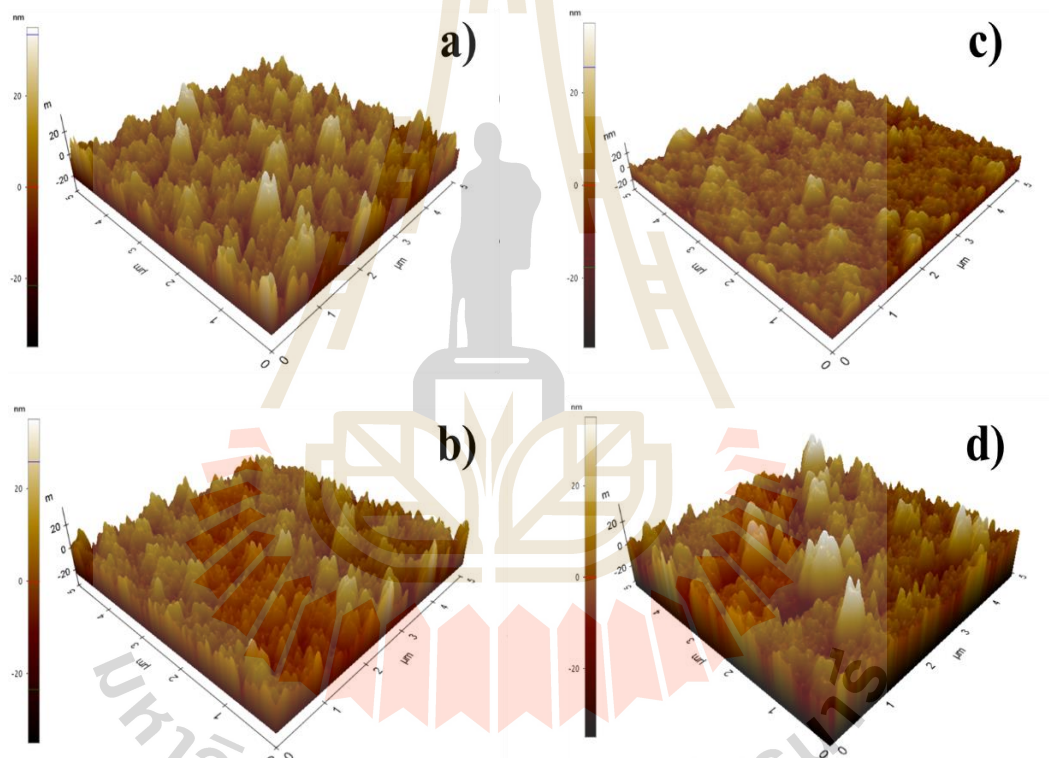


Figure 5.5 AFM images of ZnO films on Si substrates at different substrate tilt angles: (a) 0° , (b) 30° , (c) 60° , and (d) 90° deposited films. (Sonklin et al., 2022)

The optical properties of ZnO films deposited at different angles are shown in Figure 5.6a. The transmission spectra were measured from 300-800 nm and the overall transmittance was 90%. Interference fringe formation is observed for 0° , 30° , and 60° deposited films and whereas no interference fringe formation is observed for 90°

deposited film. In the case of 90° deposited film, the measured thickness (393 nm from FE-SEM measurement) is not enough to produce the fringe formation. This might be due to the high absorption of light at this nanostructure condition and similar kind of results are evident in the literature (H. F. Pang et al., 2012). On the other hand, nanostructure formation results in light scattering and traps the light in nanostructures, and thus the light trapping property with the increase in light absorption. The envelope method is used to extract the optical properties: refractive index (n), absorption coefficient (α), and bandgap energy (E_g) values from the transmission spectra from the following equation (Haneen, 2015; Tashtoush & Alkasassbeh, 2013; Zarandi & Bioki, 2017),

$$N = 2s \left(\frac{T_M - T_m}{T_M T_m} \right) + \frac{s^2 + 1}{2} \quad (5.4)$$

$$n = \sqrt{N + \sqrt{N^2 - s^2}} \quad (5.5)$$

$$\alpha = \frac{(2.303A)}{t} \quad (5.6)$$

$$(\alpha h\nu) = B(h\nu - E_g)^{\gamma} \quad (5.7)$$

where N is volume density, s is the substrate refractive index, T_M and T_m are the two adjacent maxima (or) minima of the interference fringes. Here, n is the refractive index, α is absorption coefficient, A is the absorbance, which is derived from the transmittance spectra, t is the thickness of thin-film, $h\nu$ is the photon energy, E_g is the bandgap of the material, B is the parameter of the absorption edge width. The exponent γ can be determined by the type of electronic transition. Here the superscript γ value varies from 1/2 and 2 depending on the type of direct allowed and indirect allowed transition, respectively.

The extracted optical parameters, α , E_g , and refractive index are displayed in Figure 5.6. Figure 5.6(b,d) shows the extracted absorption coefficient graphs and these values are in good agreement with the literature on ZnO films (Zarandi & Bioki, 2017).

The E_g values are extracted from extrapolating the linear part of $(\alpha h\nu)^2$ Vs. energy ($h\nu$) graphs. The extracted E_g values are 3.29, 3.25, 3.27, and 3.30 eV, respectively, for 0° , 30° , 60° , and 90° deposited films, which is in reasonable agreement with the bandgap of ZnO (H. F. Pang et al., 2012). The estimated refractive index (RI) values are 1.85, 1.82, and 1.72 with respect to tilt angles 0° , 30° , and 60° deposited films as shown in Figure 5.6c. The reduction in the refractive index, which increases substrate tilt angles, can be ascribed to the low density and increase of the porosity of deposited films (Toledano, Escobar Galindo, Yuste, Albella, & Sánchez, 2012). The variations of these refractive index and bandgap values with tilting angles could be due to the variation of film density and grain size (Hwangbo, Park, Sobahan, Nam, & Kim, 2010; Toledano et al., 2012). Therefore, the substrate tilt angle deposition plays a major role in film quality.

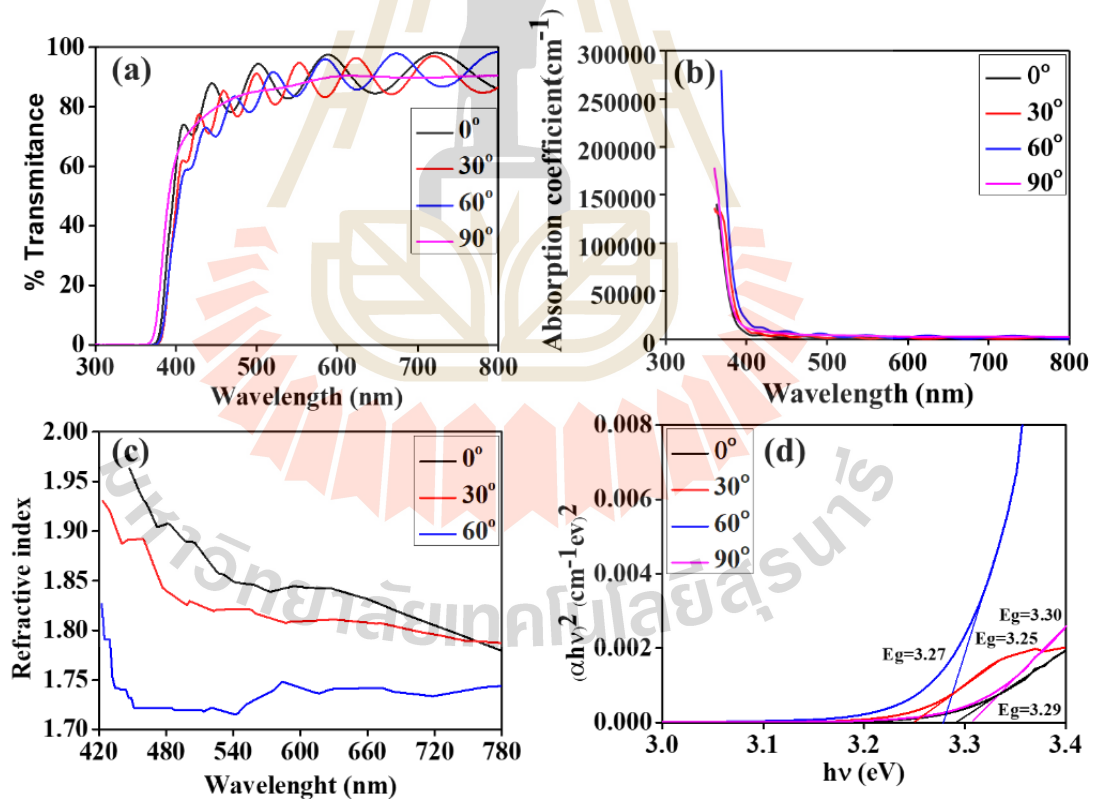


Figure 5.6 Optical properties of ZnO film, (a) transmittance spectra, (b) absorption spectra, (c) refractive index and (d) bandgap energy. (Sonklin et al., 2022)

5.4 Chapter Summary

In summary, ZnO films are deposited at different substrate tilt angles with respect to the substrate holder. The estimated crystal sizes are 21, 19, 22, and 24 nm, respectively, for 0°, 30°, 60°, and 90° substrate tilt angle deposited films. Flake-like grains are observed due to high deposition rates and this feature is observed in all the deposited films. The (002) peak shift is due to the presence of strain in ZnO films, which is generated during the deposition and might be due to the columnar grain growth. ZnO film thickness is decreased from 819 to 393 nm while increasing the substrate tilt angle from 0° to 90°, and this might be due to the shadowing effect of films. The overall columnar growth direction shift is 31° for the substrate tilt angle rotation from 0° to 90°. There is no interference fringe formation for 90 tilt angle deposition, and this might be due to the high absorption of light at this nanostructure formation. The tilt angle of columnar grain growth is due to the alignment of sputtered atoms between the target and inclined substrate. Based on the results, it can be concluded that the deposition with desirable substrate tilt angles is a crucial factor in inducing different grain shapes and orientations. In SAW applications, this developed ZnO structure remains larger grain size and lower thickness, posing a potential risk of acoustic wave loss to the substrate and potential damage to the IDT electrode.

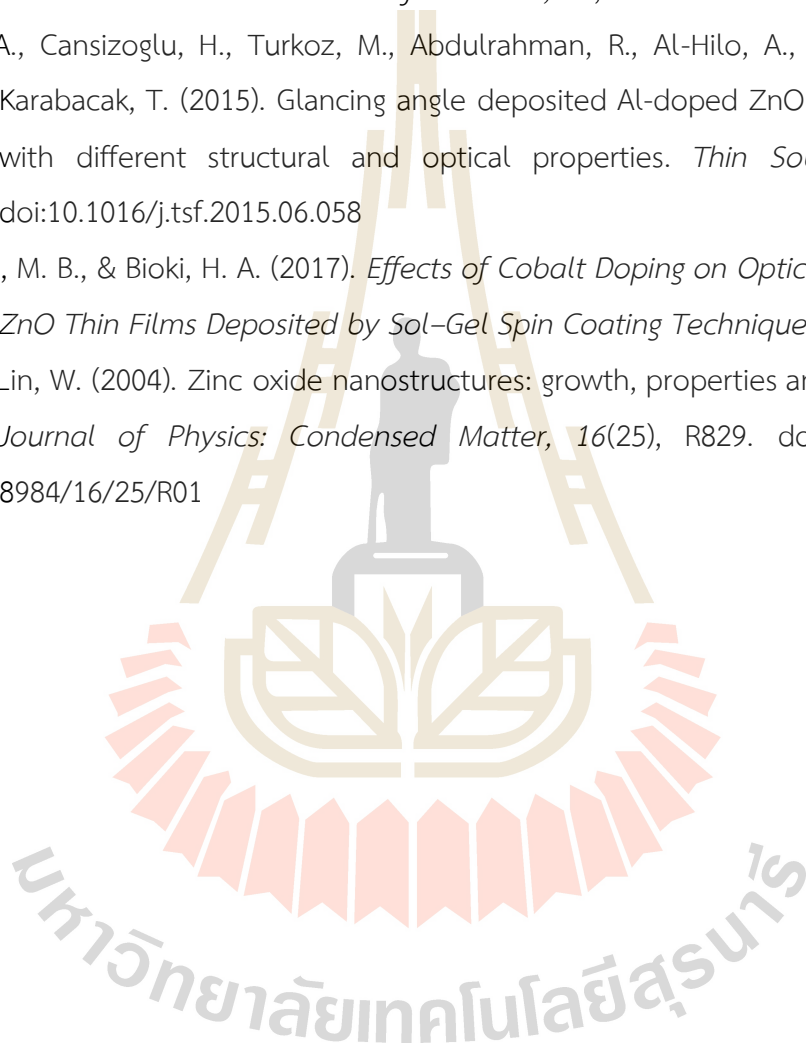
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CHAPTER VI

THE EFFECT OF ANNEALING TEMPERATURE UNDER OXYGEN GAS ON ZnO THIN FILM MICROSTRUCTURE

6.1 Introduction

In the previous chapter, the GLAD was employed to obtain a good quality of the film for SAW application. Another challenge for fabricating SAW is optimizing the annealing treatment temperature for the films to achieve high crystallinity and low defect. Several researchers have made efforts to improve film quality by inducing an annealing process after film deposition. This process offers the capability to regulate a range of factors, such as crystalline quality, crystal lattice orientation, strain, stress, morphology, crystallite and grain size, and defects. This chapter aims to investigate the effect of annealing temperature on the microstructural properties of ZnO films under oxygen gas. It provides insight into the most suitable annealing temperature for SAW applications. From the experiment, it has been observed that subjecting the film to temperature treatment under oxygen gas is a compelling method for annealing. This approach not only enhances grain size and modifies the surface structure but also induces alterations deep within the columnar structure upon reaching the appropriate temperature.

6.2 Experimental Procedure

6.2.1 Materials and Method

ZnO films were fabricated using the RF magnetron sputtering technique on commercially purchased boron-doped P-type Silicon (Si) substrates. The Radio Corporation of America (RCA) cleaning procedure was employed to remove surface contamination from the Si substrates. Initially, the Si substrates were cleaned in piranha solution at 120°C for 10 minutes to remove metal and organic residues. Subsequently, they were then immersed in buffered oxide etch (BOE) for one minute

to eliminate the native oxide, followed by soaking in 65% nitric acid (HNO₃) at 115°C for 10 minutes to form a homogeneous and uniform native oxide. Followed by, the substrates were then rinsed in deionized water and blown with nitrogen gas to remove liquid droplets on the surface. Finally, the Si substrates were transferred to an oven and dried at 200°C for 2 hours to relax the strain/stress formed during the cleaning process.

The Si substrates were transferred to the sputtering chamber and clipped to the substrate holder. The chamber was evacuated to 8.6×10^{-7} mTorr, and the deposition was carried out at 1.54×10^{-2} mTorr. A 3-inch diameter, 2 mm thick, and 99.99% pure ZnO target was used to deposit the films. Pre-sputtering was performed for 5 minutes to remove surface contamination. The deposition was carried out at 200-watt power for 3 hours to attain the desired film thickness. After the deposition, the ZnO films were annealed under oxygen gas at 150, 300, 450, 600, 750, and 900°C for 1 hour each. The deposition conditions of the ZnO thin films are listed in Table 6.1.

Table 6.1 Deposition parameters of ZnO thin film.

Sputtering parameter	Conditions
Target material	ZnO (99.999%)
Target diameter(inch)	3
Sputtering gas	Ar
Base pressure (mTorr)	8.67×10^{-7}
Working pressure (mTorr)	1.54×10^{-2}
RF Sputtering power (watt)	200
Substrate-to-target distance (cm)	6
Substrate material	100-Silicon
Deposition time	3 hours

6.2.2 Apparatus and Instrumentations

The as-deposited and annealed ZnO films were characterized using various techniques, including SEM, contact mode AFM, XRD, and FT-IR techniques.

These techniques were utilized to analyze the structural characteristics, surface morphology, surface roughness, and crystalline quality of the films.

6.3 Results and Discussion

6.3.1 XRD Results

Figure 6.1 depicts the XRD patterns of ZnO films with and without post-annealing treatment. The impact of the heat treatment on the ZnO film growth orientation is well pronounced. All the diffraction peaks exhibited a polycrystalline nature. All peak positions agree well with the standard ZnO patterns (JCPDS-PDF 03-0888). There is no discernible structure of any unrelated compound in the XRD pattern. The crystalline peak at 34.4° comprise to the predominant (002) orientation, which is clearly distinguishable. Other distinguishable peaks at 36.2° and 47.5° belonged to (101) and (102) lattice planes, respectively, which exhibited low peak intensity. This result suggests that all these films exhibited hexagonal wurtzite structure with the growth direction along the c-axis. With an increase in annealing temperature, the dominant (002) peak position slightly shifts. This might be due to the effect of residual compressive stress or strain in the film structure, which refers to the contraction of the inter-planar spacing as the annealing temperature increased. Annealing under oxygen pressure assists oxygen atoms in relocating to proper lattice sites with available kinetics (Bhattacharya, Gupta, Kahol, & Ghosh, 2010). To obtain a more detailed explanation of the (002) peaks in ZnO thin films, the XRD peak intensity, lattice parameter c, crystallite size, and lattice strain (ϵ) were calculated.

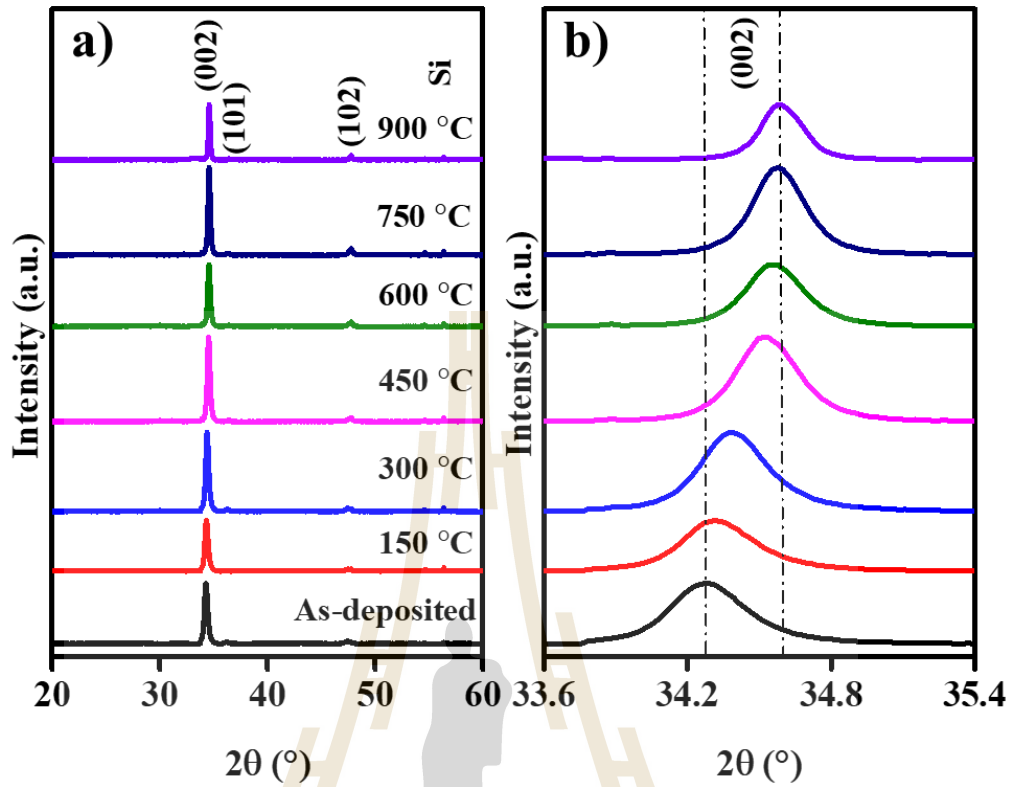


Figure 6.1 XRD pattern of ZnO thin films before and after oxygen annealing

To compare the intensity of the preferred c-axis orientation, the relative XRD peak intensity orientation of ZnO films can be calculated using the following equation (Malek et al., 2014):

$$P_{hkl} = \frac{I_{(hkl)}}{\sum I_{(hkl)}} \quad (6.1)$$

where P_{hkl} is the relative peak intensity orientation (hkl), $I_{(hkl)}$ is the intensity at (hkl) plane, and $\sum I_{(hkl)}$ is the sum of all intensities at (hkl). Table 6.2 shows the C-axis length of (002) plane ZnO thin film as increasing annealing temperature. The highest $P_{(hkl)}$ was obtained at an annealing temperature of 750 °C. The higher value of $P_{(hkl)}$, after reaching the contained temperature, indicates the improved crystalline quality.

The crystallite size (D) of the ZnO films can be calculated from the dominant (002) peak using the Scherrer equation, which is given in Eq. (4.3). The calculated crystal size and FWHM are shown in Figure 6.2. The decrease in FWHM of (002) can be observed as the annealing temperature increases. The crystallite sizes are ranged between 215 and 396 nm and it tended to increase as the annealing temperature was elevated. This result is consistent with the previous literature (Kong et al., 2013).

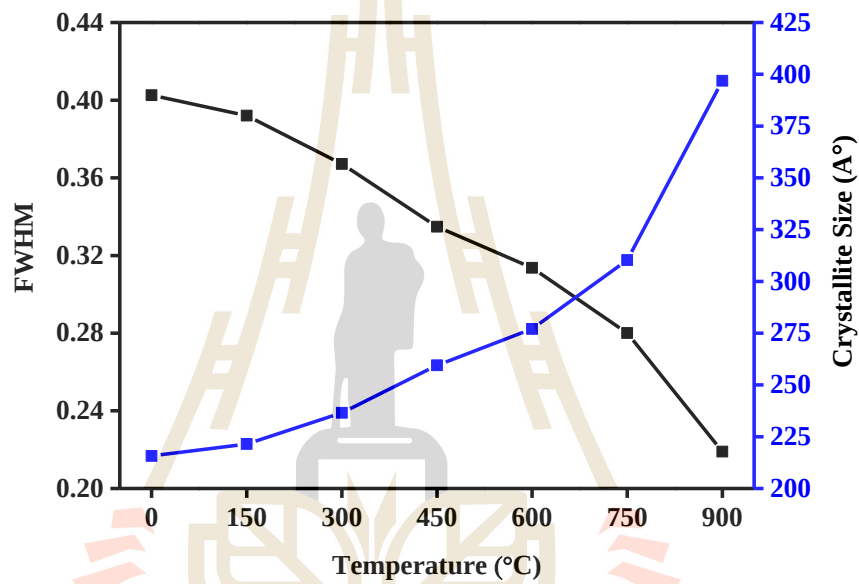


Figure 6.2 Variation of FWHM and crystallite size of ZnO thin film as a function of annealing temperature.

The lattice constant c of hexagonal wurtzite ZnO structure, can be calculated using the Eq. (5.2).

From Eq. (5.1) and Eq. (5.2), the lattice parameter c of the (002) plane can be calculated using the following equation:

$$c = \frac{\lambda}{\sin \theta} \quad (6.2)$$

the lattice parameter c decreased as the annealing temperature increased up to 450 °C, after it remained constant. This may be caused by the release of stresses within

the film's structure. During film deposition, the formation of defects such as dislocations, vacancies, and grain boundaries can contribute to the accumulation of residual stresses or strains within the film. The rearrangement of atoms within the film can be aided by annealing at high temperatures, resulting in the relaxation of these residual stresses or strains. It can lead to a reduction in the interplanar spacing and, consequently, a reduction in the lattice parameter c . In addition, the annealing under oxygen pressure can assist oxygen atoms in relocating to the correct lattice sites with the available kinetics, resulting in a change in the lattice parameter. The strain in ZnO film along the c -axis perpendicular to substrate is calculated using the Eq. (5.3).

An initial observation, it was found that the calculated strain values for a ZnO film were positive up to 300 °C, indicating a tensile strain. However, annealing the film further up to 900 °C resulted in negative strain values, indicating a compressive strain. These strain values are listed in Table 6.2. The tensile strain values decreased from 0.443% to 0.096% as the annealing temperature was increased up to 300 °C. This decrease in strain values can be attributed to the removal of intrinsic defects, such as interstitial and vacant atoms, from the crystal as the annealing temperature is increased (Seid & Dejene, 2020). Subsequently, the strain values switched to compressive with the values dropping from -0.306% to -0.421%. M.F.Malek et al. (Malek et al., 2014) demonstrated that switching between tensile and compressive strains can result in the formation of defects in the film structure, resulting in a more tense film.

Table 6.2 The intensities of (002) plane, lattice constant (c -axis), and lattice strain of ZnO thin film at different annealing temperatures under oxygen gas.

Temperature (°C)	$P_{(002)}$ peak intensity	$P_{(002)}$ peak ratio	C-axis length (Å)	Strain (%)
Pristine	0.126	1.000	5.229	0.443
150	0.105	0.833	5.223	0.327
300	0.165	1.311	5.211	0.096
450	0.177	1.401	5.190	-0.306
600	0.129	1.020	5.184	-0.421
750	0.183	1.454	5.184	-0.421
900	0.115	0.913	5.184	-0.421

6.3.2 SEM Results

Figure 6.3 shows the surface and cross-sectional morphologies of ZnO films deposited on Si substrates at different annealing temperatures. The nucleation rate can affect the formation of grain structure due to the high deposition rate (Munthala, Avasthi, Pathak, Khan, & Rao, 2018), resulting in a nano-flake-like structure in the films. The As-deposited film exhibited a flake grain size of approximately 152 nm width and 75 nm length, with a shaped edge, irregular grain distribution, and high porosities as shown in Figure 6.3a. The annealed films from 150 to 750 °C exhibited slightly merged grains, forming a denser surface with various forms of flake grain structure as shown in Figure 6.3a to 6.3g. However, these annealed films did not propose any significant changes to surface morphology from 150 to 750 °C annealing temperature whereas the films subjected to the highest annealing temperature (900 °C) demonstrated a smoother surface and larger aggregated grains without trapped pinholes on the surface. It implies that the films annealed at a high temperature may have a denser surface. The distribution size of the grains is estimated to be 160 nm width and 95 nm length as shown in Figure 6.3g. The cross-sectional images of ZnO films shows in Figure 6.3h to 6.3n. ZnO layer possess a polycrystalline columnar structure. The thickness of the ZnO films layer decreased with annealing temperature compared to the pristine film. The observed thickness ranges of approximately 640 to 814 nm. The cross-sectional view exhibited a pronounced coarse columnar structure from the pristine to 750 °C annealed films. However, a nano-crystalline structure emerged for the 900 °C annealed films as shown in Figure 6.3n. The apparent and abrupt change observed in the annealed films at 900°C may be attributed to the annihilation of defects and densification of the films.

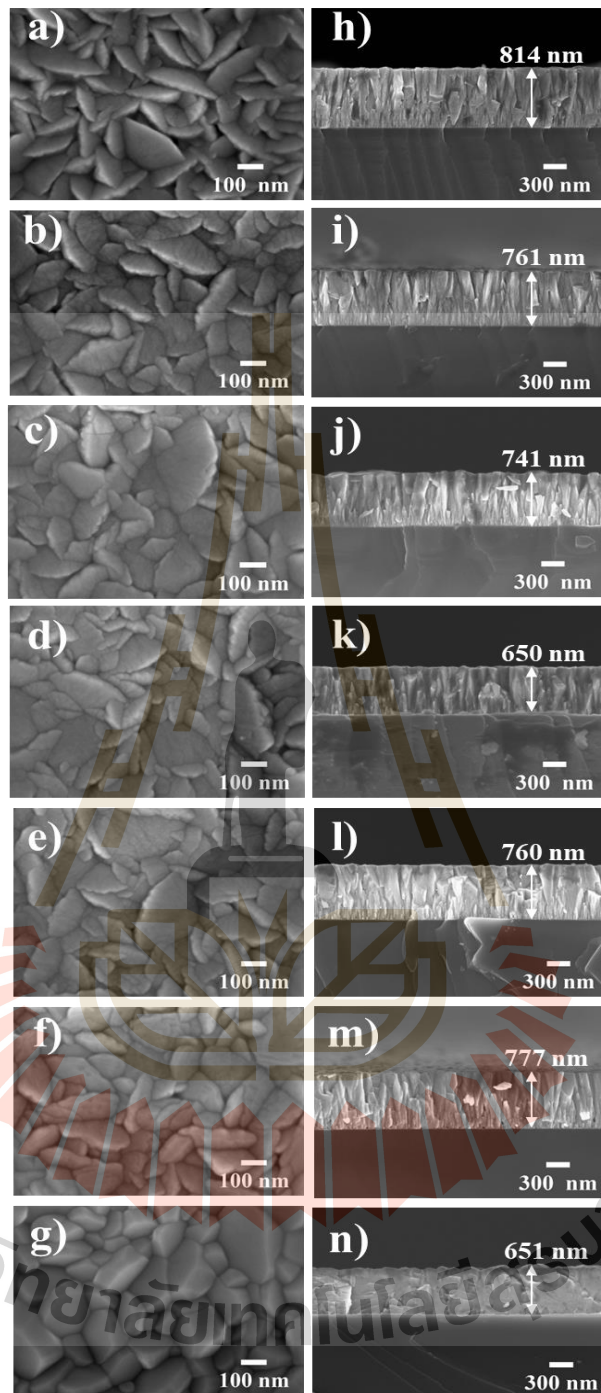


Figure 6.3 SEM images of ZnO thin films and the cross sections annealed at different temperatures. (a and h) As-deposited film, (b and i) 150 °C, (c and j) 300 °C, (d and k) 450 °C, (e and l) 600 °C, (f and m) 750 °C, and (g and n) 900 °C.

6.3.3 AFM Results

Figure 6.4 shows the AFM images of ZnO films annealed at different temperatures. The surface morphology and topography of the films slightly changed during the annealing process. The Root means square (RMS) values of ZnO films are 6.3, 6.4, 7.8, 6.7, 6.7, 6.7, and 11.2 nm for pristine, 150, 300, 450, 600, 750, and 900 °C annealed films, respectively. It is noteworthy that the RMS values do not differ much for the various annealed films, except for the one annealed at 900 °C. As shown in the cross-sectional FE-SEM images, the columnar structure is present up to 750 °C and absent for 900 °C. The much variation of RMS values (11.2 nm) is attributed to the morphological changes in the ZnO film annealed at 900 °C. As seen in Fig 4a and 4f, the presence of columnar structure in AFM images indicates that the majority of these films are orientated along the c-axis, but there is no apparent columnar structure for the 900 °C annealed sample, which is consistent with the FE-SEM results.

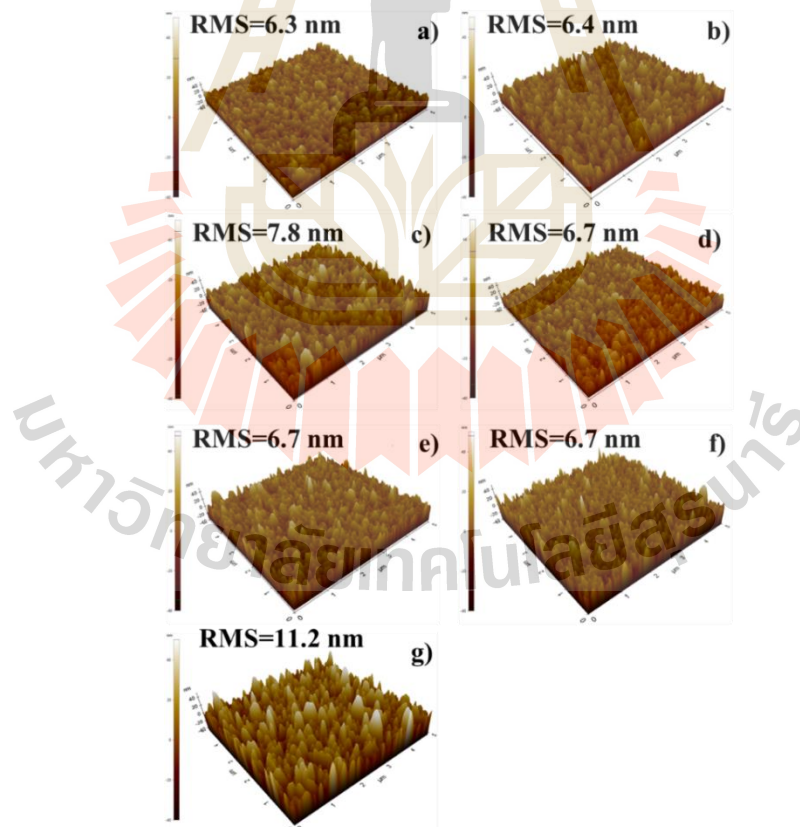


Figure 6.4 AFM images of ZnO films annealed at different temperatures. (a) As-deposited film, (b) 150 °C, (c) 300 °C, (d) 450 °C, (e) 600 °C, (f) 750 °C, and (g) 900 °C.

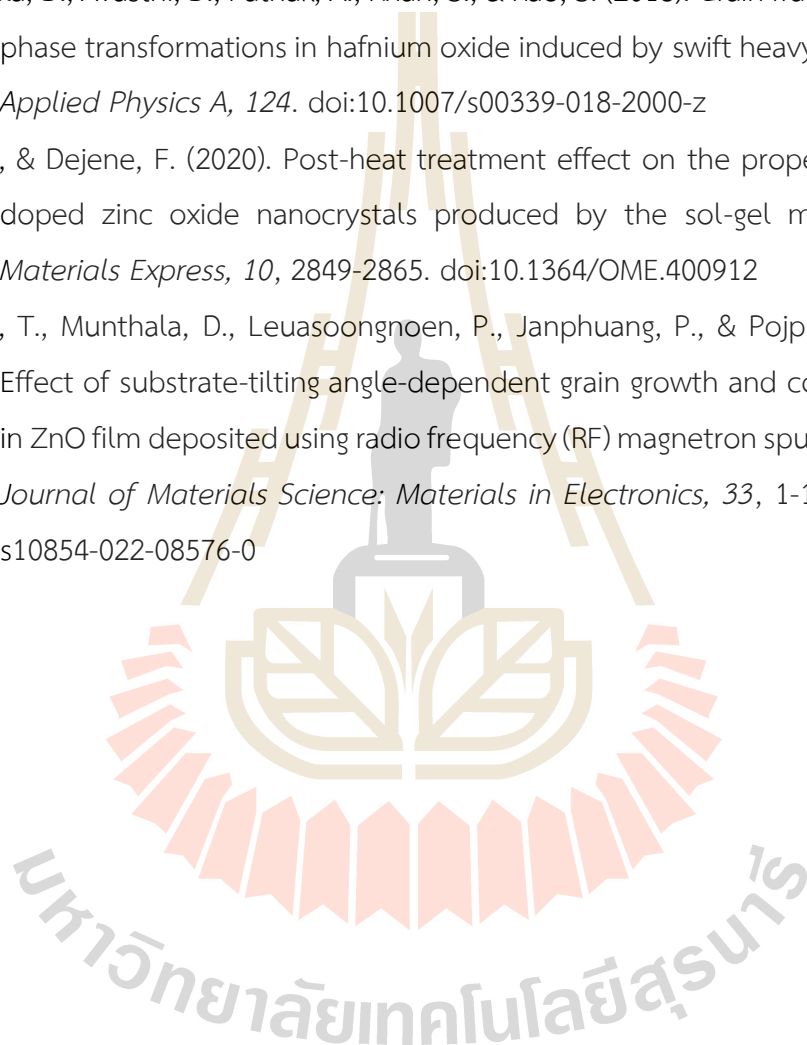
6.4 Chapter Summary

In summary, the ZnO films on Si substrates were deposited by using the RF sputtering method and followed by post-annealing treatment from 150 to 900 °C. The films exhibit hexagonal wurtzite phase. The highest intensity of (002) peak refers to crystalline quality of the film and improving appears in an annealed sample at 750 °C. A decrease in FWHM and a corresponding increase in the crystalline size has been observed with increased annealing temperature from 150 to 900 °C. The peak shift at (002) plane and the changing from tensile strain to compressive strain are also observed with increasing annealing temperature. The obtained nano-flakes shape is attributed to a high deposition rate. The obtained columnar structures are observed at temperature treatments below 750 °C. Additionally, AFM data are in agreement with SEM result which confirms the changing of columnar structure at elevated to particular annealing temperature. Based on these results, it can be concluded that temperature treatment under oxygen gas is a significant factor for film annealing. This process enhances grain size, and modifies the structure. Nonetheless, During the fabrication and annealing processes, persistent issues arise regarding the uniformity and random size distribution of the grains. Fine-tuning the adjusting parameters is essential to achieve optimal film quality for SAW application.

6.5 References

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CHAPTER VII

EXPERIMENTAL AND SIMULATION STUDY OF ZnO/SiO₂/Si AND ZnO /Si PIEZOELECTRIC FILM-BASED SURFACE ACOUSTIC WAVE WITH/WITHOUT GOLD SENSING LAYER

7.1 Introduction

In Chapter 5 and 6, GLAD and temperature manipulation were employed to enhance the microstructure of ZnO for SAW applications. This chapter focuses on depositing ZnO on Si-based substrates (Si and SiO₂), with microstructure refinement achieved through prolonged deposition times and reduced deposition power to attain a smoother surface and mitigate flake-like structures. A two-port SAW device will be constructed on ZnO films featuring Au/Ti delay line electrodes, serving as a sensitive layer for specific biomolecule binding. Both experimental and simulation approaches will be utilized for device fabrication and performance prediction. The influence of the sensing area on acoustic wave mode propagation will be investigated, employing a two-dimensional (2D) frequency-domain FEM to analyze scattering parameters. Additionally, a three-dimensional (3D) FEM analysis will be employed to assess eigenfrequency and mode shape. Films deposition will be employed using RF sputtering technique, with the IDT comb-like structure developed in this study. In summary, successful fabrication of a two-port SAW device on ZnO films with Au/Ti delay line electrodes has been achieved. By integrating experimental and simulation approaches, this study facilitates precise device fabrication and accurate performance prediction. The consistency observed between simulation and experimental results not only validates the research but also enhances a more understanding of SAW device fabrication and operation. These results reveal substantial promise in crafting SAW devices with sensing regions for biological recognition. These could profoundly elevate biosensing capabilities, enabling the detection of biomarkers and other related applications.

7.2 Experimental Procedure

7.2.1 COMSOL Simulation

The COMSOL Multiphysics 5.3a was supported by University of Hyderabad, India. A systematic study of ZnO/SiO₂/Si and ZnO/Si structures, with and without a sensing layer, was conducted using the FEA, along with the Perfectly Matched Layer (PML) technique. The schematic diagram depicted in Figure 7.1 illustrates the use of a one-dimensional (2D) frequency-domain FEM to evaluate resonance frequency, velocity, and propagate loss. A three-dimensional (3D) FEM analysis was applied to assess eigenfrequency and mode shapes.

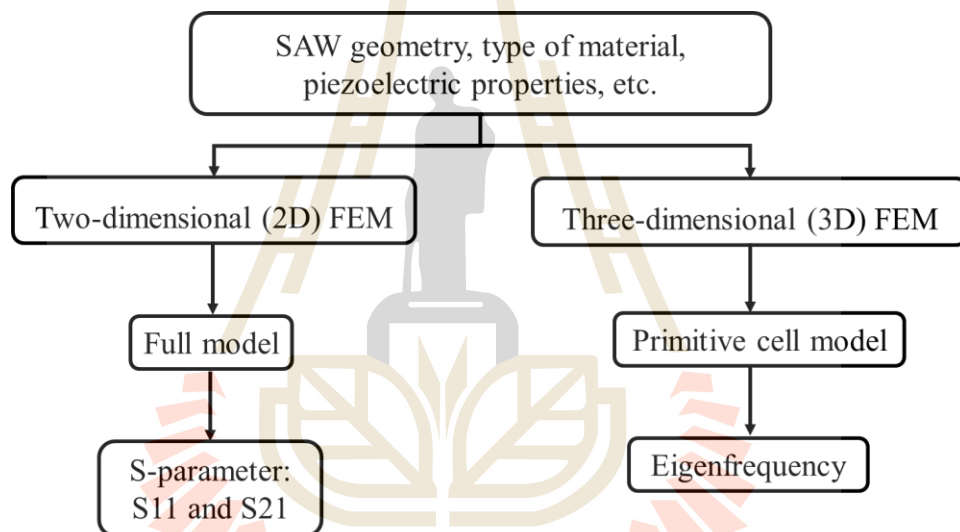


Figure 7.1 A chart displays the FEA model approaches for predicting the frequency response, along with relevant simulated results of SAW device in 2D and 3D geometries.

7.2.1.1 Geometry

Figure 7.2 depicts a schematic representation of a layered SAW device consisting of ZnO coated over Silicon (Si) based substrates (Si and SiO₂/Si). The schematic includes the 3D primitive cell and the 2D full model. The thickness of ZnO, SiO₂, and Si is defined by t_{ZnO} , t_{SiO_2} , and t_{Si} , respectively. The Gold (Au) is utilized for the IDT, acting as the transducer with two interlocking comb-shaped structures. In the

simulation, the SAW structure is covered with a Perfect Matched Layer (PML) that facilitates simulation with open boundaries, allowing electromagnetic and acoustic waves to pass without reflection (Timoshenko, Kalinchuk, & Shirokov, 2018). The film boundary is denoted by Γ , and the PML thickness is denoted by t_{PML} . For the 3D primitive cell, the length is denoted by L_a . The IDTs are positioned at the top of the film surface, with one IDT at port 1 serving as a transmitter for generating acoustic signals, and another IDT at port 2 functioning as a receiver for capturing signals. The distance between the two IDTs is defined by L_{IDT} . The delay lines consist of IDTs with an acoustic wavelength (λ) of 30 μm , and additional parameters are provided in Table 7.1.

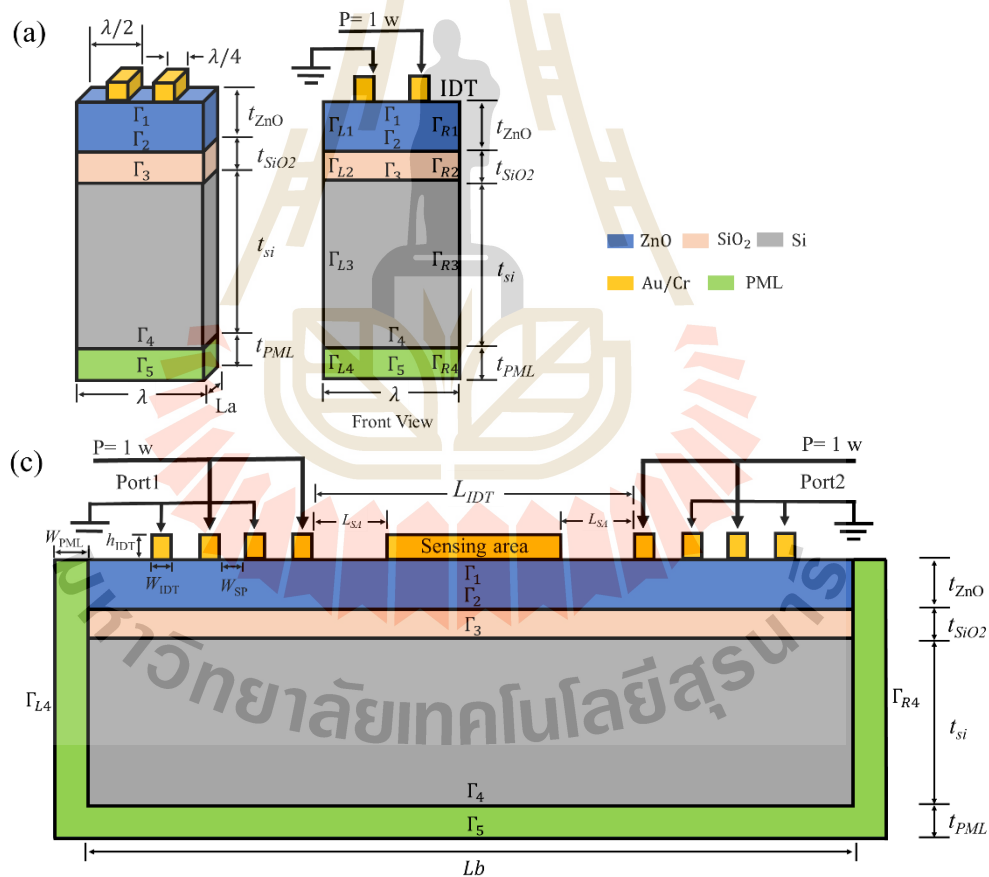


Figure 7.2 Structure of (a) 3D primitive cell and side view and (b) 2D full body for simulation

Table 7.1 The parameter of the simulation model.

Parameter	Value	Description
t_{ZnO}	1.3 μm	Film 's thickness
t_{SiO_2}	1 μm	SiO ₂ substrate thickness
t_{Si}	525 μm	Si substrate 's thickness
t_{PML}	100 μm	PML thickness
W_{PML}	100 μm	PML width
L_b	3.01 mm	Length of SAW device
W_{IDT}	7.5 μm	IDT finger width
h_{IDT}	200 nm	IDT finger height
W_{SP}	7.5 μm	Electrode spacing
L_{IDT}	1.810 mm	Length between electrode
L_{SA}	7.5 μm	Length between sensing area (delay line) and IDT electrode
N_{IDT}	20 pairs	IDT finger number
λ	30	wavelength
L_a	$\lambda/3$	Depth of 3D primitive cell

7.2.1.2 Modelling

Piezoelectric materials serve as substrates in SAW devices for the excitation and detection of SAWs. The mathematical models for SAW sensors are derived from the piezoelectric constitutive equation, Newton's second law governing mechanical behavior, and Maxwell's equations governing electrical behavior (Cheng, Lu, Yang, Gong, & Ke, 2023). The analysis involves a piezoelectric material, requiring the user to set two distinct sets of boundary conditions. First is the mechanical boundary conditions and second is the electrical boundary. The related equation may be defined in the weak form or be implemented in COMSOL™ by 'Solid Mechanics (solid)', 'Electrostatics (es)', and 'Piezoelectric Effect (pze)' physics interfaces. The

Physics-controlled mesh was selected and extremely fine was chosen as element size. The COMSOL software default for material parameters used in the simulation are listed in Table 7.2 and Table 7.3 for non-piezoelectric material and ZnO piezoelectric material respectively.

Table 7.2 Non-piezoelectric material parameter used in the simulations.

Materials	Au	Si	SiO ₂
Density, ρ (kg/m ³)	19300	2330	2200
Young's modulus, E (Pa)	70E9	69E9	70E9
Possion's ratio, ν	0.44	0.22	0.17

Table 7.3 Parameters of piezoelectric materials used in the simulation model.

Piezoelectric material	ZnO
Density, ρ (kg/m ³)	5680
Relative Permittivity (Dielectric constant), ϵ	$\begin{pmatrix} 8.5446 & 0 & 0 \\ 0 & 8.5446 & 0 \\ 0 & 0 & 10.204 \end{pmatrix}$
Coupling matrix, e (C/m ²)	$\begin{bmatrix} 0 & 0 & 0 & 0 & -0.48050 \\ 0 & 0 & 0 & -0.4805 & 0 & 0 \\ -0.567 & -0.567 & 1.320 & 0 & 0 & 0 \end{bmatrix}$
Elastic matrix, C_E (GPa)	$\begin{bmatrix} 209.7 & 121.1 & 105.4 & 0 & 0 & 0 \\ 121.1 & 209.7 & 105.4 & 0 & 0 & 0 \\ 105.4 & 105.4 & 211.2 & 0 & 0 & 0 \\ 0 & 0 & 0 & 42.4 & 0 & 0 \\ 0 & 0 & 0 & 0 & 42.4 & 0 \\ 0 & 0 & 0 & 0 & 0 & 42.4 \end{bmatrix}$

7.2.2 Fabrication and Characterization of ZnO Film

ZnO thin films were fabricated using the RF magnetron sputtering method at beamline 6 (BL-6), Synchrotron Light Research Institute (SLRI), Thailand. The films were deposited on commercially purchased boron-doped P-type Si (100) and

SiO₂/Si substrates with resistivity ranging from 1 to 10 ohm-cm, on 1×1 cm². For the deposition process, a 99.99% pure ZnO sputter target was utilized. The target had a diameter of 3 inches and a thickness of 3 mm. Before deposition, the Si substrates underwent a thorough cleaning process using Radio Corporation of America (RCA) method. The sputter deposition conditions are provided in Table 7.4. The IDTs were deposited onto ZnO film by a conventional lithography (UV, Maskless) technique, as shown in Figure 7.3a. The design of IDT with and without the presence of a sensing area (SA) is shown in Figure 7.3b. Au was selected as the material for the IDT electrodes.

Table 7.4 Sputtering parameter

Sputtering parameter	Conditions
Target material	ZnO (99.999%)
Target diameter(inch)	3
Sputtering gas	Ar (10 -11 sccm)
Base pressure (mTorr)	7.40×10^{-7}
Working pressure (mTorr)	$1.37 - 1.40 \times 10^{-2}$
RF Sputtering power (watt)	150 W
Substrate-to-target distance (cm)	7
Substate material	Silicon and SiO ₂ /Si
Deposition time (hours)	16

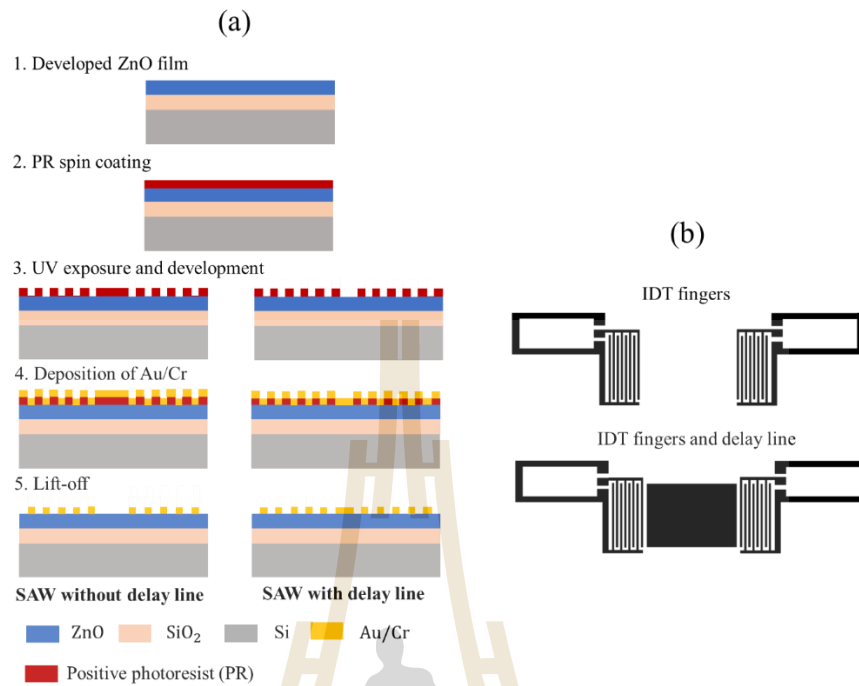


Figure 7.3 (a) Process flow for ZnO-SAW fabrication, (b) Schematic of IDT of ZnO-SAW device.

7.3 Results and Discussion

7.3.1 Simulation Study

7.3.1.1 Two-Dimension (2D) FEM Analysis: Full Model

To investigate scattering (S) parameters, a 2D full-body model was employed. A parametric sweep study named 'PortName', labelled as 1 and 2 was defined via COMSOL simulation. The simulation study was conducted in the frequency domain, with frequencies specified in MHz. The range chosen was 100 to 300 MHz. Electrostatics, Terminal, and S-parameter (dB) data were selected for the y-axis of a 1D plot group for S_{11} (es.S11dB) and S_{12} (es.S21dB) simulations.

Table 7.5 illustrates detailed boundary condition settings for the 2D primitive cell. The performance of the ZnO SAW device was evaluated through S-parameter vs. frequency curves (S_{11} and S_{21}), depicted in Figure 7.4. Two prominent acoustic modes were identified at center frequencies of 145 MHz and 234 MHz for ZnO/SiO₂/Si, both with and without the presence of the SA. Additionally, a single

dominant acoustic mode at 149 MHz was observed for ZnO/Si, again with and without SA, as illustrated in Figure 7.5.

The wave propagation behavior, with or without the SA, was elucidated in terms of the S-parameter, as shown in Figure 7.6. The S_{21} parameter, transmission between incident power wave and power wave of a network system, was examined. It was noted that the presence of SA slightly enhanced transmission by approximately 2 dB, and no significant change in the S_{21} signal was observed.

Table 7.5 Mechanical and electrical boundary conditions used in 2D Full simulation model of figure 7.2a

Boundary	Mechanical boundary conditions (Solid Mechanics, solid)	Electrical boundary conditions (Electrostatics, es)
Γ_1	Free	Ground (Port 1 and Port 2) Terminal 1 (P = 1 w) Terminal 2 (P = 1 w)
$\Gamma_{L4}, \Gamma_{R4}, \Gamma_5$	Low-Reflecting	Zero charge
Γ_2	Piezoelectric Material	Charge Conservation, piezoelectric
$\Gamma_3, \Gamma_4, \Gamma_5$	Linear electric material	Charge Conservation

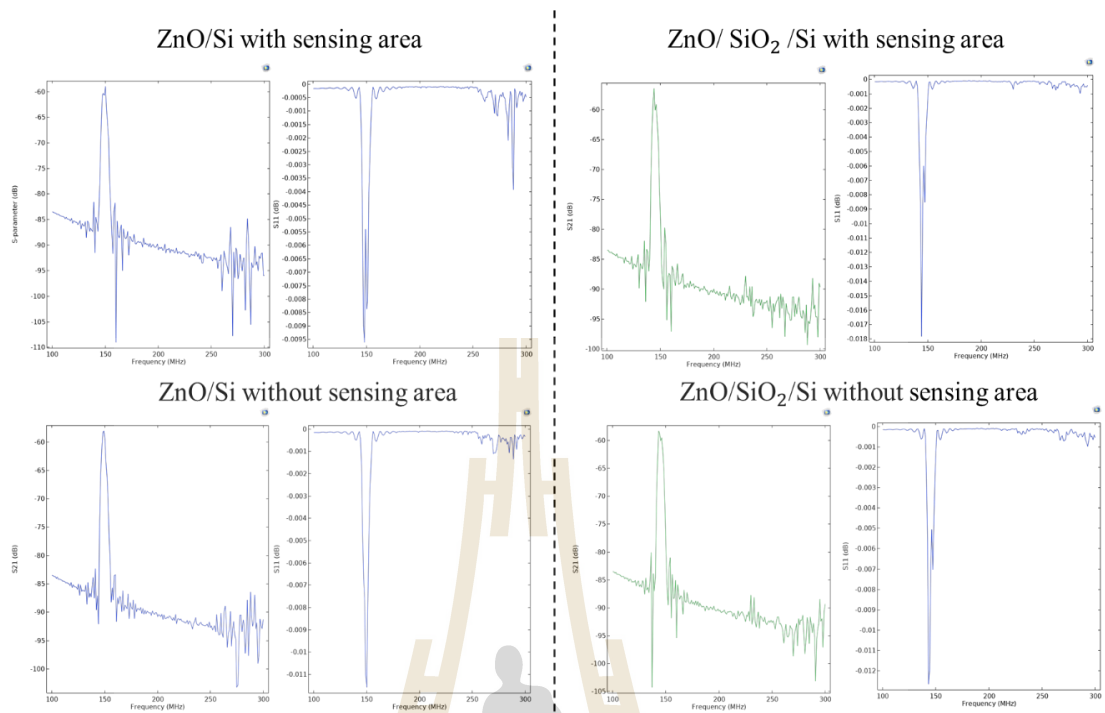


Figure 7.4 The simulation results of the 2D full model are presented, showing S_{11} and S_{21} with and without the sensing area.

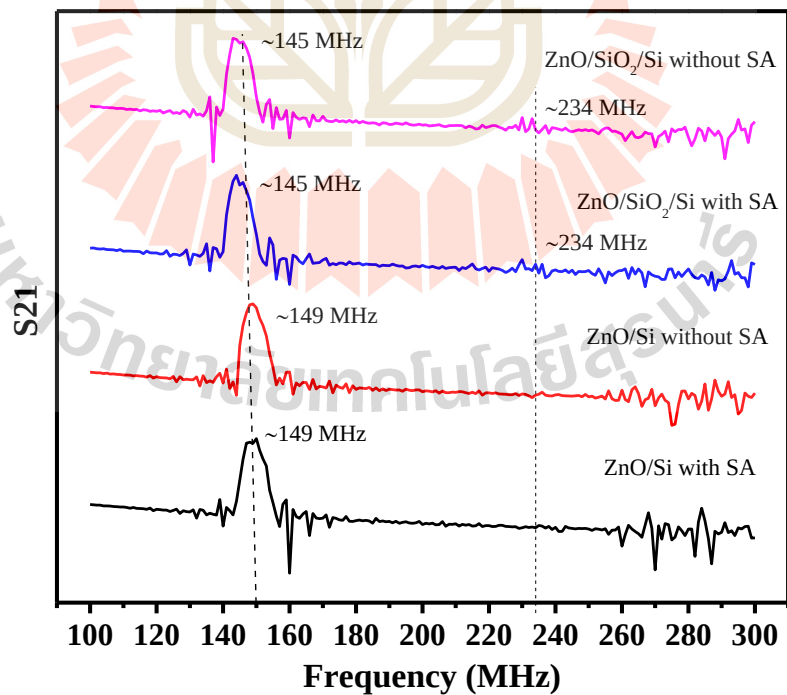


Figure 7.5 The center frequencies of the Simulation result of the 2D full model of S_{21} .

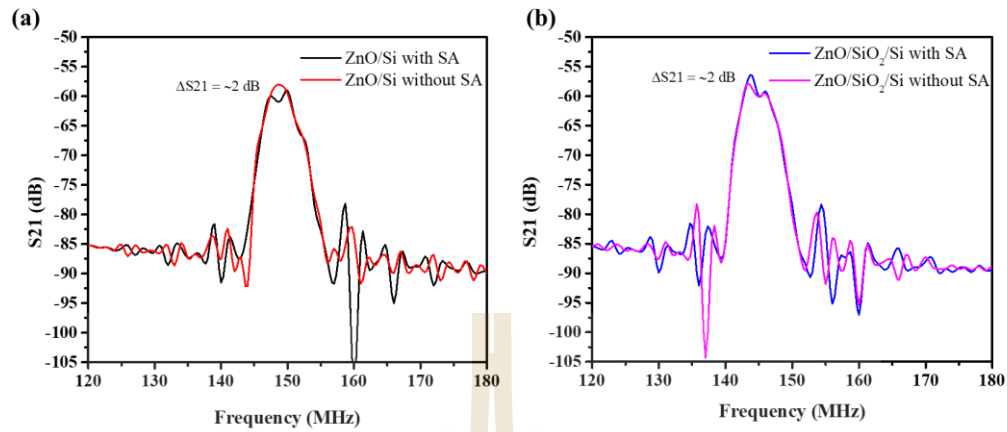


Figure 7.6 The change in S_{21} was observed under the condition with and without SA.

7.3.1.2 Three-Dimension (3D) FEM Analysis: Primitive Cell

The propagation characteristics of the ZnO SAW device were examined through 3D FEM analysis for an eigenfrequency study, focusing on the vibration patterns of particles. Rayleigh and Sezawa mode SAW occurrence and resonance frequencies were identified by analysing mode shapes of total displacement profiles at various eigenfrequencies. Detailed boundary condition settings for the 2D primitive cell are provided in Table 7.6. Figures 7.7-7.8 depict the surface displacement at each frequency for ZnO-based SiO₂/Si and Si, respectively. The resonant frequency for both ZnO-based Si and SiO₂/Si were explored. From the simulation ZnO/Si results as shown in Fig.7.7, Rayleigh mode frequencies were found at 135.6 MHz (Anti-symmetric resonance frequency), as shown in Fig.7.7a. And 143 MHz (Symmetric resonance frequency), as shown in Fig.7.7b. For ZnO/SiO₂/Si, only Rayleigh mode frequencies were observed at 130 MHz (Anti-symmetric resonance frequency), as shown in Fig.7.8a and 140 MHz (Symmetric resonance frequency), as shown in Fig.7.8b. It can be seen that the resonance frequency of the SAW device with ZnO/Si film is larger than that of the SAW device with ZnO/SiO₂/Si.

Table 7.6 Mechanical and electrical boundary conditions used in 3D primitive cell simulation model of figure 7.2b.

Boundary	Mechanical boundary conditions (Solid Mechanics, solid)	Electrical boundary conditions (Electrostatics, es)
Γ_1	Free	Zero charge Ground (IDT _{Ground}) Terminal 1 (IDTP = 1 w)
Γ_2	Piezoelectric Material	Charge Conservation Periodic Condition 2: Continuity
$\Gamma_3, \Gamma_4, \Gamma_5$	Linear Elastic Material Periodic Condition 2: Continuity	
$\Gamma_{L1}, \Gamma_{L2}, \Gamma_{L3}, \Gamma_{L4}$ $\Gamma_{R1}, \Gamma_{R2}, \Gamma_{R3}, \Gamma_{R4}$	Periodic Condition1: Continuity	

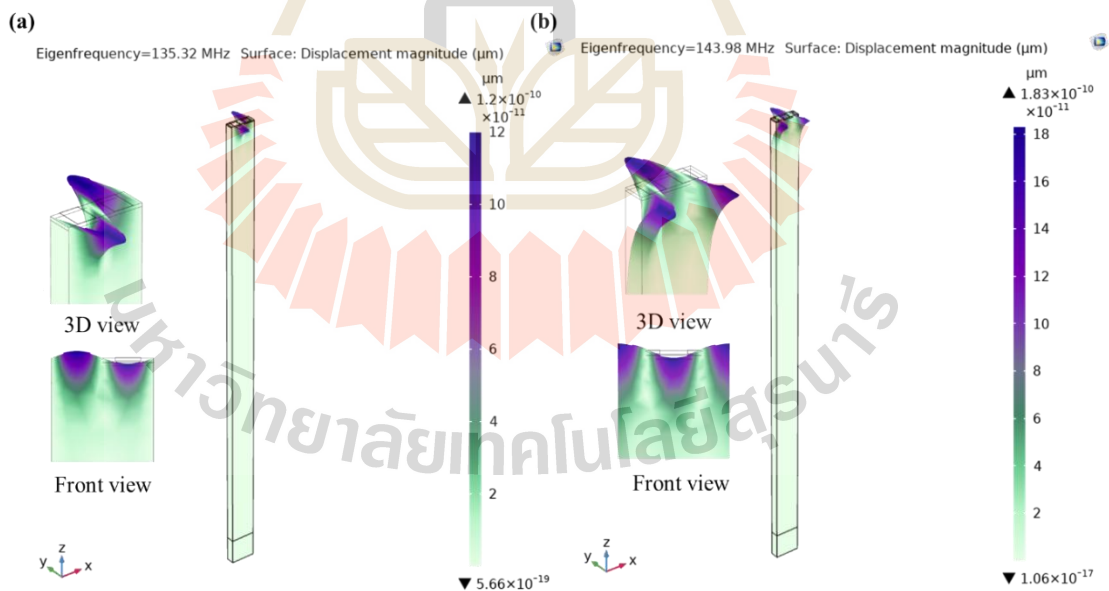


Figure 7.7 Eigenfrequency modes of the ZnO/Si SAW structure.

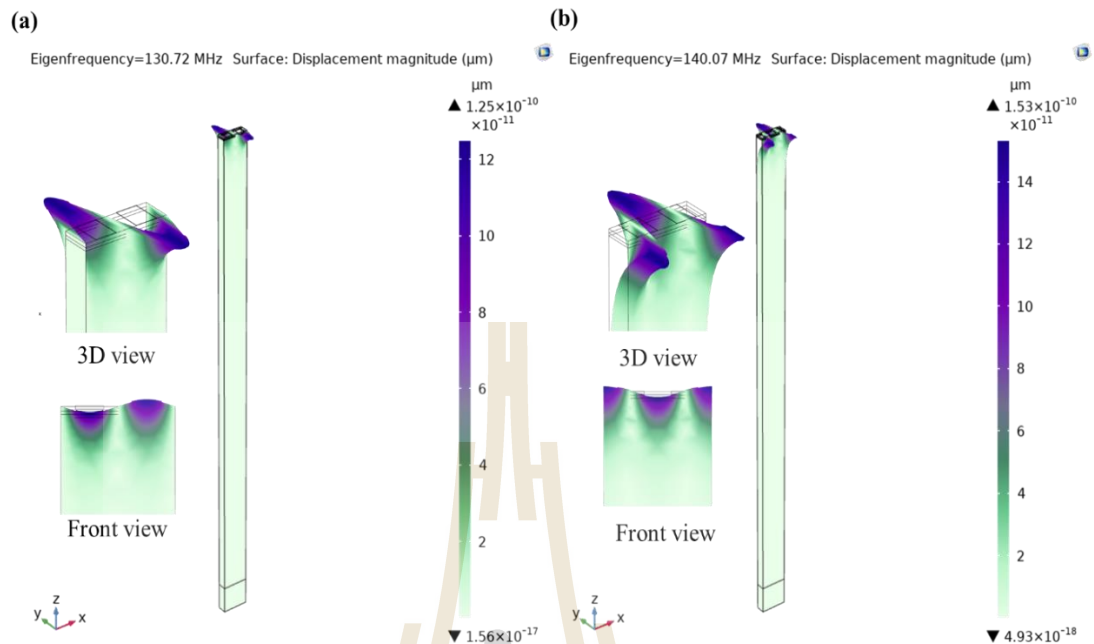


Figure 7.8 Eigenfrequency modes of the ZnO/SiO₂/Si SAW structure.

7.3.2 Morphology and Structure Characterizations

Figure 7.9 presents a surface and cross-sectional SEM image of the SAW device based on ZnO/SiO₂/Si and ZnO/Si structure. The surface morphology of the deposited film shows a relative homogeneous size distribution and shaped grains of size up to 110 nm, mixed with small irregular flakes of approximately the grains of size up to 140 nm. The thickness of the ZnO film on SiO₂/Si and Si substrate are approximately 1.3 μm.

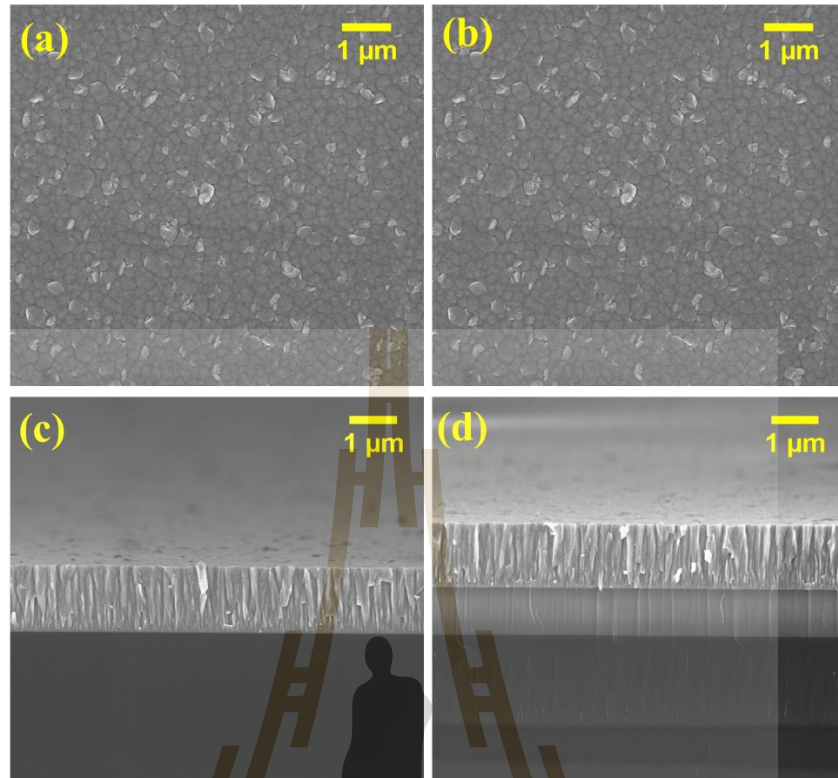


Figure 7.9 (a,b) Surface and (c,d) Cross-sectional image of ZnO/SiO₂/Si and ZnO/Si respectively.

7.3.3 Experimental Results

The IDTs was fabricated using a Micro Writer ML3 Durham (Markless), as depicted in Figure 7.10a. The design after the lift-off process is shown in Figure 7.10b. The S_{21} of the ZnO SAW devices was measured using a grounded-signal-ground (GSG) probe connected to a VNA, as illustrated in Figure 7.10 (c to d). Prior to measuring the scattering parameters (S parameters) of the fabricated SAW device, the VNA underwent calibration using a two-port calibration standard, including open circuit, short circuit, and load calibration, to minimize the impact of parasitic parameters generated by the RF cable and probe test equipment.

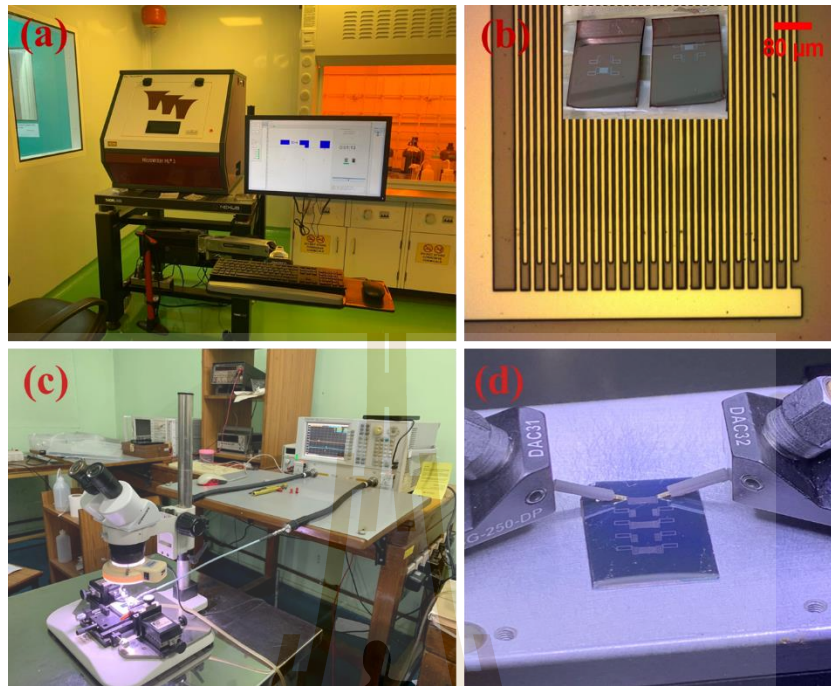


Figure 7.10 (a) Photolithography instrument, (b) Optical image of IDT line after developed, (c) Network analyzer connected with wafer probe station, and (d) ZnO SAW device with measurement setup.

Figure 7.11 presents the experimental frequency response, denoted as S_{21} , of two-port ZnO-SAW devices measured using a VNA. The resulting ZnO/SiO₂/Si SAW devices exhibit a resonant frequency of 145 MHz, corresponding to a wavelength of 30 μm, which aligns well with simulation results ($f_0 = 145$ MHz). The phase velocity of the Rayleigh wave was calculated to be 4350 m/s. Additionally, the Sezawa mode is detected at a frequency of 238 MHz.

Another SAW resonator fabricated on ZnO/Si demonstrates a resonant frequency of 163 MHz without any discernible peak in higher modes, representing a slight deviation of 14 MHz from the simulated frequency results ($f_0 = 145$ MHz). The Rayleigh wave's phase velocity on ZnO/Si is calculated at 4320 m/s. The result indicates that the presence of a sensing area layer slightly enhances transmission by approximately 2 dB, consistent with the simulation results. The appearance of the Sezawa mode is anticipated when the phase velocity of sound, determined from the resonance frequency, is greater in the substrate

than in the overlayer. The confirmation that the second mode is the Sezawa mode results from the S-parameter analysis from simulation and experimental results.

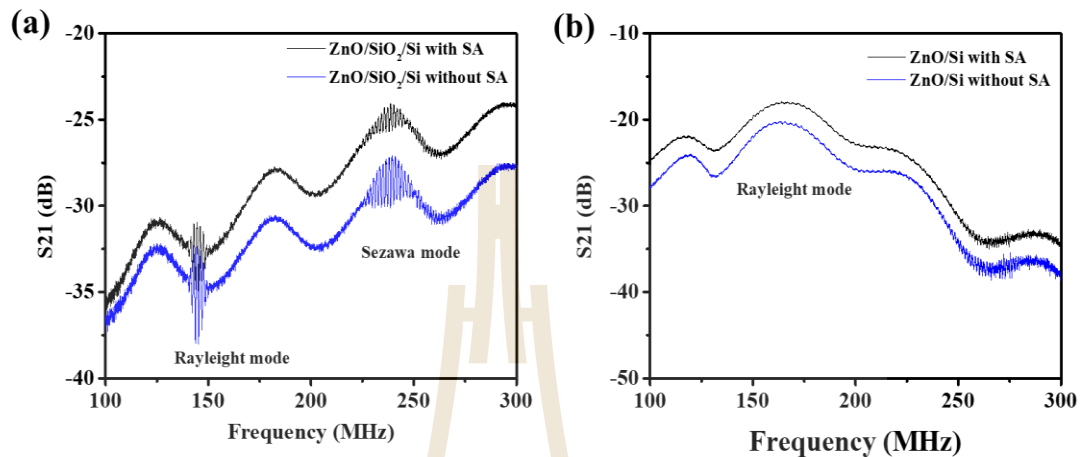


Figure 7.11 The measured S_{21} results obtained via a VNA instrument for ZnO SAW devices.

7.4 Chapter Summary

The author has proposed an approach for designing SAW structures utilizing ZnO/SiO₂/Si and ZnO/Si configurations, with and without a sensing area. This study integrates simulation, morphology analysis, and experimental data to evaluate ZnO-SAW devices. Simulations reveal acoustic modes and enhancements in S_{21} attributed to the sensing layer. Morphological analysis confirms that the prolongation and low power deposition help achieve uniform grain distribution. The experimental findings closely align with simulated resonance frequencies, deepening our comprehension of SAW device efficacy with a sensing area integrated into the structure. This suggests promising avenues for biosensing applications. The S_{21} results from both approaches align well with each other and with simulations and experiments, showing a resonant frequency of 145 MHz of Rayleigh mode, with the detection of the Sezawa mode at 238 MHz, closely matching the simulated 234 MHz. The Sezawa waves represent higher-order SAWs propagating on layered SAW devices where the substrate's acoustic velocity exceeds that of the deposited piezoelectric thin film. Despite S_{21} values, both structures (with and without a sensing area) exhibit low power transfer to the receiving

IDT, indicating significant power absorption in the designs. This issue requires attention in future iterations to improve wave propagation with minimal insertion loss from the input to the output power port. Nevertheless, the approaches for designing SAW devices enable quick and accurate prediction of frequency responses during the simulation stage, reducing the need for experimental iterations and enhancing development efficiency.

7.5 References

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CHAPTER VIII

CONCLUSION AND FUTURE PERSPECTIVES

The conclusion of the research work and the perspectives of the potential future research for improving the biosensors are presented in this chapter.

8.1 General Conclusions

This research focused on developing piezoelectric acoustic biosensors, specifically QCM and ZnO-SAW sensor, for cancer biomarker detection. The aim is (1) to employ a piezoelectric QCM sensor for cancer detection and improve its performance by integrating nanomaterials, and (2) to create an alternative piezoelectric sensor for incorporation into SAW devices. The study explored the impact of nanomaterials on the sensitivity for detecting the PSA biomarker for PCa. It also investigated the effects of GLAD and post-annealing temperature on the piezoelectric ZnO films with Si-based substrates. Furthermore, the ZnO-SAW results obtained through modeling using COMSOL Multiphysics and experimental fabrication were analyzed.

8.2 Summary of Outcomes and Implications

Based on the studies outlined in the previous five chapters, the main thesis conclusions are summarised for the detection of PCa via PSA biomarker using a piezoelectric biosensor, the response of resonance frequency signal through inducing nanostructures, and the effect of chemical treatment on the piezoelectric biosensor electrode surface, the optimization of ZnO for SAW using GLAD and post-annealing, and the simulation integrated with experimentation for SAW fabrication.

The initial hypotheses for this study, are used as the basis for this section. These are:

(1) PSA could be detected through resonance frequency shift measurements using the piezoelectric biosensor QCM.

(2) The output frequency could be influenced by nanomaterials, leading to enhanced resonance frequency shifts detected via frequency shift measurements.

(3) The size of nanomaterials can impact the sensor's performance in biomarker detection. QCM-based nanomaterials could provide better signals than pristine QCM sensors.

(4) Chemical treatments can influence the efficiency of biomarker detection.

(5) The microstructure of ZnO for SAW devices could be affected by GLAD and annealing temperature.

(6) SAW could be modeled via COMSOL Multiphysics, and the simulated results align well with experimental data.

The experimental results obtained from the thesis work corroborate the hypothesis, as delineated below.

8.2.1 Detection of PSA Biomarker via Nanostructure-Modified Piezoelectric QCM Biosensors

The preliminary results presented in Chapter 3 demonstrate the enhanced detection of the PSA biomarker through surface-modified QCMs using the DC electrodeposition technique. It was observed that increasing the electroplating time led to higher surface roughness and surface area values, thereby improving the sensitivity of detection compared to the pristine QCM. These results support the hypothesis that the PSA could be detected through resonance frequency shift measurements using the piezoelectric QCM biosensor. The hypothesis that the output frequency could be influenced by nanomaterials, leading to enhanced resonance frequency shifts detected via frequency measurements is confirmed to be true by the results and analysis presented in Chapter 3 and Chapter 4. From the overall results of Chapter 3, the introduction of Gold (Au) nanostructures significantly enhanced resonance frequency shifts, which was confirmed through frequency shift measurements and changes in surface morphology. SEM and AFM analysis confirmed the formation of nanostructures. Specifically, the sensitivity of QCMs with electrodeposited surfaces for 30 and 60-minutes was found to be 3 and 5 times higher than the pristine QCM sample, respectively. Notably, QCMs with a 60-minute deposition exhibited exceptional properties, achieving the highest resonance

frequency shift ($|\Delta f|$) for PSA detection at 483 Hz. However, it was noted that the DC electroplating technique led to the continuous deposition of Au ions onto the QCM electrode surface, resulting in significant frequency shifts. Specifically, observations of 255 kHz and 592 kHz of $|\Delta f|$ were recorded after 30- and 60-minute deposition times, respectively. Furthermore, there were instances where the nanostructures deposited on the Au-QCM were noted to detach from the quartz electrode surface during the cleaning procedure, which was addressed and presented in Chapter 4.

8.2.2 Improvement of AuNPs-modified Piezoelectric QCM Biosensors for PSA Biomarker Detection

Chapter 4 provides further support for the hypothesis that the size of nanomaterials can impact the sensor's performance in biomarker detection and the QCM-based nanomaterials may offer superior signals compared to pristine QCM sensors. PCD was introduced to address challenges associated with excessive nanomaterial loading. This innovative approach creates a distinct structure with minimal nanoparticle mass loading on the QCM electrode surface. Analysis using XRD, AFM, SEM, and XPS confirmed the formation of gold with a unique nanospike (AuNPs) structure, free from contamination by other elements. The size of nanostructures varied depending on the duty cycle of PCD, with higher duty cycles resulting in larger nanostructures. Improved Au formations on the QCM maintain a similar crystal structure while creating exceptional nanostructures on the electrode surfaces, demonstrating minimal mass loading post-electrodeposition. Frequency shifts of 15% and 20% were observed at 20% and 80% duty cycles, respectively, compared to the total frequency shift (100%) observed for the 30-minute DC deposition in Chapter 3. Under PSA detection, the 20% and 80% duty cycles of PCD exhibited larger frequency shifts than the pristine samples at the same PSA concentration. This underscores the potential of QCM-based nanomaterials to provide better signals than pristine QCM sensors. The observed frequency shift demonstrates the ability of the AuNPs-modified QCM biosensor at 20% duty cycle to detect PSA at various concentrations, with a remarkable LOD of 24 pg mL^{-1} and a linear range of $0.1\text{-}100 \text{ ng mL}^{-1}$. Additionally, the hypothesis that chemical treatments can influence biomarker detection efficiency, and the impact of increased surface area on functionalized QCM electrodes on PSA

detection, is confirmed by the results from FT-IR and wettability analyses. These analyses provided insights into chemical treatments on QCM electrode surfaces for biomolecule immobilization, confirming the presence of functional groups for further bio-immobilization. Wettability studies confirmed chemical treatment on electrode surfaces, modifying their hydrophobic properties to aid bio-immobilization by enhancing their hydrophilic properties.

8.2.3 Optimization of ZnO Piezoelectric Films via GLAD and Post-annealing Temperature.

Chapters 5 and 6 affirm the research hypothesis that the microstructure of ZnO for SAW devices is influenced by GLAD and annealing temperature.

In Chapter 5, the significant impact of GLAD on the microstructure of ZnO films is demonstrated. Under the GLAD condition, ZnO films are deposited at various substrate tilt angles with respect to the substrate holder. Different substrate tilt angles induce varied grain shapes and orientations. Flake-like grains are observed due to high deposition rates, a feature observed in all deposited films. The (002) peak shift is attributed to strain in ZnO films generated during deposition, possibly due to columnar grain growth. The tilt angle of columnar grain growth arises from the alignment of sputtered atoms between the target and inclined substrate. In SAW applications, this developed ZnO structure retains larger grain size and lower thickness, posing a potential risk of acoustic wave loss to the substrate and potential damage to the IDT electrode.

Chapter 6 provides further support for the hypothesis that the microstructure of ZnO for SAW devices could be affected by GLAD and annealing temperature. The annealing under specific conditions enhances the crystalline quality and grain size, although issues persist regarding grain uniformity. ZnO films on Si substrates were deposited using the RF sputtering method with varying post-annealing treatments from 150 to 900 °C under oxygen gas. The films exhibit a hexagonal wurtzite phase, with the highest intensity of the (002) plane observed in an annealed sample at 750 °C, indicating improved crystalline quality. A decrease in full width at half maximum (FWHM) and an increase in crystalline size are observed with increased annealing temperature from 150 to 900 °C. Peak shift at the (002) plane and a change

from tensile strain to compressive strain is also observed with increasing annealing temperature. Nano-flake shapes are attributed to high deposition rates, with columnar structures observed at temperature treatments below 750 °C. Additionally, AFM data corroborating with SEM results confirms the change in columnar structure at elevated annealing temperatures.

Based on these results, it can be concluded that temperature treatment under oxygen gas is an influential factor. Annealed ZnO thin films show remarkable performance, with improved grain size and enhanced crystalline quality. Nonetheless, issues persist regarding the uniformity and random size distribution of grains during fabrication and annealing processes. Fine-tuning adjusting parameters is essential to achieve optimal film quality.

8.2.4 ZnO-SAW Modeling and Experiment

Chapter 7 confirms the research hypothesis that SAW can be effectively modeled using COMSOL Multiphysics, with simulated results demonstrating strong alignment with experimental data. The author proposes an approach for designing SAW structures utilizing both ZnO/SiO₂/Si and ZnO/Si film, with and without a sensing area. This comprehensive study integrates simulation, morphology analysis, and experimental data to evaluate ZnO-SAW devices. Simulations uncover acoustic modes and enhancements in S_{21} attributed to the sensing layer, while morphological analysis confirms the importance of prolongation and low-power deposition in achieving uniform grain distribution. Experimental findings closely align with simulated resonance frequencies, deepening our understanding of SAW device efficacy when a sensing area is integrated into the structure. This suggests promising avenues for biosensing applications. The S_{21} results from both approaches closely match each other and simulations and experiments, demonstrating a resonant frequency of 145 MHz for the Rayleigh mode and the detection of the Sezawa mode at 238 MHz, in close agreement with the simulated results at 145 MHz and 234 MHz, respectively. Sezawa waves represent higher-order SAWs propagating on layered SAW devices, where the substrate's acoustic velocity exceeds that of the deposited piezoelectric thin film. Based on these results, it can be concluded that the proposed approach for designing ZnO-based SAW structures, integrated with sensing areas, exhibits a strong correlation

between simulated and experimental results. The simulations effectively predict frequency responses, thus reducing the need for extensive experimental iterations and improving development efficiency for SAW device. However, the S_{21} values being one factor to consider, both structures, with and without a sensing area, show low power transfer to the receiving IDT, indicating significant power absorption in the designs. This issue requires attention in future iterations to enhance wave propagation with minimal insertion loss from the input to the output power port. A comparison between the QCM and the developed SAW device is presented in Table 8.1.

Overall, this research highlights the potential of surface-modified QCMs for sensitive biomarker detection. It underscores the importance of optimizing nanomaterial characteristics and device structures to enhance bio-detection performance. Additionally, the study emphasizes the development of ZnO-based silicon substrates for SAW devices to advance biomarker detection further.

Table 8.1 Comparison of the QCM and the developed SAW device.

	Commercial QCM	The developed ZnO-SAW
Principle	Piezoelectric effect	Piezoelectric effect
Materials	Quartz	ZnO
Electrode substrate	Au - Ti substrate	Au - Cr substrate
Dimension	~ 13.9 mm diameter	~ 6.6 mm width ~ 2 mm height
Thickness	~ 160 μm	~ 527 μm
Fabrication system	N/A	RF magnetron sputtering
Applicable frequency range	10 MHz	145 MHz for Rayleigh mode 238 MHz for Sezawa mode
Measured factor	Resonance Frequency shift	S-parameter shift
Sensitivity enhancement	Nanomaterials modified QCM (e.g., Gold (Au) Nanostructure and Gold Nanospikes (AuNPs) structure)	Further study
Applications	PCa detection via PSA biomarker	Further study

8.3 Future Perspectives

The research outlined in this study aims to advance piezoelectric acoustic devices for future advancements in biosensor technology and cancer biomarker detection. These approaches set the stage for the development of customized gold nanostructure (AuNS)-QCM biosensors for specific biosensing applications in future research. Further investigations into the size of the AuNS structure and fine-tuning the relationship between the developed Au structure on the QCM electrode surface and the sizes of AuNS are essential to enhance QCM efficiency for biomarker detection. Building upon the findings and insights gained, several avenues for further exploration and development could emerge:

8.3.1 Nanomaterial Optimization:

Continued research into optimizing nanomaterial characteristics, such as size, shape, and surface properties, holds the potential for further enhancing biosensor sensitivity and specificity. Exploring novel nanomaterials and fabrication techniques could lead to more efficient detection platforms. A study on the specific nanomaterial size for capturing particular biorecognition molecules is promising for achieving the better detection.

8.3.2 Integration of Multi-Sensing Approaches:

Investigating the integration of multiple sensing modalities, such as optical, electrical, and mechanical, could offer complementary information and improve overall detection accuracy. Combining piezoelectric biosensors with other sensing technologies may enable comprehensive profiling of cancer biomarkers.

8.3.3 Clinical Validation:

Transitioning from commercial biomarker testing experiments to real sample studies represents a crucial step toward clinical translation. Further research is needed to validate biosensor performance in complex biological environments and assess their feasibility for real-time monitoring in clinical settings.

8.3.4 Miniaturization and Portability:

Shrinking biosensor platforms and integrating them into portable devices could revolutionize point-of-care diagnostics. Future efforts should focus on

developing miniaturized, low-cost biosensors that are user-friendly and suitable for widespread deployment, particularly in resource-limited settings.

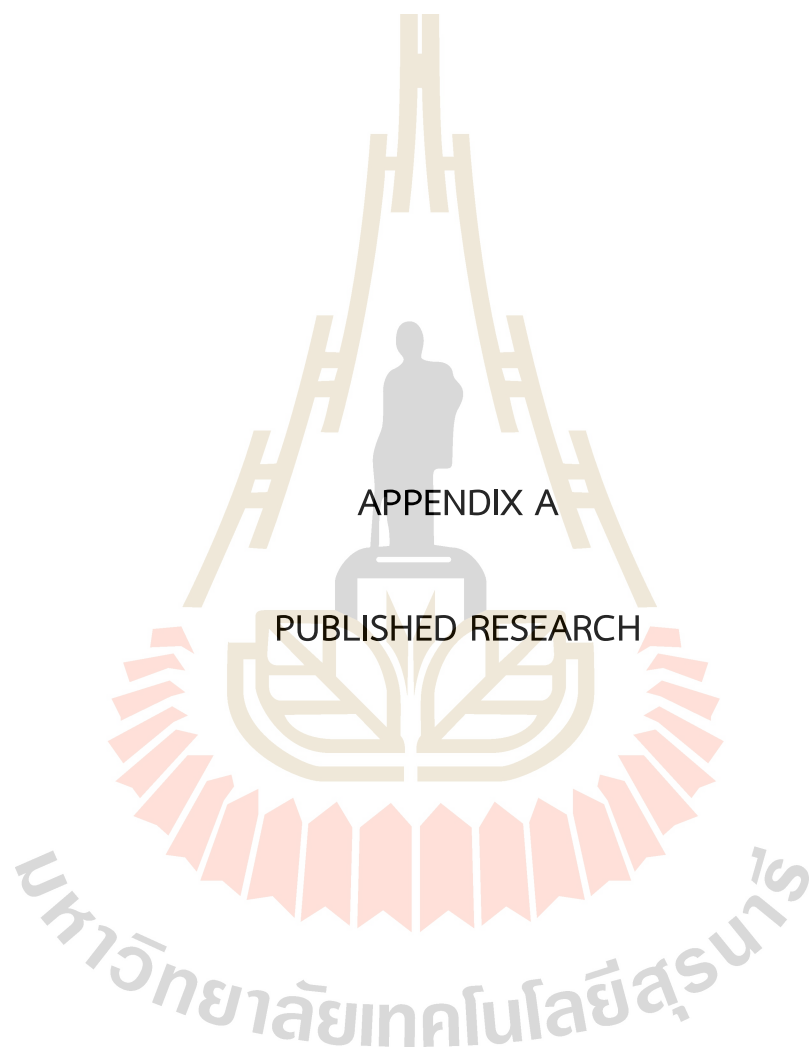
8.3.5 Biocompatibility and Long-term Stability:

Addressing concerns regarding biocompatibility and long-term stability is essential for ensuring the reliability and safety of biosensor devices. Research into biocompatible materials could prolong device lifespan and minimize adverse reactions in biological environments.

8.3.6 Reduction of insertion loss in ZnO SAW device:

Addressing the challenges of low power transfer and high insertion loss is crucial for enhancing the signal response of SAW devices.

Finally, the author hopes that the research presented in this study will lead to advances in the development of piezoelectric acoustic devices for biomarker detection. In particular, the knowledge gained on nanomaterials based on piezoelectric acoustic devices will aid in the roadmap, understanding of chemical interactions, fabrication, characterization, optimization of these materials. Ultimately, the future of biosensor technology for cancer biomarker detection is bright, with opportunities for innovation and impact across various domains.



List of Publication

Sonklin, T., Munthala, D., Suksaweang, S., Janphuang, P., & Pojprapai, S. (2022). Effect of Surface Modified Quartz Crystal Microbalance (QCM) on Detection Sensitivity for Prostate Specific Antigen (PSA). *Integrated Ferroelectrics*, 222, 93-101. doi:10.1080/10584587.2021.1961519

Sonklin, T., Munthala, D., Leuasongnoen, P., Janphuang, P., & Pojprapai, S. (2022). Effect of substrate-tilting angle-dependent grain growth and columnar growth in ZnO film deposited using radio frequency (RF) magnetron sputtering method. *Journal of Materials Science: Materials in Electronics*, 33, 1-10. doi:10.1007/s10854-022-08576-0

Sonklin, T., Tongpeng, S., Munthala, D., Suksaweang, S., Janphuang, P., Bharti, A., Pojprapai, S. (2024). Development of gold nanospikes-modified quartz crystal microbalance biosensor for prostate specific antigen detection. *Surfaces and Interfaces*, 45, 103877. doi:10.1016/j.surfin.2024.103877





Effect of Surface Modified Quartz Crystal Microbalance (QCM) on Detection Sensitivity for Prostate Specific Antigen (PSA)

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ABSTRACT

In this work, the surface-modified quartz crystal microbalance (QCM) was utilized for prostate cancer biomarker detection. The electroplating method was highly utilized to modify the QCMs. The influence of electroplating time, corresponding nanostructure formation and surface-modified electrode sensitivity are investigated. The estimated frequency shift for unmodified QCM is 39 Hz, 30-minute modified QCM is 213 Hz, and 60-minute modified QCMs is 483 Hz. This method is practically convenient to improve the mass sensitivity of the PSA detector. The mass sensitivity of QCM increased with electroplating time.

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Quartz crystal microbalance (QCM); electroplating; surface modification; PSA

1. Introduction

The biosensor is a miniaturized device composed of a sensor for a physio-chemical transducer [1]. The biological components like antibody, antigen, enzyme, nucleic acids (DNA, RNA), and tissues are the biosensor analytes [2]. Clark and coworkers developed the first biosensor in the early 1960s [3]. Later, many biosensors evolved like voltammetric, potentiometric, optic, and piezoelectric techniques based on the analyte, requirement, and kind of transducer [4]. In this work, the piezoelectric sensor, quartz crystal microbalance (QCM), is reviewed as a suitable tool for label-free direct assay application. Quartz crystal (QC) is used as a piezoelectric resonator in QCM. The resonance frequency change with a small mass (usually in nanograms) is the core technology in QCM biosensing [5]. QCM sensors have been extensively used in various real-time biosensing applications, such as the health sector [6], environment monitoring [7, 8], food inspection [3], clinical diagnostic [9], etc. Theoretically, the resonance frequency shift and the mass sensitivity of QCM can be estimated by using Sauerbrey's equation [10] as given below:

$$\delta f = -\frac{2f_0^2}{\sqrt{\mu_p \rho_q A}} \delta m \quad (1)$$

Here f_0 is the resonance frequency, δf is change in resonance frequency (or) frequency shift (Hz), δm is mass change (ng), A is active area of the QCM electrodes, ρ_q is the density of quartz (2.648 g cm^{-3}), and μ_q is the shear modulus of quartz (for AT-cut quartz, $\mu_q = 2.947 \times 10^{11} \text{ g cm}^{-1} \text{ s}^{-1}$), respectively. In this equation, the resonance frequency shift is proportional to the addition of mass (δm) and inversely proportional to the area under the working electrode. The sensitivity of QCM electrode can be improved by (1) decreasing the quartz crystal thickness, (2) decrease the active electrode area of quartz crystal, and (3) nanostructuring by surface modification method. Recently, several researchers reported that the nanostructuring of QCM electrodes improves the QCM sensitivity [11–15].

In this work, the sensitivity of QCM after electrode surface modification by electroplating was investigated. The effect of surface modification (improved surface area and roughness) will be discussed in detail. Prostate-specific antigen (PSA) and its counterpart antibody are the standard biomarkers of prostate cancers. Since decades, prostate cancer is the leading cause of cancer-related death in men. The early detection of this anomaly is one way to improve the diagnostic ability, and addressing this issue is highly needed. Atomic force microscopy (AFM), scanning electron microscopy (SEM), and x-ray diffraction (XRD) techniques were utilized to characterize the surface modified QCM electrode.

2. Materials and Method

The QCM kit and standard AT-cut quartz crystals (QCs) were purchased from Novaetech S.r.l. (Italy) to conduct the proposed experiment. The QCs specifications are 10 MHz resonance frequency, 13.9 mm disk (quartz crystal) diameter, and 6 mm electrode diameter. Before the electroplating, QCs were cleaned by using piranha solution (70% of H_2SO_4 , 30% of H_2O_2 , and DI-water in 3:1:1 ratio) for 5 min, followed by deionized (DI) water several times and finally dried with nitrogen (N_2) gas blow. The chemical solutions: Sulfuric acid (H_2SO_4 , Anapure, New Zealand), Hydrogen peroxide (H_2O_2 -30%, Qrec, Nong Khaem 10160, Bangkok), were purchased from the referenced companies. In order to modify the surface area (by electroplating method), a Gold (Au) electrolyte (IntelliChems, Bangkok, Thailand) was used. During the electroplating, both QC (as a cathode) and platinum grating mesh (as an anode) were immersed in the electrolyte solution for 30 and 60 min and applied a 20 mA/cm^2 current density. The sketch diagram of the whole electroplating process is shown in Fig. 1. Electroplating of QCs performed at Micromachining Laboratory, Synchrotron Light Research Institute (SLRI), Thailand.

Soon after the electroplating, a linker group solution (10 μl of protein-A with 1 mg/ml concentration) was added to the electrode surface and incubated for 24 hrs. After Protein-A incubation, 2 μl of PSA antibody (0.5 mg/ml) was added to the QCs and then incubated for 1 hr. A blocker solution (20 μl of 3% BSA) was added to the QCs to block the nonspecific bindings and incubated for 2 hr. Finally, 2.5 ng/ml of PSA (in 1X diluted PBS) was added to the QCs and incubated for 1 hr at ambient temperature to allow antibody-antigen interaction. Polyclonal PSA (Saint Louis, MO 63103, USA), protein A (Saint Louis, MO 63103, USA), PSA antibody (Assaypro, St. Charles, MO, US), Bovine

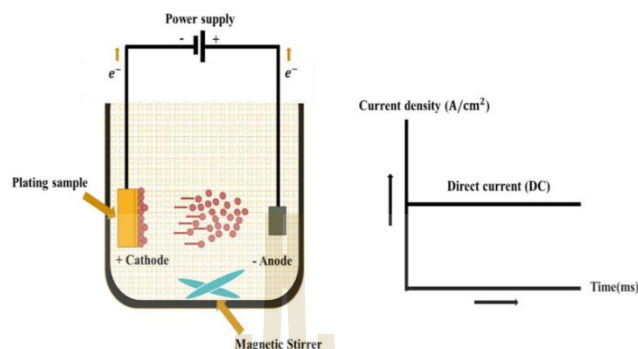


Figure 1. Schematic representation of the electroplating method.

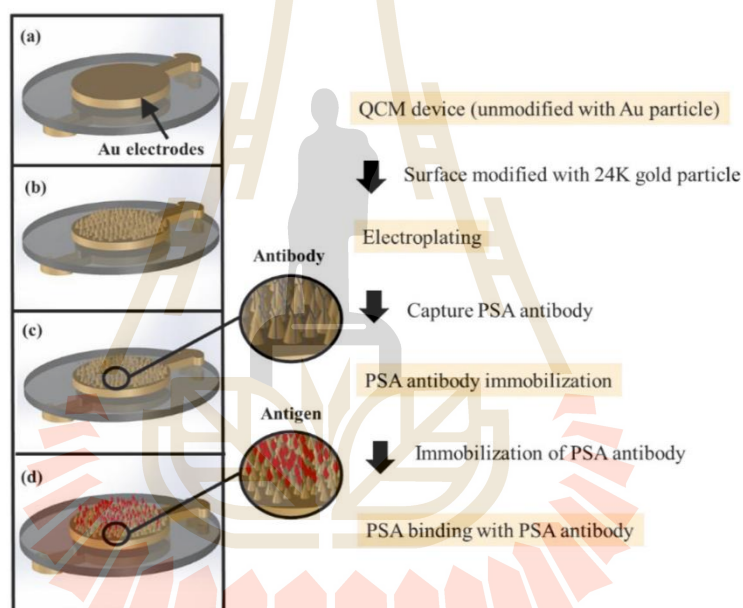


Figure 2. The surface modification and PSA antibody-PSA interaction on QCs: (a) pristine QCs device, (b) deposition of gold through electroplating to develop gold on QCs electrode surface, (c) PSA antibody immobilization, and finally (d) PSA binding with PSA antibody.

Serum Albumin (Billerica Ma, USA), and Phosphate-buffered saline (Calbiochem, La Jolla, CA) were purchased from Sigma Aldrich. After every incubation process, QCs were washed in DI water carefully to remove the excess biological parts. The electroplating process, mobilization, and immobilization of antibody-antigens are represented in a schematic diagram as shown in Fig. 2. Figure 2(a) describes a pristine quartz crystal with an unmodified electrode surface; Fig. 2(b) presents the surface modified QC by the electroplating method; Fig. 2(c) illustrates the mobilization of the PSA antibody onto the modified electrode surface; and Fig. 2(d) shows the immobilization of the PSA.

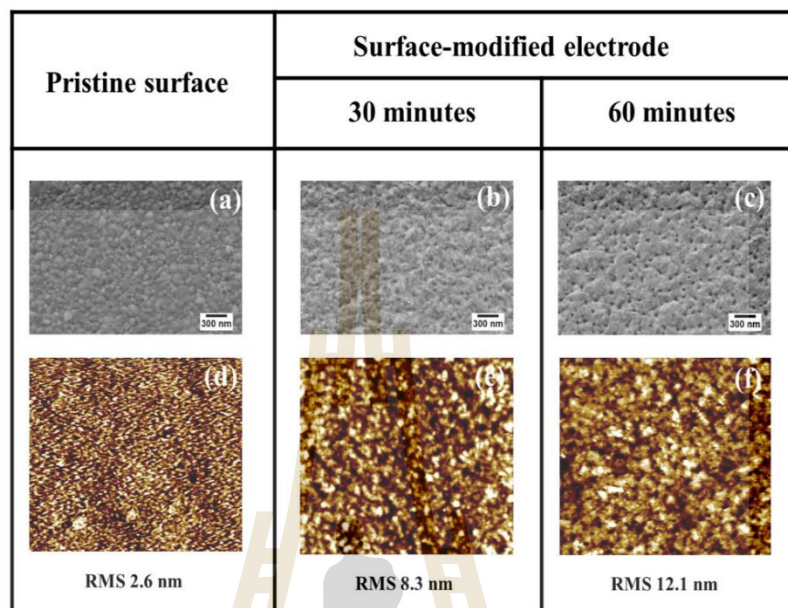


Figure 3. Surface morphology of electroplated QCs (SEM and AFM images): (a, d) pristine QCs, (b, e) 30-minute electroplated QCs, and (c, f) 60-minute electroplated QCs.

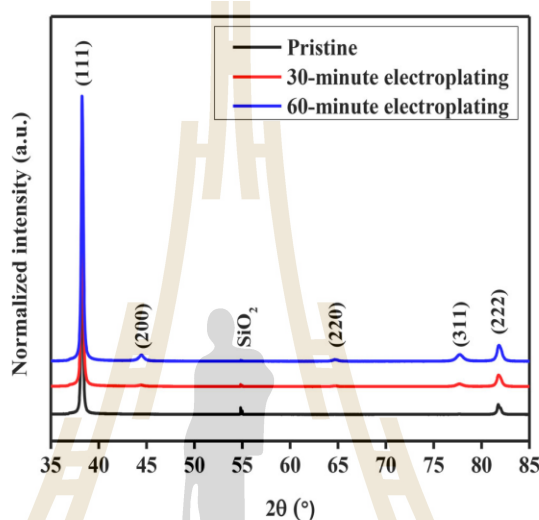
Scanning electron microscope (SEM, JEOL JSM-6010LV), Atomic force microscope (AFM, Park Systems AFM XE-120), and X-ray diffraction (XRD, Bruker D8 ADVANCE) techniques were employed to characterize the modified and unmodified QCs. The resonance frequency of both modified, unmodified QC was recorded using the OpenQCM analyzer and analyzed using openQCM software.

3. Results and Discussion

The surface morphology, the surface roughness of unmodified and modified QCs were characterized using SEM and AFM techniques, as shown in Fig. 3. Unmodified QC electrode surface showed more uniform nanostructure, smoother, relatively smaller grain sizes than that of surface-modified, as shown in Fig. 3(a)–(c). During the electroplating, the Au ions in the electrolyte get deposited on the QC electrode surface, and consequently, the nanostructure formed on the surface. With increasing electroplating time from 30- to 60-minute, the nanostructure formation increases. The measured root mean square (RMS) values of unmodified QC, 30-minute electroplated QC, and 60-minute electroplated QC are 2.6, 8.3, and 12.1 nm, respectively. It is seen that the roughness increased with electroplating time, as shown in Fig. 3(d)–(f). The surface area of nanostructured QCs was calculated from measured RMS roughness values. The estimated surface area values of unmodified QC, 30- and 60-minute electroplated QCs are 25.07, 25.58, and 26.53 nm², respectively, with respect to the increase of electroplating time. The measured RMS roughness and surface area values are also depicted in Table 1.

Table 1. The summary of RMS and surface area values estimated from the AFM topographical images.

Sample and condition	RMS (nm)	Surface area (nm ²)
Pristine	2.6	25.1
30-minute	8.3	25.6
60-minute	12.1	26.5

**Figure 4.** XRD patterns of pristine and electroplated QCs.

The XRD spectra of unmodified and modified QCs electrode surfaces are shown in Fig. 4. The XRD scan range was from 20° to 80° of 2θ , and the resolution was 0.02° . The diffraction pattern peaks at 38.2° and 81.8° of 2θ correspond to the (111) and (222) crystal planes. These XRD diffraction patterns are matched with JCPDS file 00-004-0784 for Gold (Au). The peak at 54.9° corresponds to (222) plan and is assigned to the hexagonal phase of SiO_2 (Quartz) and is well-matched with JCPDS file 00-02-0458. Due to electroplating, a significant enhancement in the (111), (200), (220), (311), and (222) diffraction peak which belongs to Au and the intensity of peaks increased with electroplating time. The preferential growth (peak at 38.2° of (111) plane) was observed after the electroplating process.

The resonance frequencies, corresponding shifts of 30- and 60-minute electroplated QCs are mentioned in Table 2 and shown in Fig. 5. Owing to the increase of the electroplating time (30- and 60-minute), the resonance frequency shift increased, and it is in agreement with the Sauerbrey equation. A -255 kHz of frequency shift was observed for the 30-minute electroplated QC, and -592 kHz of frequency shift was observed for 60-minute electroplated QC, compared to the resonance frequency of the pristine QC.

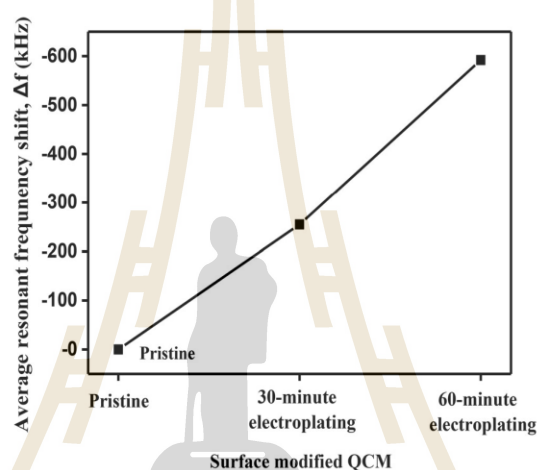
Further, PSA antibody was added to the protein-A incubated pristine QCs and surface modified QCs (of both 30- and 60-minute) and incubated for 1 hr. The corresponding frequencies are -262 Hz , -255206 and -491415 Hz with respect to the pristine resonance frequency. Further, 2.5 mg/ml of PSA has added to the antibody incubated

Table 2. The resonance frequency shifts at different stages of the experiment.

Sample and condition	(1) Frequency shift after surface modification (kHz) ^a	(2) Resonance frequency after adding PSA Antibody (Hz)	(3) Resonance frequency after adding PSA (Hz)	(4) Frequency shift due to Antigen-Antibody interaction (Hz) ^b
Pristine	0	10,008,940.1	10,008,901.0	−39
30-minute	−255	9,760,151.3	9,759,937.5	−213
60-minute	−592	9,515,700.4	9,515,217.3	−483

^aThe resonance frequency of the surface modified samples compared to that of pristine.

^bThe resonance frequency of (3) minus (2).

**Figure 5.** Resonance frequency shifts of electroplated QCs at different times.

QCs and incubated for 1 hr. The corresponding frequencies are -302 , -255419 and -491897 Hz with respect to the pristine resonance frequency. All the corresponding frequency shifts are shown in Fig. 6. The estimated frequency shifts from antibody to antigens are 39 Hz (in case of pristine QCs), 213 Hz (in case of 30-minute QCs), 483 Hz (in case of 60-minute QC), and the same values are mentioned in Fig. 7. These obtained results suggest that the sensitivity of 30- and 60-minute modified QCs is 3 times and 5 times greater than pristine QC. The large surface area with the nanostructured surface might be facilitated the high binding ability between antibody-antibody.

4. Conclusion

In summary, the surface-modified QCs were successfully prepared by using the electroplating method. The surface roughness and the surface area values are increased with the electroplating time. Characterization techniques, SEM, AFM, and XRD, were utilized to confirm the surface morphology, roughness, and crystal structure. The sensitivity of QC of 30-, 60-minute QCs is 3 and 5 times that of pristine QC. These positive results suggest that the surface modification of QCM electrode can be a promising candidate for biomarkers detection.

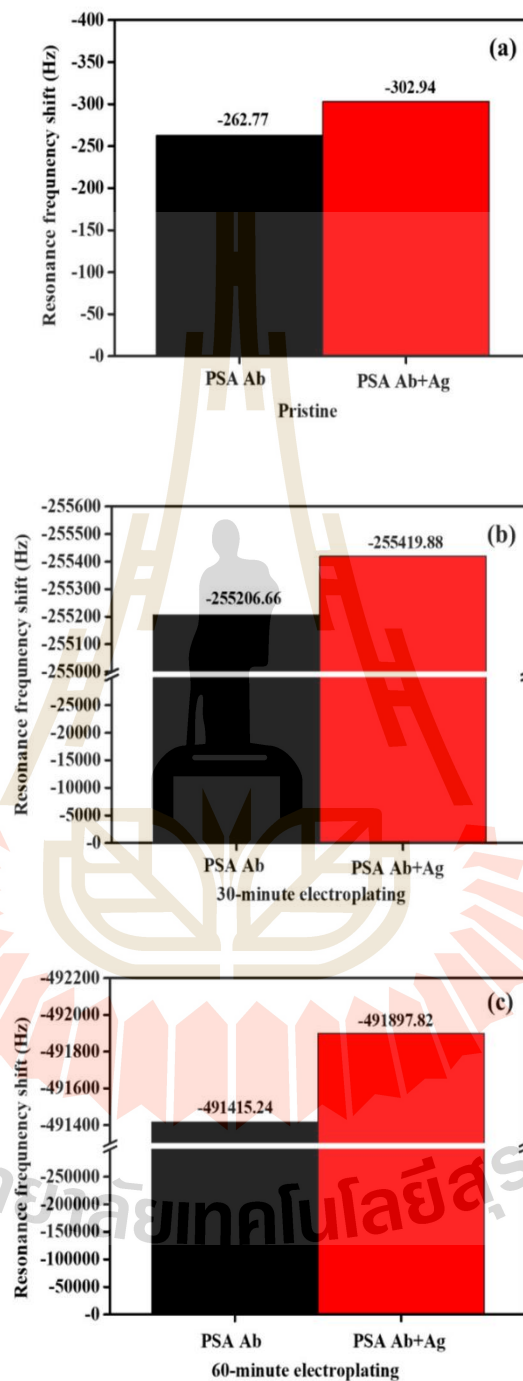


Figure 6. The resonance frequency shifts with respect to PSA antibody and PSA.

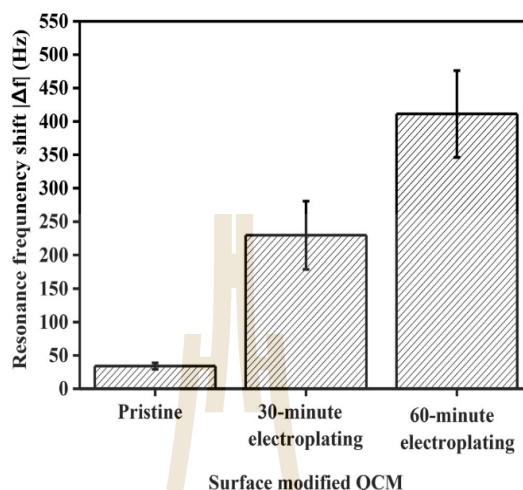


Figure 7. The average of resonance frequencies shifts caused by antigen and antibody binding on the pristine, 30-minute electroplated, and 60-minute electroplated. The test was all at a concentration of 2 mg/ml of PSA.

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Effect of substrate-tilting angle-dependent grain growth and columnar growth in ZnO film deposited using radio frequency (RF) magnetron sputtering method

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ABSTRACT

The present work reports the result of substrate-tilt angle-dependent deposition of Zinc Oxide (ZnO) films using Radio Frequency (RF) magnetron sputtering method. The substrate angle and deposition rate played an important role in the formation of columnar growth direction and grain growth. The ZnO film surface roughness, morphology, crystallinity, and optical properties were studied using Field Emission Scanning Electron Microscope (FE-SEM), Atomic Force Microscopy (AFM), X-ray Diffraction Spectroscopy (XRD), and Ultraviolet-Visible Spectroscopy (Uv-Vis), respectively. The present study reveals that the glancing angle deposition is of paramount importance, which leads to the different growth orientations on ZnO film. This knowledge can apply to ZnO film fabrication to improve the film quality.

1 Introduction

Zinc Oxide (ZnO) is a well-known piezoelectric material that has unique properties, such as wide bandgap energy (3.37 eV), high excitation binding energy, and good piezoelectric properties [1–3]. Therefore, it has been found in various commercial applications, such as gas sensors [4], solar energy conversion [5], ultrasonic transducers [6], ultraviolet laser [7], MEMS actuators [8], and surface acoustic wave devices [9]. There are several deposition

methods that are used to deposit the ZnO films, like physical vapor, chemical vapor, sol-gel, atomic, and molecular deposition techniques [10–14]. Among these, radio frequency (RF) magnetron sputtering [15–17] is suitable for large-scale deposition, ease of use, and useful for mass production. For piezoelectric-based applications, preferred c-axis orientation (002) and low leakage currents are necessary to ensure high-output performance [18]. The best piezoelectric properties can be obtained when all the microcrystals are aligned with the c-axis. However,

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differently oriented grains or c-axis orientation can deteriorate acoustic wave propagation velocity and piezoelectric response [19].

The alternative deposition techniques enable the different growth-oriented nanostructure of films to meet the requirements. Incorporating the substrate-to-target variations, substrate-tilt angle and substrate rotation speed prevalently change the film's physical and chemical properties. Especially glancing angle depositions (GLAD) ensure the flux direction and adatoms motion to grow differently oriented nanostructures. There are several reports on tilt (or) [20–22]. The geometric shadow effect and surface diffusion are the expected mechanisms to form the nanostructures in the above method [23]. The GLAD method provides different nanostructures that can induce the material to alter its properties and these structures can be very useful for a wide range of applications, such as biosensors [24], humidity sensors [25], sensor optics [26], and solar cells [27].

This work attempts to fabricate the films at different deposition angles by varying the substrate angle with respect to the substrate holder. ZnO films are fabricated using RF magnetron sputtering method at different glancing angles from 0° to 90° with respect to the substrate holder. Expect that the specific role of oblique angle deposition influences on growth direction, phase formation, formation of nanostructure, and surface morphology of deposited films.

2 Experimental details

ZnO thin films were deposited on commercially purchased boron-doped P-type Si (100) with a 1–10 Ω -cm resistivity and 1×1 cm² commercially purchased quartz substrates using RF magnetron sputtering method, facilitated at beamline 6a (BL-6a), Synchrotron Light Research Institute (SLRI), Thailand. A 99.99% pure, 3 inch diameter, and 3-mm-thick commercially purchased ZnO sputter target was used to deposit the ZnO films. The substrates were thoroughly cleaned using the Radio Corporation of America (RCA) process before the prior depositions [28]. The pre-cleaned Si substrates and quartz substrates were clamped to the custom-made holder at different tilt angles (0°, 30°, 60°, and 90°) with respect to the substrate holder and the custom-made holder was mounted to the substrate holder in the sputtering chamber. The schematic representation

of the experimental setup is illustrated in Fig. 1a, and sputter deposition conditions are mentioned in Table 1. The optical properties were measured using ultraviolet–visible spectroscopy (UV–Vis, Agilent Cary300). Scanning electron microscope (SEM, JEOL JSM-6010LV) was performed to reveal the surface morphology of ZnO films. The surface topology was investigated using Atomic force microscope (AFM, Park Systems AFM XE-120) in contact mode with a scan area 5×5 μ m². X-ray diffraction (XRD, Bruker D8 ADVANCE) technique was performed to study the crystallite size and crystalline structure.

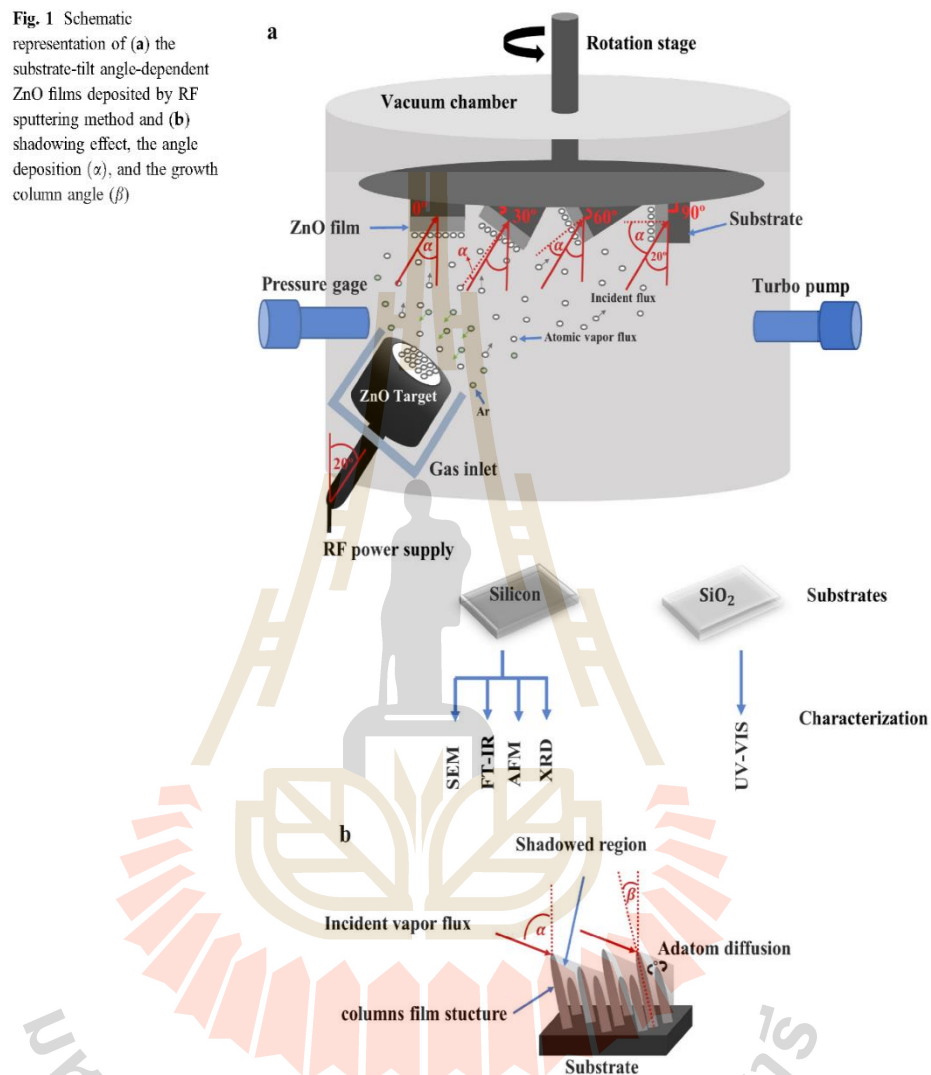
3 Results and discussion

Figure 2 shows the XRD patterns of glancing angle-deposited ZnO films on Si substrates. The scan range of each XRD spectra was measured from 20 to 60° of 2θ . The intensity of diffracted peaks typically depends on the film thickness and crystalline quality of films. The XRD peak positions at 34.4°, 36.1°, and 47.5° of 2θ correspond to the (002), (101), and (102) directions of hexagonal wurtzite ZnO structure. The above peaks are well matching with the ICDD file (PDF# 03-0888). The peak intensities and FWHM are measured to estimate the crystallite size and the same values are plotted in Fig. 3 in terms of deposition angle. The following Debye–Scherrer equation is used to calculate the crystallite size (D) [29],

$$D = \frac{K\lambda}{\beta \cos \theta} \quad (1)$$

where K is the shape factor, taken as 0.94, λ is the incident X-ray wavelength ($\lambda = 1.5406$ Å), β is the full width at half maximum of (002) plane, and θ is the diffraction angle. The calculated crystal sizes are 21, 19, 22, and 24 nm, respectively, for 0°, 30°, 60°, and 90° of substrate-tilt angles deposited film. Seems to be both intensity and FWHM values are high for 30° tilt angle-deposited films compared to other tilt angle depositions. Both values slightly decreased from 0° to 30° and further increased as the deposition angle increased to 90°. As seen above, the crystal size is less for 30° deposition. This might be due to the directional grain growth effect and selectively less for 30° tilt angle. The hexagonal lattice constant (c) and interplanar spacing (d) are calculated by the following equations [30],

Fig. 1 Schematic representation of (a) the substrate-tilt angle-dependent ZnO films deposited by RF sputtering method and (b) shadowing effect, the angle deposition (α), and the growth column angle (β)



$$n\lambda = 2d_{hkl} \sin \theta \quad (2)$$

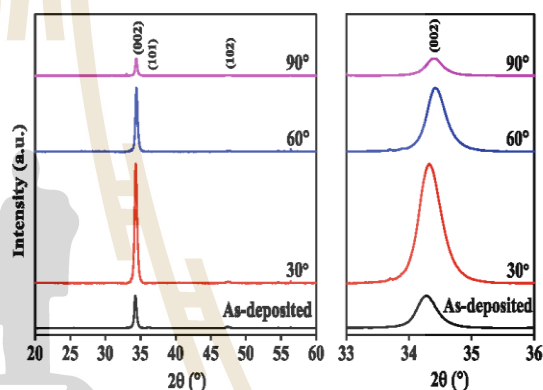
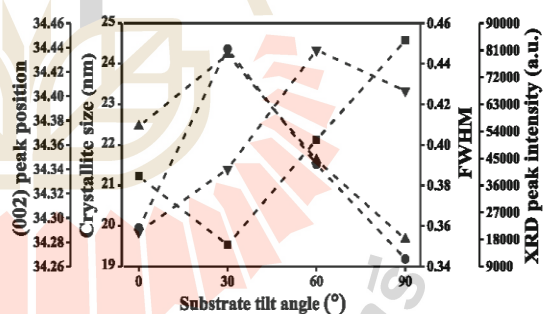
$$\frac{1}{d_{hkl}^2} = \frac{4}{3} \left(\frac{h^2 + hk + k^2}{a^2} \right) + \frac{l^2}{c^2} \quad (3)$$

where h, k, l are the miller index, a and c are the lattice parameters, d_{hkl} is the interplanar spacing, θ is the incident angle, and λ is the incident X-ray wavelength (1.5406 Å). In this case, (hkl) values are considered from (002) peak since it is the dominant peak in XRD

spectra. The estimated hexagonal lattice constant values are 5.226, 5.218, 5.204, and 5.209 Å, respectively, for 0°, 30°, 60°, and 90°-deposited films. Minor changes are observed in the estimated hexagonal lattice constant and its value is decreased with an increase of tilt angle. Also, (002) peak position is shifted for all tilt angle depositions and this might be due to the stress/strain formation in the films [31].

Table 1 RF sputter deposition conditions

Sputtering parameter	Conditions
Target material	ZnO (99.999%)
Target diameter(cm)	3
Sputtering gas	Ar
Base pressure (mTorr)	8.67×10^{-7}
Working pressure (mTorr)	1.54×10^{-2}
RF Sputtering power (Watt)	200
Substrate-to-target distance (cm)	6
Substrate materials	100 P-type Silicon and quartz substrate
Deposition time (hours)	3

Fig. 2 XRD patterns of ZnO films deposited at different substrate-tilt angles**Fig. 3** XRD peak intensities, peak positions, FWHM, and crystallite size values of ZnO films deposited at different substrate-tilt angles

The strain (ϵ) present in the ZnO films can be calculated by the following equation [30],

$$\epsilon(\%) = \frac{c - c_0}{c_0} \times 100 \quad (4)$$

where c is the calculated lattice parameter of the film and c_0 is the lattice parameter of ZnO in bulk (5.206 Å). The estimated strain values are 0.39, 0.24, −0.03, and 0.06, respectively, for 0°, 30°, 60°, and 90°-deposited ZnO films. The above-estimated values are

also mentioned in Table 2. The Lattice strain is related to film stress along the c -axis. A negative value corresponds to the tensile stress, while a positive value denotes the film's compressive stress. A decreasing lattice strain related to film stress implies that a greater number of defects are formed in the ZnO films with an increasing substrate-tilt angle [32].

The surface morphology, grain size, grain shape, film thickness, and growth direction of ZnO films are

analyzed using FE-SEM images and the same images are displayed in Fig. 4. An initial observation shows that the film grains are flake-like structures, and these structures are observed for all the deposited substrate angles. The flake-like structures are generally observable at high sputtering powers and at higher deposition rates. Due to high powers, highly energized Ar ions can provide translational energy to the adatoms, providing high nucleation rates and irregular grain growth [33–35]. It is found that the flake-like grains have sharp edges; grain distribution is irregular, and the average grain size is 156 nm in length and 73 nm in width for 0° tilt angle deposition, as shown in Fig. 4a. In the case of 30° tilt angle deposition, grains merged together and formed a dense surface, and the average grain size is 124 nm in length and 75 nm in width, as shown in Fig. 4b. Similarly, when the substrate is 60° with the substrate holder, the average grain size varies from 55 to 96 nm and apparently, high grain density compared to other depositions is observed, as shown in Fig. 4c. Interestingly, the grain size dropped sharply and sharp edges are not visible in this case of 60° angle deposition. In the case of 90°-deposited films (i.e., the angle between the substrate-to-substrate holder is 90°), the grains are nearly the same as 0°-deposited film, and the observed grain size varied from 145 nm in length and 80 nm width, as shown in Fig. 4d. It is clearly noticeable that the porosity is high for 0° tilt angle deposition, less for 30° tilt angle deposition, and moderate for other depositions. The above behavior of film surfaces depends on nucleation rates and diffusion of adatoms.

A cross-sectional view of angle-dependent deposited ZnO films is analyzed to see growth direction and film thickness, and the corresponding images are shown in Fig. 4e–h. The estimated film thicknesses are approximately 819, 731, 427, and 393 nm, for 0°, 30°, 60°, and 90°-deposited films, respectively. The slight difference in the distance from the target to inclined substrates might induce the difference in incoming flux directions of sputtered atoms on the

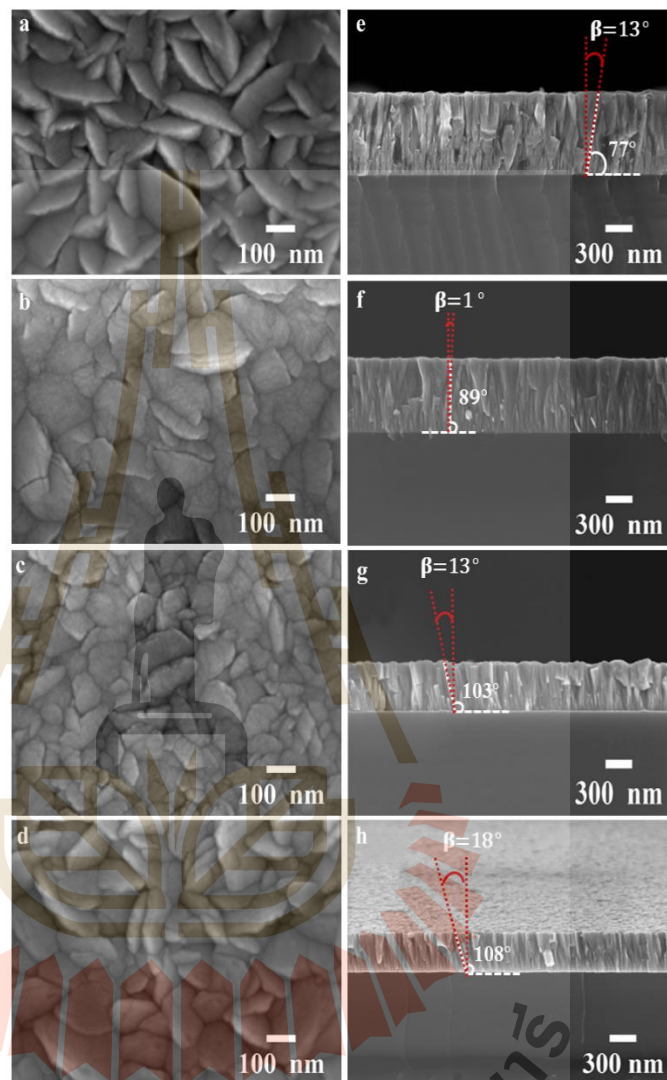
substrates, resulting in film thickness variation. Film thickness is drastically decreased with an increase in deposition angle. At an initial stage, evaporated atoms reach the substrate and condense on the substrate surface to spontaneously form nucleation. The nucleation rate and diffusion length of adatoms provide a necessary situation to create valleys and hillocks. The newly formed valleys and hillocks obstacle for the incoming flux to behind regions and form a tilt columnar nanostructure on the substrate. The valleys are shadowed by neighboring hillocks and are caused by receiving insufficient vapor flux [36]. Moreover, the movement of atom vapor during the substrate-tilt angle along the deposition process is insufficient to reach the substrate surface, leading to a decrease in the film thickness [37].

At the shadow regions, incident vapor atoms arriving to the substrates with deposition angle (α) are prevented by growing peak regions where did not occur nanostructure growth. On the contrary, the peak regions receive enough adatoms flux, resulting in a tilt column nanostructure with different growth angles (β). A schematic representation of the so-called shadowing effect is explained in Fig. 1b. Meanwhile, the surface diffusion and the film surface oppose nanostructure formation by providing smooth surfaces that might decrease the columnar branching [38]. In the present work, the incoming flux direction of deposition angle (α) is approximately 30° with respect to the substrate holder. In the case of 0° tilt angle deposition, the substrate normal is 60° inclined with the incoming flux direction. In this case, as observed from the FE-SEM (Fig. 4e) image, the columnar growth direction is about 13° only. When it comes to the 30° tilt angle deposition, the substrate normal is parallel to the flux direction and the columnar growth direction is almost 90° (as observed in Fig. 4f). Similarly, in the case of 60° tilt angle deposition, substrate normal is 60° inclined with the incoming flux direction and the columnar growth direction is about 13°, as shown in Fig. 4g. Similarly, in the case of 90° tilt angle deposition, the substrate

Table 2 Extracted ZnO films' lattice parameters, strain, film thickness, and RMS roughness values

Tilted angle (°)	C-axis length (Å)	Strain (%)	Thickness (nm)	RMS (nm)
0	5.226	0.39	819	5.40 ± 0.26
30	5.218	0.24	731	4.38 ± 0.21
60	5.204	− 0.03	427	4.73 ± 0.21
90	5.209	0.06	393	5.85 ± 0.07

Fig. 4 The FE-SEM images of ZnO films: **a, b, c, d** surface images belong to 0° -, 30° -, 60° -, and 90° -deposited films. **e, f, g, h** Cross-sectional images belong to 0° -, 30° -, 60° -, and 90° -deposited films



normal is 60° inclined with the incoming flux direction and the columnar growth direction is approximately 18° , as shown in Fig. 4h. The shift of normal substrate-tilt angles provides the columnar inclination. Compared to 0° tilt angle with 90° tilt angle depositions, the total columnar growth rotation is 31° , as observed from FE-SEM images.

The surface roughness of ZnO thin films was analyzed using a contact mode AFM, and the

measurement area was $5 \times 5 \mu\text{m}^2$ (as shown in Fig. 5). The measured root mean square (RMS) values are 5.40, 4.38, 4.73, and 5.85 nm, respectively, for 0° -, 30° -, 60° -, and 90° -deposited ZnO films. The minimum RMS value was observed for 30° , and the maximum RMS value was observed for 90° -deposited film. The decrease and increase of RMS values might be due to growth direction-induced surface roughness. The growing film surface depends

on the substrate-tilt angle and flux direction, influencing the c-axis orientation of sputter-deposited ZnO thin films.

The optical properties of ZnO films deposited at different angles are shown in Fig. 6. The transmission spectra were measured from 300 to 800 nm and the overall transmittance was 90%. Interference fringe formation is observed for 0°-, 30°-, and 60°-deposited films, whereas no interference fringe formation is observed for 90°-deposited film. In the case of 90°-deposited film, the measured thickness (393 nm from FE-SEM measurement) is not enough to produce the fringe formation. This might be due to the high absorption of light at this nanostructure condition and similar kind of results are evident in the literature [32]. On the other hand, nanostructure formation results in light scattering and traps the light in nanostructures and thus the light trapping property with the increase in light absorption. The envelope method is used to extract the optical properties: refractive index (n), absorption coefficient (α), and

bandgap energy (E_g) values from the transmission spectra from the following equation [39–41],

$$N = 2s \left(\frac{T_M - T_m}{T_M T_m} \right) + \frac{s^2 + 1}{2} \quad (5)$$

$$n = \sqrt{N + \sqrt{N^2 - s^2}} \quad (6)$$

$$\alpha = \frac{(2.303A)}{t} \quad (7)$$

$$(xh\nu) = B(h\nu - E_g)^\gamma \quad (8)$$

where N is the volume density, s is the substrate refractive index, and T_M and T_m are the two adjacent maxima (or) minima of the interference fringes. Here, n is the refractive index, α is absorption coefficient, A is the absorbance, which is derived from the transmittance spectra, t is the thickness of thin-film, $h\nu$ is the photon energy, E_g is the bandgap of the material, and B is the parameter of the absorption edge width. The exponent γ can be determined by the type of electronic transition. Here the superscript γ value varies from 1/2 and 2 depending on the type of direct allowed and indirect allowed transition, respectively.

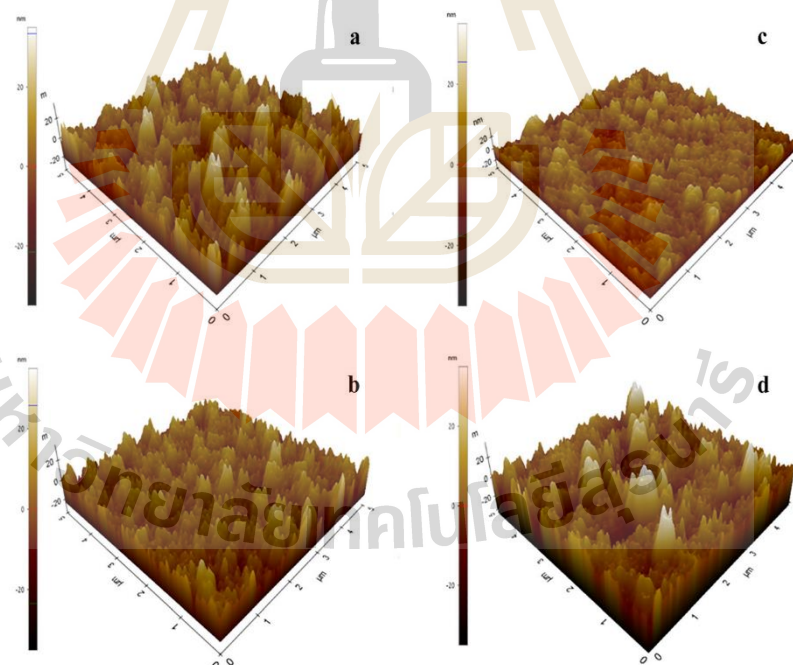


Fig. 5 AFM images of ZnO films on Si substrates at different substrate-tilt angles: a 0°, b 30°, c 60°, and d 90°-deposited films

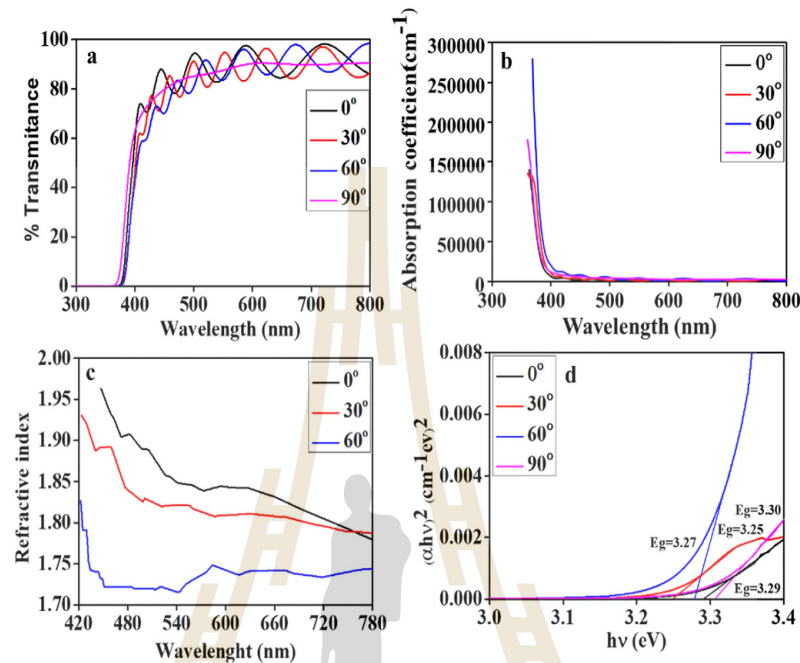


Fig. 6 Optical properties of ZnO film, a transmittance spectra, b absorption spectra, c refractive index, and d bandgap energy

The extracted optical parameters, absorption coefficient (α), bandgap energy (E_g), and refractive index are displayed in Fig. 6. Figure 6b and d shows the extracted absorption coefficient graphs and these values are in good agreement with the literature on ZnO films [40]. The E_g values are extracted from extrapolating the linear part of $(\alpha h\nu)^2$ Vs. energy ($h\nu$) graphs. The extracted E_g values are 3.29, 3.25, 3.27, and 3.30 eV, respectively, for 0°, 30°, 60°, and 90°-deposited films, which are in reasonable agreement with the bandgap of ZnO [32]. The estimated refractive index (RI) values are 1.85, 1.82, and 1.72 with respect to tilt angles 0°, 30°, and 60°-deposited films as shown in Fig. 6c. The reduction in the refractive index, which increases substrate-tilt angles, can be ascribed to the low density and increase of the porosity of deposited films [42]. The variations of these refractive index and bandgap values with tilting angles could be due to the variation of film density and grain size [42, 43]. Therefore, the substrate-tilt angle deposition plays a major role in film quality.

4 Conclusion

In summary, ZnO films are deposited at different substrate-tilt angles with respect to the substrate holder. The estimated crystal sizes are 21, 19, 22, and 24 nm, respectively, for 0°, 30°, 60°, and 90° substrate-tilt angle-deposited films. Flake-like grains are observed due to high deposition rates and this feature is observed in all the deposited films. The (002) peak shift is due to the presence of strain in ZnO films, which is generated during the deposition and might be due to the columnar grain growth. ZnO film thickness is decreased from 819 to 393 nm while increasing the substrate-tilt angle from 0° to 90°, and this might be due to the shadowing effect of films. The overall columnar growth direction shift is 31° for the substrate-tilt angle rotation from 0° to 90°. There is no interference fringe formation for 90 tilt angle deposition, and this might be due to the high absorption of light at this nanostructure formation. The tilt angle of columnar grain growth is due to the alignment of sputtered atoms between the target and inclined substrate. Based on the results, it can be

concluded that the deposition with desirable substrate-tilt angles is a crucial factor in inducing different grain shapes and orientations.

Author contributions

All authors are contributed equally to this study. Material preparation, data collection, and analysis were performed by TS. The writing of original draft was performed by TS and DM. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability

This manuscript has no associated data. All data generated or analyzed during this study are included in this published article.

Declarations

Conflict of interest The authors declare that they have no financial and personal relationship with other people or organizations that could inappropriately influence the work reports in this paper.

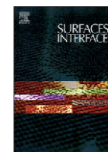
Ethical approval We, the undersigned, declare that this manuscript is original, has not been published before, and is not currently being considered for publication elsewhere.

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Development of gold nanopikes-modified quartz crystal microbalance biosensor for prostate specific antigen detection

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ABSTRACT

Prostate-specific antigen (PSA) screening is vital for the early detection and assessment of prostate cancer treatment efficacy. Integrating nanotechnology into biosensor development allows for targeted biomarker detection. In this study, we present the fabrication of gold nanopikes (AuNS) structures on a quartz crystal microbalance (QCM) using the pulse current electrodeposition (PCD) technique. By varying the duty cycles of PCD, we achieved the desired nanostructure. The characteristics of the AuNS-modified QCM electrode were thoroughly investigated using various micro-structural characterization techniques such as x-ray diffraction (XRD), field emission scanning electron microscope (FESEM), atomic force microscope (AFM), X-ray photoelectron spectroscopy (XPS) and cyclic voltammetry (CV). Prior to biomolecule immobilization, each chemical treatment was verified through contact angle and FT-IR analyses. The performance of the developed QCM biosensor was evaluated using PSA at various concentrations. The results revealed that the frequency variation of the QCM biosensor was directly proportional to the PSA concentration. Notably, the AuNS-modified QCM biosensor exhibited the highest frequency shift at a 20 % duty cycle, demonstrating a linear detection range of 0.1–100 ng mL⁻¹ and a limit of detection (LOD) of 24 pg mL⁻¹. The developed QCM biosensor significantly enhances detection efficiency by increasing the number of binding sites for biomolecules through an enlarged surface area. The demonstrated sensitivity of the AuNS-modified QCM biosensor indicates its potential to address the increasing demand for early diagnosis and screening in healthcare systems. This innovative biosensor technology holds promise for improving prostate cancer detection and has broader implications for the development of highly sensitive biosensors for various biomarkers.

1. Introduction

Nanotechnology has garnered significant attention in 21st-century technology, and researchers are actively exploring its innovative applications, especially in medical research. Nanotechnology-based diagnostic methods are being investigated as potential tools for real-time, convenient, and cost-effective cancer diagnosis and detection [1]. Nanomaterials offer an opportunity to advance early diagnosis, improve patient prognoses, and enhance treatment monitoring, ultimately

resulting in improved patient outcomes. The use of nanomaterials underscores the potential for progress and improved patient care. Currently, cancer diagnostics are gradually shifting from invasive and complex procedures toward the adoption of highly sensitive point-of-care liquid biopsies [2], where nanomaterials have demonstrated remarkable effectiveness in detecting cancer biomarkers. A key advantage of using nanomaterials in cancer detection is their large surface area-to-volume ratio compared to bulk materials. This characteristic allows nanomaterial surfaces to be densely coated with

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antibodies and other small molecules capable of identifying specific cancer molecules, thereby enhancing assay specificity and sensitivity [3, 4].

Gold (Au) has emerged as a remarkable class of nanomaterials, possessing properties that make them highly desirable for various applications, such as biomedicine and biosensors. These unique properties include controllable synthesis steps, low toxicity, and high biocompatibility [5]. The combination of the general properties of nanomaterials with the unique properties of gold (Au) enables them to offer an enhanced surface for the immobilization of biomolecules, making them exceptional candidates for the development of highly sensitive and selective biosensing platforms. In various sensor designs, gold (Au) has been used to facilitate the immobilization of recognition elements, amplify signal transduction, and improve the limit of detection for biomarkers.

Detecting overexpressed unique biomarkers in serum holds great promise for cancer prognosis, diagnostics, and therapies. Prostate cancer (PCa) is the most prevalent malignancy diagnosed in men worldwide, with 1.41 million cases reported by the World Health Organization in 2020. Approximately 1 in 8 men will be diagnosed with PCa during their lifetime, and older men have a higher risk for PCa, with ~99 % of patients aged >50 years [6]. It is estimated that 1 in 41 men die from PCa. Early diagnosis and screening for malignant growths significantly improve prognosis, survival rates, and overall understanding of personal well-being. Biomarker detection at an early stage enables prompt initiation of treatment, resulting in increased effectiveness of drug therapy, reduced risk, and decreased patient mortality [7]. Prostate-specific antigen (PSA) is among the biomarkers utilized in hospitals and clinics for prostate cancer screening and health check-ups. In 1994, the US Food and Drug Administration authorized PSA as a marker for the detection of malignant prostate growth [8]. PSA can be detected in males' blood, urine, and saliva [9]. In clinical practice, a serum PSA concentration is essential for guiding patient care in various situations, spanning from an initial detection of prostate cancer to post-treatment monitoring. After obtaining a PSA value, an additional patient-clinician encounter is often required, making the point-of-care test potentially simplify patient care. Furthermore, the POC allows for immediate discussions about the next steps, such as whether to proceed with a digital rectal examination (DRE), transrectal ultrasound (TRUS), positron emission tomography/computed tomography (PET/CT) scan, magnetic resonance imaging (MRI), or other diagnostic procedures [10].

Nowadays, current medical instruments for detecting PSA have proven to be highly beneficial. However, they have certain limitations due to their sophisticated nature, time-consuming operation, slow response time, high cost, laborious procedures, and lack of portability for POC scenarios. To overcome these challenges, biosensors present several advantages, such as cost-effectiveness, heightened sensitivity, ease of use, portability, real-time analysis, and the capability to offer POC diagnosis. Various types of biosensors, including electrochemical, optical, surface plasmon resonance, and piezoelectric [11–14], have been developed with a specific focus on detecting PCa biomarkers, aiming to enhance the effectiveness of cancer tumor screening, monitoring, and diagnosis.

Among these biosensors, the piezoelectric quartz crystal microbalance (QCM) biosensor has been reviewed as an attractive device for label-free biomarker applications, particularly in tumor screening, monitoring, and diagnosis. This is because the QCM offers fast detection, high sensitivity, and the ability to be integrated with cost-effective electronic devices, making it a viable alternative POC to traditional methods. The QCM consists of a specialized quartz crystal (QC) oriented for precision cutting, along with metallic electrodes. The QC is sandwiched between two electrodes, one on the top and the other on the bottom side. The orientation of the quartz crystals cut play a crucial role in its piezoelectric properties. The QCM functions as a high-resolution mass sensing technique. It can operate under various conditions, including liquid, gas, and vacuum environments [15,16]. The principle

of QCM relies on the piezoelectric effect, which exhibits an indirect effect. When an external electric field is applied to the QC, it induces mechanical stresses within the QC. An alternating voltage creates mechanical oscillations in the direction perpendicular to the QC surface, and a resonance frequency is recorded when the crystal is placed in an oscillation circuit [17,18]. The QCM measures mass variation per unit area by detecting changes in the frequency of a quartz crystal resonator. The introduction or removal of a small mass influences the resonance frequency. When target analytes such as antibody, antigen, enzyme, nucleic acid (DNA, RNA), and tissues are captured by the recognition elements on the QCM sensor electrode, it induces a change in the mass and/or viscoelastic properties of the QCM, resulting in a shift in its resonance frequency. The resonance frequency shift and mass sensitivity of the QCM can be determined using Sauerbrey's equation, which is expressed as follows:

$$\Delta f = -\frac{2f_0^2}{\mu_q p_q A} \Delta m \quad (1)$$

where f_0 is the resonance frequency (Hz), Δf is the change in resonance frequency, Δm is the mass change (ng), A is the active surface area of the QCM electrodes, p_q is the density of quartz (2.648 gcm^{-3}), and μ_q is the shear modulus of quartz (for AT-cut quartz, $\mu_q = 2.947 \times 10^{11} \text{ gcm}^{-1} \text{ s}^{-2}$), respectively.

According to Sauerbrey's equation, the resonance frequency shift is proportional to adding mass (m) and inversely proportional to the surface area of the working electrode. To enhance the sensitivity of a QCM electrode [19], either of these three methods can be employed: (1) reducing the thickness of the quartz crystal, (2) enlarging the surface area for attachment of a greater quantity of biomolecule, and/or (3) incorporating nanoparticles on the sensor electrode. Several researchers have reported that the combination of nanotechnology with QCM electrodes enhances the capabilities of biosensing platforms by increasing the biomolecule binding sites and signal amplification. Various nanomaterials, including nanotubes, nanorods, and nanoparticles, are being studied for use in immunological sensing systems. For instance, Pohanka et al. [20] developed an approach that involved using Gold (Au) nanoparticles-conjugated antibody to improve the sensitivity of QCM detection of PSA, resulting in a simple, selective, and fast detection method that is suitable for POC applications. Uludag et al. [21] described a sandwich hybridization assay based on antibody-modified nanoparticles amplification and signal enhancement for POC immunosensor of PSA detection. Asai et al. [22] modified the active surface area of QCM by introducing Anodic Aluminum Oxide nanostructures. This modification was done to enhance antibody-antigen interactions and improve mass sensitivity. Pirich et al. [23] utilized a bacterial cellulose nanocrystal thin film-coated QCM electrode to increase sensitivity and create an adapted interface for the attachment of captured immunoglobulin G. In this regard, exploring a signal amplification strategy with nanotechnology would be valuable for the development of a highly effective QCM biosensing platform.

We have recently utilized a direct current (DC) electroplating technique to create gold (Au) nanostructures on the active area of the QCM as a signal amplification strategy, with Anti-PSA serving as the recognition element for detecting PSA. Protein A was chosen as a bridging linker for the gold electrode surface through physical adsorption [24]. Our results demonstrated that increasing the electroplating time resulted in a larger surface area, consequently enhancing the mass sensitivity of the PSA detector [25]. Our previous work demonstrated the feasibility of utilizing Au nanostructures on a QCM as an alternative biosensing platform for PSA detection. Nonetheless, there is a continued need to investigate PSA detection at various concentrations on a functionalized QCM electrode and enhance the detection procedure. Additionally, specific issues demand consideration. For example, the surface modification of the QCM created through the Au structures in the Au micro-particles electrolyte lacked significant distinctiveness from the pristine

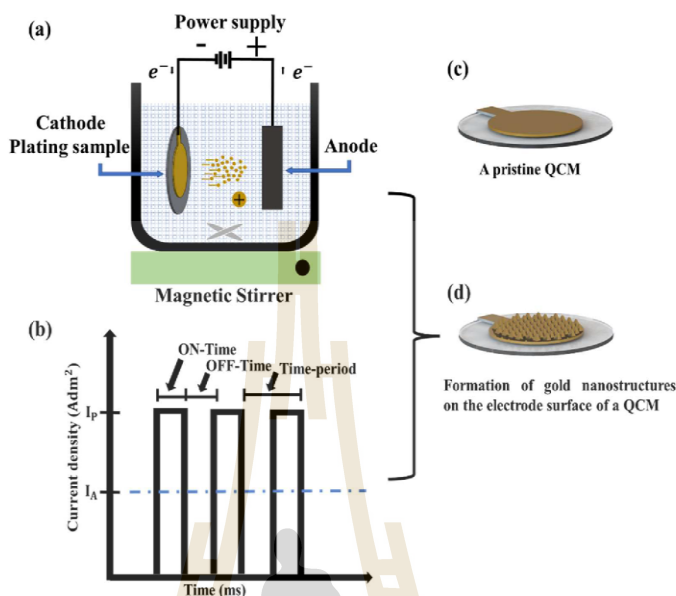


Fig. 1. (a) working principle of a pulse current deposition technique used to deposit AuNS on QCM electrode surfaces, (b) a typical pulse current waveform, (c) the as-received QCM, and (d) the nanostructure-modified QCM electrode surface.

surface. Moreover, the DC electroplating technique resulted in the continuous deposition of Au ions from the electrolyte onto the QCM electrode surface. This excessive Au deposition caused a significant frequency shift in the QCM, with a shift of ~ 255 kHz observed after a 30-minute deposition time, sometimes exceeding the measurement range. There were also instances when the deposited nanostructures on the Au-QCM detached from the quartz substrate during the cleaning process. Addressing these challenges is essential to enhance the overall effectiveness of this biosensing platform.

In this study, we introduce a new approach, pulse current electrodeposition (PCD), to enhance a method by creating a distinctive structure with minimal nanoparticle mass loading on the QCM electrode surface. Furthermore, our research broadens to explore the detection of the PSA biomarker at varying concentrations. We also investigate the impact of various parameters related to the chemical treatment on the QCM electrode surface through wettability characterization and the identification of functional groups to confirm the surface's suitability for biomolecule immobilization. In contrast to our previous method, this study employs L-cysteine and EDC/NHS as linkers instead of protein A, resulting in increased physical strength due to the adhesive properties of L-cysteine, which forms covalent bonds, and the use of EDC/NHS, which enhances the efficiency of the cross-linking reaction.

2. Experimental

2.1. Materials and reagents

All chemicals used in this work were of analytical grade and were used as received, without further purification. A Quartz Crystal Microbalance (QCM) with a 13.9 mm diameter and a 160 μm thickness, operating at 10 MHz, was obtained from Novaetech (Pompeii, Italy). The crystal's electrodes have a 200 nm thick gold (Au) layer deposited on a 10 nm titanium (Ti) adhesive layer. Piranha solution was made by combining a 70:30 v/v solution of 96 % sulfuric acid (H_2SO_4 , Anapure, New Zealand) and 30 % hydrogen peroxide (H_2O_2 , Qrec, New Zealand).

99.9 % ethanol was purchased from Merck (Darmstadt, Germany). Human PSA antibody was purchased from Assaypro (St. Charles, MO, US) and PSA antigen was purchased from Sigma Aldrich (Saint Louis, US). Phosphate-buffered saline (PBS, pH 6.4) was obtained from Calbiochem (La Jolla, CA, US). Tween 20 was from Chem-Supply (Gillman, SA, Australia) and Au electrolyte was supplied by IntelliChem (Bangkok, Thailand). 1-Ethyl-3-(3-dimethylamino propyl) carbodiimide (EDC), N-Hydroxy succinimide (NHS), and L-cysteine were provided by NPKChemicals (Bangkok, Thailand). Potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$] and potassium ferrocyanide [$\text{K}_4\text{Fe}(\text{CN})_6$] were obtained from Fisher Scientific (Gujarat, India). In all experiments, autoclaved deionized (DI) water was used. The QCMs were electrodeposited in the Micro-machining Laboratory of the Synchrotron Light Research Institute (SLRI), Thailand.

2.2. Fabrication of gold nanostructures on QCM electrode

Pulse current electrodeposition was utilized for the formation of a nanostructured thin film on the QCM electrode surface. The experimental setup is depicted in Fig. 1a. Au nanostructures were prepared by immersing a pristine QCM in an Au electrolyte solution. The QCM functioned as a cathode and a platinum grating mesh functioned as an anode. A magnetic stirrer was utilized, rotating a stir bar to facilitate the dispersion of the Au electrolyte solution. An electrical current was applied between the electrodes, resulting movement of electrons from anode to cathode. The Au ions are formed on the QCM electrode. Pulse current electrodeposition (PCD) involves three variable parameters: pulse ON-time (t_{on}), pulse OFF-time (t_{off}), and peak current (I_p). The ON-time corresponds to when a positive current is applied, and the OFF-time occurs when no current is applied, with both contributing to the total time period with an average current (I_a) [26].

The t_{on} and t_{off} are described in terms of a duty cycle that can be expressed as follows [27]:

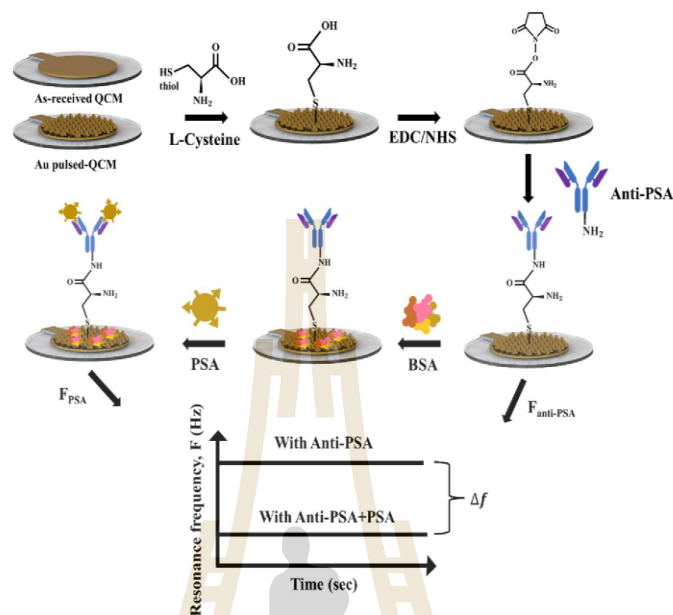


Fig. 2. Schematic illustration of the QCM biosensor construction for the PSA detection procedure.

$$\text{Duty cycle}(\%) = \left[\frac{t_{\text{on}}}{t_{\text{on}} + t_{\text{off}}} \right] \times 100 \quad (2)$$

The diffusion layer, grain size, and nucleation rate can be controlled by modulating the duty cycle. During the t_{on} , a negatively charged layer is formed around the cathode, which is discharged during the t_{off} . The periodic ON and OFF times not only provide an easier path for ion transfer to the cathode but also allow for relaxation and rearrangement of the deposited atoms, resulting in a more uniform ion distribution on the surface of the cathode. As a result, the deposition uniformity is improved, and the porosity of the deposited surface is reduced [28]. In this study, the electrical current was maintained at 0.5 mA for 30 min with a duty cycle of 20 % and 80 %.

2.3. Characterization of gold nanostructures on QCM electrode surface

The morphology of Au structure coated QCM electrode was observed by field emission scanning electron microscopy (FESEM, JEOL JSM-6010LV) at an acceleration voltage of 20 kV and a working distance of 5.5 mm revealed the structures at magnifications of 30,000x and 100,000x. The topographical image was obtained using atomic force microscopy (AFM, Parker, USA) in C-AFM mode with a scan rate of 0.3 Hz over a $3 \times 3 \mu\text{m}$ area. X-ray diffraction (XRD) patterns were obtained utilizing a Bruker D8 Advance X-Ray diffractometer with Cu-K α 1 radiation ($\lambda = 0.15406 \text{ nm}$), operating at 40 kV and 40 mA. Scans were conducted from 35° to 80° in 0.02° increments to investigate the crystal structure and analyze crystallite size. Cyclic voltammetry measurements were conducted using an electrochemical analyzer (CHI660D, CH Instruments Inc., United States) with a conventional three-electrode system (Ag/AgCl reference electrode, platinum wire counter electrode, and QCM as the working electrode) to investigate an effective surface area. X-ray photoelectron spectroscopy (XPS) was performed using ESCA-LAB250Xi X-ray photoelectron spectrometer (Thermo Scientific, UK) with a monochromated Al K alpha excitation source operating at 120 W (13.8 kV $\times$ 8.7 mA), maintaining a base pressure better than 2×10^{-9}

mbar.

2.4. Electrode surface treatments and immobilization steps of PSA

Gold nanostructure-coated QCM electrodes were cleaned with piranha solution via a shaker at 20 revolutions Per Minute (rpm) for 30 min. Subsequently, the QCMs were cleaned in DI-water, followed by ethanol and dried under ambient temperature. The cleaned QCMs were then placed in a custom-made moisture chamber to minimize the evaporation of the reagents on the QCM electrodes. L-cysteine was used as the linker due to its ability to form Gold-Sulfur (Au-S) covalent bonds. The electrodes were further incubated with 30 μL of 1 M L-cysteine for 2 h at room temperature. The electrodes were further activated using EDC/NHS which enhances the effectiveness of cross-linking reaction. To activate carboxyl group and increase the effective antibody immobilization, 50 μL of 0.1 M EDC/NHS was added to the electrode surface for 2 h. Then, 10 μL of anti-PSA antibody at a concentration of 0.408 mg mL^{-1} in PBS with a pH of 7.4, 50 % Glycerol (v/v), and 0.02 % (w/v) Sodium Azide (NaN_3) was added and incubated on the electrode overnight at 20°C . To prevent non-specific binding, the surface was treated with 2.5 % BSA in 1X PBS and 0.05 % Tween for 1 hour. To investigate the response of the QCM signal, various concentrations of PSA (0.1, 1, 10, 100, and 1000 ng mL^{-1}) were utilized in this work. 30 μL of PSA was bound with anti-PSA antibody for 1 hour at room temperature. And the signal response was recorded for 3 min at each concentration. All experimental steps were performed using the drop-casting method. During each incubation step, the QCM was extensively rinsed with autoclaved DI water to eliminate any unbound molecules from the electrode surface. Subsequently, the drying process was carried out at room temperature before recording the frequency response. The schematic representation of the immobilization step is provided in Fig. 2.

2.5. QCM measurements

The frequency response of the QCM before and after immobilization

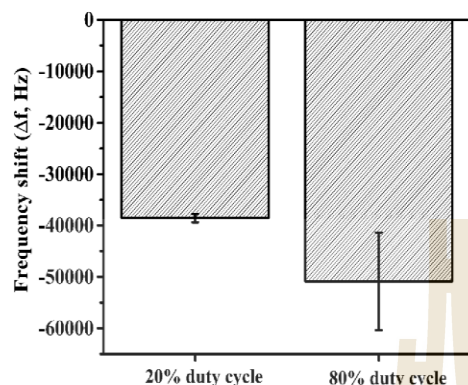


Fig. 3. Resonance frequency shift resulting from the development of AuNS structures on the QCM electrode.

was evaluated using a portable QCM instrument (Surazense, Thailand). The electronic module, designed and developed at Suranaree University of Technology, Thailand, incorporated an open-cover fluidic cell purchased from Novaetech (Pompei, Italy). Data acquisition was performed with the Surazense software. The measurements were conducted under dry conditions at room temperature to mitigate the changes in the viscosity of the assay media on the QCM. The frequency was recorded before and after immobilizing PSA on the Au nanostructure-coated QCM. For each measurement, the average QCM frequency was recorded over 3 min.

2.6. Characterization of chemical treatments on QCM electrode

The wettability properties resulting from the chemical treatment of the QCM surface were determined through contact angle (CA) measurements. These measurements were conducted using a custom-made water drop test station equipped with Dino-Lite digital microscopes (Netherlands). The contact angle of water on the QCM electrode surface with various chemical treatments was recorded using Dino Capture 2.0 software. The CA was measured by placing a 10 μ L water droplet from a syringe needle onto the various chemically treated QCM surfaces along the Y-axis. Fourier-Transform Infrared (FTIR) spectroscopy (Bruker, Tensor 27) was employed to analyze the QCM electrodes and identify the functional groups involved in the chemical treatment of the QCM surfaces.

3. Results and discussion

3.1. Resonance frequency response after surface modification on the QCM electrode with different %duty cycles

The presence of Au structures on the QCM electrode after electro-deposition was confirmed by measuring the frequency shift (Δf) before and after the deposition ($F_{\text{after deposition}} - F_{\text{before deposition}}$), as shown in Fig. 3. The results demonstrated that the Δf value for the pulse-deposited Au with a 20 % duty cycle was approximately $-38,560 \pm 836$ Hz, while for the 80 % duty cycle, it was $-50,863 \pm 9480$ Hz. It is indicated that the mass loading of the Au structures on QCM under the 80 % was higher compared to the 20 % duty cycle. This result is consistent with the theory of pulse electrodeposition, where a higher duty cycle, indicating a longer duration of the ON-time, typically leads to a greater mass deposition, as described by Eq. (2).

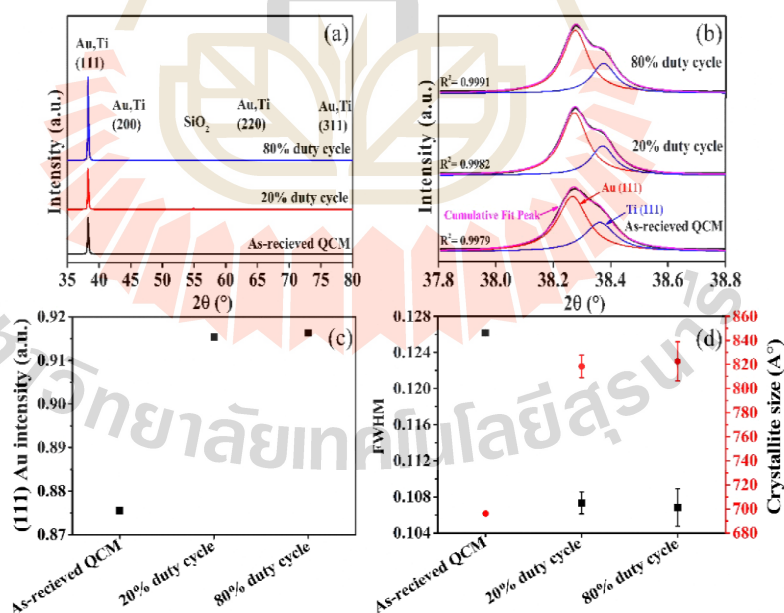


Fig. 4. (a) XRD patterns, (b) XRD peak intensities, (c) fitting result of (111) Au peaks, (d) FWHM and crystallite size values of QCM pristine and different percentages of duty cycle.

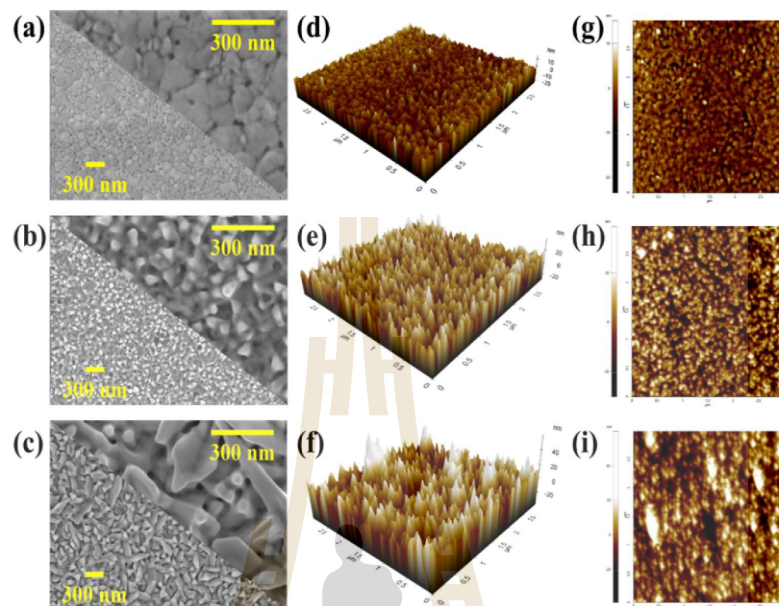


Fig. 5. SEM and AFM images of the QCM working electrode surface: (a,d,g) As-received QCM, (b,e,h) AuNS-modified QCM with 20 % duty cycle, and (c,f,i) AuNS-modified QCM with 80 % duty cycle.

3.2. XRD spectra of Au-pulsed QCM with different duty cycles

XRD spectra of the QCM electrode with/without the formation of Au structure via electrodeposition was obtained to investigate surface morphology, grain size, grain shape, and growth direction. The XRD diffraction patterns are matched with JCPDS file 00-004-0784 for Gold (Au) and JCPDS file 01-088-2321 for Titanium (Ti). The XRD spectra have been assigned to the (111), (200), (220), and (311) peaks of Au/Ti, with the dominant peak corresponding to (111) as shown in Fig. 4a. It is observed that the (111) intensity increases as the portion of duty cycle increases, indicating the Au/Ti of pulse-deposited Au on QCM electrode grew preferentially in the (111) orientation. The predominant peak spectra can be fitted using the Lorentz spectral function. The correlation coefficient (R^2) was greater than 0.99, and the (111) peak was deconvoluted into peaks near 38.2° and 38.6° , corresponding to Au (111) and Ti(111), as depicted in Fig. 4b. The (111) peak intensity, crystallite size, and Full Width at Half Maximum (FWHM) of the Au (111) were investigated. The Debye-Scherrer equation for calculating the crystallite size (D) is given by [29]:

$$D = \frac{K\lambda}{\beta \cos \theta} \quad (3)$$

where K is the shape factor (taken as 0.94), λ is the incident X-ray wavelength ($\lambda=1.5406 \text{ \AA}$), β is the FWHM (in radians) of the (002), and θ is the diffraction angle.

Compared to the pristine QCM, the extracted (111) Au peak revealed a significant increase in intensities when applying 20 % duty cycle and 80 % duty cycle of Au deposition on the QCM electrode, as shown in Fig. 4c. The FWHM decreases when the electrode was controlled at 20 % duty cycle, followed by a gradual decline in FWHM with 80 % duty cycle. The calculated crystallite size is inversely proportional to FWHM. The crystallite size is about 695, 807, 811 nm of pristine QCM, 20 % duty cycle and 80 % duty cycle Au modified-QCM, respectively, as depicted in Fig. 4d. From this result, it can be observed that an increase in the

Table 1

Surface area, roughness average, root-mean-square roughness values from AFM and effective surface area values from CV for the as-received, 20 % duty cycle pulse-deposited Au, and 80 % duty cycle pulse-deposited Au on the QCM.

Sample	Effective surface area (cm ²) from CV	Surface area (nm ²) from AFM	Roughness average (nm)	Root-mean-squared roughness (nm)
As-received QCM	0.167	9.0951	3.1	4.2
AuNS-modified QCM (20 % duty cycle)	0.234	9.5833	7.7	9.5
AuNS-modified QCM (80 % duty cycle)	0.169	9.262	8.1	10.5

percentage of duty cycle leads to the agglomeration of Au particles on the QCM electrode, resulting in an increase frequency shift, the crystallite size and the intensities of Au, particularly in the (111) plane.

3.3. Surface characterization of the Au-QCM electrodes

FESEM micrographs were used to analyze the surface morphology. Fig. 5(a-c) displays FESEM images of the as-received QCM, 20 % duty cycle Au-modified QCM, and 80 % duty cycle Au-modified QCM. As displayed in Fig. 5a, the as-received QCM exhibited a relatively smooth and flattened surface. After inducing the electrodeposition, the formation of gold nanospikes (AuNS) was observed on the QCM electrode surface. As depicted in Fig. 5b, the 20 % duty cycle showed a well-

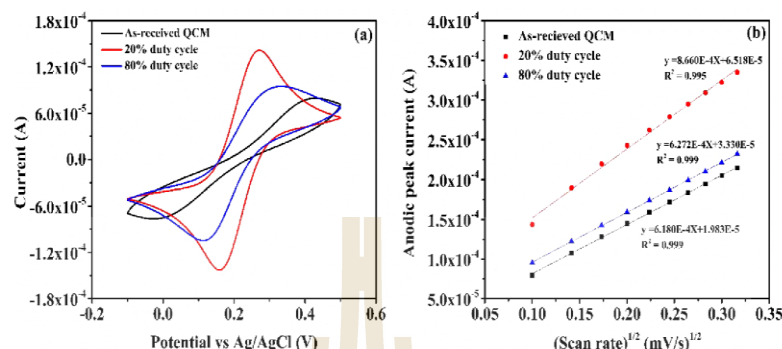


Fig. 6. Electrochemical results of (a) CV plot within a scan range from -0.1 V to 0.5 V at a scan rate of 10 mV s^{-1} and (b) the relationship between anodic peak current and the square root of the scan rate at different scan rates of as-received QCM, 20 % duty cycle, and 80 % duty cycle of AuNS-modified QCM.

formed nanopike structures with small grains. The 80 % duty cycle demonstrated bigger nanopike structures of Au, as shown in Fig. 5c. The results indicate that during the 80 % duty cycle of deposition, a higher aggregation of Au nanoparticles occurs on the electrode. This suggests that the shape of the nanostructure is dependent on the adjusted percentage of the duty cycle. AFM measurements were carried out on the QCM's working electrode surface to explore the surface's 2D and 3D profile, as shown in Fig. 5 (d-i). The analysis of 2D AFM images using XEI software yielded numerical data concerning roughness parameters and surface area, as detailed in Table 1. An average (Ra) and Root Mean Square (RMS) roughness values exhibited a concurrent increase with the elevation in the % duty cycle. The initial observation from the AFM image of the As-received QCM, as illustrated in Fig. 5 (d, g), showed the distinctive grainy structure typical of gold. This surface exhibited a relatively flat profile with an RMS roughness measuring 3.1 nm and a surface area of $9.0951 \mu\text{m}^2$. Following the deposition of AuNS at 20 % (Fig. 5 (e,h)) and 80 % duty cycle (Fig. 5 (f,i)), a noticeable and

significant transformation took place on the surface, resulting in observable alterations in grain shape. This alteration led to a surge in the RMS roughness values, reaching 9.5 nm and 10.5 nm, while the surface area increased to $9.5833 \mu\text{m}^2$ (5 % increase compared to the pristine state) and $9.282 \mu\text{m}^2$ (2 % increase compared to the pristine state), respectively.

3.4. Electroactive surface area

The cyclic voltammetry (CV) experiments were performed to examine the impact of PCD technique on the enhancement of the QCM electrode's surface area. The CV was conducted in a three-electrode configuration, with a gold electrode as the working electrode, an Ag/AgCl electrode as the reference electrode, and a platinum wire as the counter electrode. The electrolyte chosen for this study was in PBS (0.1 M, pH 6.4), containing $5 \text{ mM } [\text{Fe}(\text{CN})_6]^{3-/4-}$. Fig. 6a shows CV results of 10 mV s^{-1} for the as-received QCM and the AuNS-modified QCM

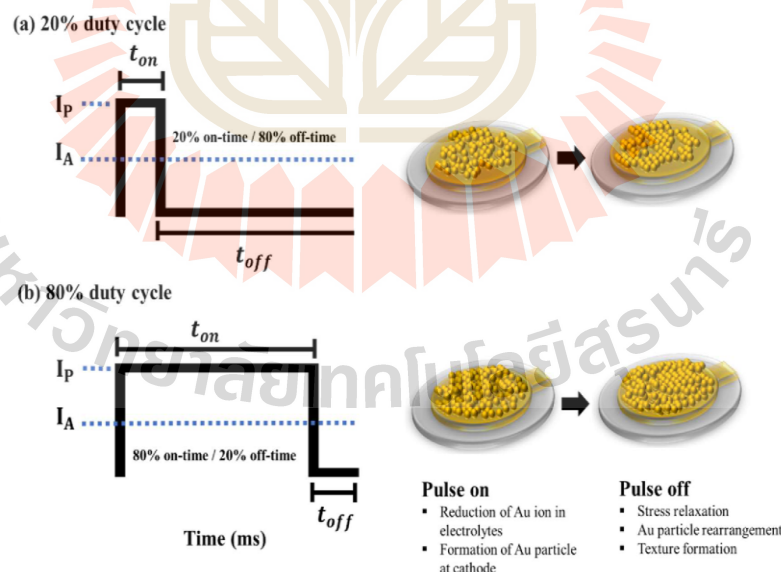


Fig. 7. Schematic representation of Au deposition mechanism on a QCM during electrodeposition with various the percentage of duty cycle.

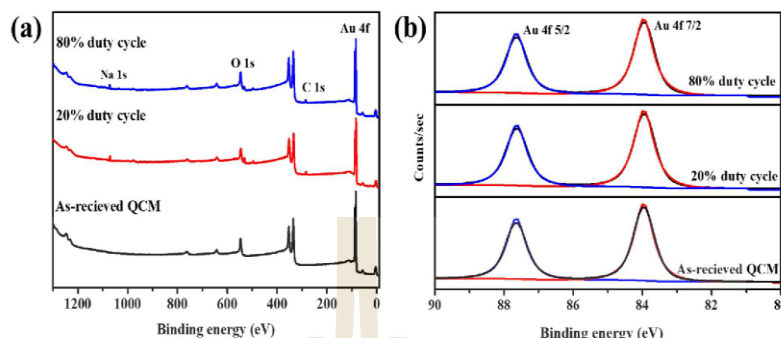


Fig. 8. (a) XPS survey spectra and (b) narrow region XPS spectra for Au 4f.

electrodes. The result reveals an increase in peak current when comparing the pulse conditions to the as-received QCM electrode. This increase suggests a reduction in electron-transfer barriers attributed to the presence of nanoparticles. However, at 80 % duty cycle, a decrease in voltammetric peak currents is observed, indicating lower conductivity compared to the 20 % duty cycle. This decrease in peak current at 80 % duty cycle can be attributed to the larger size of the AuNS formed during the QCM deposition process. The electroactive surface area (A) can be expressed by Randles-Sevcik equation [30]:

$$A = \frac{I_{pa}}{2.69 \times 10^5 n^2 C_0 D_r^{1/2} \nu^{1/2}} \quad (4)$$

where A is the effective surface area (cm^2), I_{pa} is anodic peak current (A), n is number of electrons participating in the redox reaction (taken as 1), C_0 is concentration of $\text{Fe}(\text{CN})_6^{3-}$ (mol cm^{-3}), D_r is diffusion coefficient of $7.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, and ν is the scan rate (V s^{-1}). The CV was measured within a potential range of -0.1 to 0.5 V at different scan rates. The I_{pa} was found to be proportional to the square root of the sweep rate, and the slope was utilized to calculate the effective surface area, as shown in Fig. 6b. Under 20 % duty cycle, the highest I_{pa} was observed. According to Randles-Sevcik equation, the estimated effective surface areas are displayed in Table 1. The surface area of the as-received QCM and the AuNS-modified QCM electrodes with 20 and 80 % duty cycle were calculated to be 0.167 cm^2 , 0.234 cm^2 , and 0.169 cm^2 , respectively. Interestingly, a 20 % duty cycle results in the highest surface area, whereas 80 % duty cycle results in the lower surface area. Fig. 7 presents a schematic diagram depicting a random Au particle formed around the QCM electrode during pulse current deposition with 20 and 80 % duty cycle. A low duty cycle results in a short On-time, limiting the movement of Au ions and leading to less Au deposition on the electrode surface, as illustrated in Fig. 7a. On the other hand, a higher duty cycle with a longer On-time promotes higher ion mobility, causing Au particles to merge and aggregate on the electrode, as shown in Fig. 7b. During the OFF-time period, stress relaxation, atom rearrangement, and texture formation were occurred. A longer OFF-time, indicating lower duty cycle, provide sufficient time for stress relaxation, rearrangement of the deposited atoms. The surface area results obtained from AFM and CV offered significant insights into the correlation between the geometric surface area, determined by topographical features, and the electrochemically active surface area. These findings substantiated a consistent trend within the QCM samples, affirming similar correlations concerning the % duty cycle. The PCD technique for AuNS formation leads to an increased surface area and a distinctive surface morphology. These results are consistent with the work done by Kamnerdkhag et al. [31], which demonstrates that an increase in the duty cycle promotes larger crystallite growth, leading to a decrease in surface area. During pulse deposition, the growth of larger crystallites is

Table 2

XPS Au 4f binding energies, FWHM, peak area, and peak position for as-received QCM, 20 % duty cycle, and 80 % duty cycle of AuNS-modified QCM.

Sample	Position	FWHM	Peak area (counts)	Atomic %
As-received QCM	83.96	0.66	99,931.88	90.56
20 % duty cycle	83.96	0.66	83,913.89	41.68
80 % duty cycle	83.96	0.69	95,548.39	50.22

correlated with a decrease in the surface area. It can be concluded that improving the Au formation process on the QCM maintains a similar crystal structure while creating exceptional nanostructures on the QCM electrode surfaces. Furthermore, this improvement demonstrates minimal mass loading after electrodeposition, as evidenced by frequency shifts of 15 % and 20 % at 20 % and 80 % duty cycles, respectively, compared to the frequency shifts observed in the previous work [25].

3.5. XPS analysis

XPS analyses were performed to acquire precise information regarding the elemental composition of AuNS and to reveal the chemical state of gold, as illustrated in Fig. 8. While preceding AFM and electrochemical analyses provided information solely about the surface structure, XPS is an inherently surface-sensitive technique that probes to a depth of approximately $\sim 10 \text{ nm}$. Fig. 8a displays a wide-ranging survey scan of the XPS spectrum of the pristine and AuNS-modified QCM. This spectrum showed prominent peaks of Au 4f, C 1s, O 1s, and Na 1s elements in increasing binding energy (BE) order. It primarily confirms the presence of carbon (C), oxygen (O), and gold (Au) atoms in all the samples. The presence of Na 1s observed in the AuNS-modified QCM suggested that it may originate from residual sodium cyanide solution used in Au electrolyte during the PCD process. A high-resolution narrow scan of the predominant peak Au 4f is displayed in Fig. 8b. The measured spectrum is in a solid black line and the fitted spectrum is in a dashed line. The XPS spectrum within the Au 4f region reveals distinct doublets at approximately ~ 83.96 and 87.65 eV , exhibiting a characteristic energy separation (ΔE) of approximately 3.69 eV . Deconvolution of these doublet peaks indicates the presence of bands centered at ~ 83.96 and 87.65 eV , corresponding to 4f 7/2 and 4f 5/2 Au⁰, respectively. These results primarily denote the presence of neutral Au⁰ atoms on the surface of the As-received QCM and AuNS-modified QCM, remaining entrapped within the QCM electrode. Table 2 presents the Au atomic percentages, FWHM, binding energies, peak area, and peak position for each sample condition. The oxidation state significantly influences the binding energy and peak position. Atoms with higher positive oxidation states exhibit peaks at higher binding energies due to interactions between photoemitted electrons and the ion core [32].

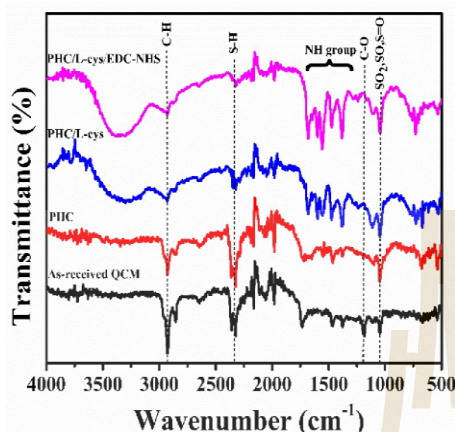


Fig. 9. FT-IR spectra for stepwise chemical treatment on the Au-QCM surface. Piranha solution: PHC.

Despite this, there is no significant change observed in the peak position among the three conditions, suggesting that the oxidation states of the gold nanoparticles remained consistent throughout the process of modifying Au on the working electrode. The oxygen (O1s) and carbon (C1s) peaks observed on the gold QCM surface are attributed to contaminants originating from atmospheric exposure, the cleaning procedure, or the manipulation of the substrates.

3.6. FT-IR bands detected the various chemical treatment on QCM electrode surface

Fig. 9 shows the molecular vibration characteristics of the QCM surface treated with various chemicals, as measured by FTIR spectra in the wavelength range of 400–4000 cm^{-1} . The QCM was used separately for each chemical treatment and cleaning process. The initial observation, the transmittance bands were observed near 1180 cm^{-1} , which correspond to C–O bonds, were associated with organic residues and carbonates. The absence of these bands after the cleaning process suggests that the piranha solution was effective in eliminating organic contamination [33] from the Au surface. After adding L-cysteine, the bands were observed in the range between 1681–1380 cm^{-1} which are correlated with Amide I (C=O, C–N), (N–H), Amide II (C–N, N–H), and Amide III (C=O), (C–N), (N–H), (O=C–N). Additionally, the decreased band was observed at wavenumber 2341–2550 cm^{-1} , indicating S–H stretching vibration of the thiol group. The reduction of bands of thiol group can be attributed to the splitting of the thiol group in the L-cysteine molecule, which is the prerequisite for forming Au–S bonding. According to Li et al. [34], a bifunctional ligand of L-cysteine can provide a thiol group to form a chemical bond on nanoparticle surfaces. The bands of the amide group tend to clearly visible after adding EDC/NHS, indicating their strong bond of amide group and carboxyl acid groups on the surface. The main functional EDC/NHS containing amines and carboxyl acid groups [35] thus allows the appearance transmittance bands in this range. The chemical-treated Au surface demonstrates the influence of chemical activity on chemical bonding and molecule vibrations. This result confirms the successful chemical treatment of the QCM surface, making the electrode ready for antigen-antibody immobilization.

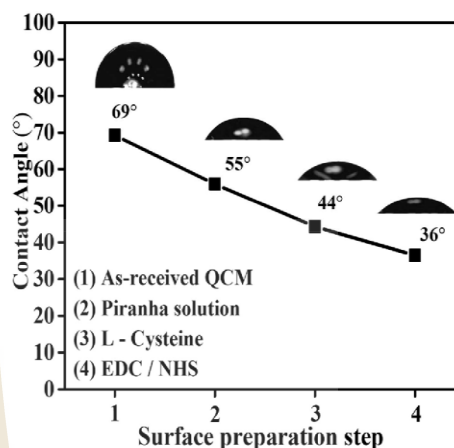


Fig. 10. Contact angle of liquid on the Au-QCM surface that was cleaned with cleaning solutions, treated by L-cysteine, and EDC/NHS.

3.7. Wettability of the various chemical treatment on QCM electrode

Before biomolecule immobilization, contact angle (CA) to investigate the wettability of the electrode surface after chemical treatment were measured. The wettability of a surface depends on the affinity between the material's surface and the liquid, which can be classified into two main classes: hydrophobic and hydrophilic. While, extreme of wettability are referred to its superhydrophobic and superhydrophilic nature. According to wettability theory, it is based on CA measurement, which is strongly influenced by liquid surface tension [36]. The relationship between CA and the surface tension is described by Young's equation [37], which can be expressed as follows:

$$\cos\theta = \frac{\gamma_{SG} - \gamma_{SL}}{\gamma_{LG}} \quad (5)$$

where γ_{SG} , γ_{SL} , and γ_{LG} is solid-vapor, solid-liquid, and liquid-vapor interfacial tension, respectively, and θ represent the CA. Therefore, the CA of a droplet of liquid on a solid surface is determined by the equilibrium of these three interfacial tensions.

For a hydrophobic surface, the CA $> 90^\circ$, whereas for a hydrophilic surface, the CA $< 90^\circ$; for a superhydrophobic surface, the CA $> 150^\circ$, whereas for a superhydrophilic surface, the CA $< 50^\circ$ [38]. The wettability of a surface is a critical factor in influencing the biosensor. The bioreceptor surfaces need to be hydrophilic in order to facilitate easy interaction with the analyte biomolecule [39]. The result is presented in Fig. 10. The captured snapshots indicate a reduction in droplet size, which is consistent with a decrease in the CA over the chemical-treated surface. The CA measured was 69° for the untreated Au-QCM surface. After the cleaning process with piranha solution, the CA decreased to 55° , and the CA value consecutively decreased from 44° of the QCM surface treated with L-cysteine to 36° of the QCM surface treated with EDC/NHS. The QCM surfaces were cleaned with the piranha solution, exhibited hydrophilic character that might be an effect of a thin native layer on the surface and plenty of hydroxyl (–OH) [40]. After treating the Au surface with L-cysteine, there was a transfer from hydrophilic to superhydrophilic character, resulting in the reduction of the CA by 11° . Interestingly, a significant decrease in the CA value of the Au surface of the QCM was observed when the QCM was cleaned, L-cysteine and EDC/NHS were added. This suggested that the chemical treatment helps to increase the hydrophilic nature and. This ensures the potential effective attachment of the analyte biomolecule on the Au-QCM surface.

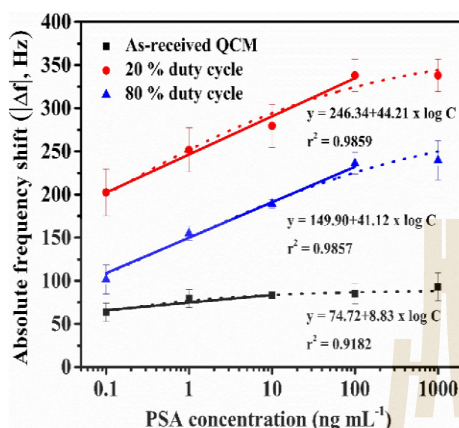


Fig. 11. Resonance frequency response with PSA at various concentration.

3.8. Performance of QCM biosensor for PSA detection

To examine the sensitivity and quantitative detection range of the QCM, various concentrations of PSA were applied to the pristine QCM and the AuNS-modified QCM with 20 and 80 % duty cycles. The frequency shift as a function of the logarithm of PSA concentration is shown in Fig. 11. The frequency shift observed on the QCM with AuNS structures were higher compared to the pristine QCM when PSA was loaded. This difference in frequency shift indicated that the AuNS structures amplifies the signal and improves the detection of the PSA biomarker. The enhanced detection can be attributed to the facilitated attachment of biomolecules onto the nanospoke structures, resulting in a stronger interaction between the biomarker and the AuNS-modified QCM electrode surface.

The initial observation of the relationship between the resonance frequency shift and the various duty cycles of the nanospoke structures formation, it was observed that the shift in frequency during the antibody-antigen interaction, at the same concentration, was not directly correlated to the increase of percentage of duty cycle of the Au pulsed-QCM. However, the surface morphology from FESEM and the surface area obtained from CV results revealed a significant correlation with the resonance shift. The highest resonance shift was observed in the 20 % duty cycle, which exhibited the highest surface roughness and the smallest grain size. This observation suggested that the attachment of antibody and antigen on the electrode surface was enhanced by the increased surface area created by the nanospikes on the QCM electrode surface. The presence of nanospoke structures with a larger surface area could offer additional sites for biomolecule attachment, facilitating more effective interactions between biomolecules and the electrode surface. The limit of detection (LOD) for the highest frequency shift was calculated using the following equation. [41]:

$$LOD = \frac{3S}{K} \quad (6)$$

where S is the standard deviation and K is the slope of the calibration curve. The limit of detection (LOD) depicted in Table 3. It was found to be 24 pg mL^{-1} with a linear range of detection range $0.1\text{--}100 \text{ ng mL}^{-1}$ at 20 % duty cycle of pulse-deposited Au. These conclusions support the idea that the immobilization surface plays a significant role in determining the frequency shift during the antibody-antigen interaction. A larger surface area provides more binding sites for biomolecules, resulting in stronger interactions between the antibody and antigen and leading to an enhanced resonance shift. In addition, we compared the sensitivity of our method with previously work that utilize QCM with various surface modifications for signal amplification. The results of this comparison are presented in Table 3.

4. Conclusion

In summary, the incorporation of gold nanospikes (AuNS) into the QCM biosensor shows promise for point-of-care PSA diagnosis. This approach is convenient for biomedical research and can the potential to extend its use for analyzing other cancer-related biomolecules. This developed biosensor enhances recognition element immobilization, amplifies signal transduction, and improves detection limits. It holds significant implications for early cancer detection and screening, addressing the growing demand for improved healthcare systems. The results confirm the successful deposition of gold nanostructures, increasing the effective surface area. The various chemical treatments provided in this work have improved surface properties suitable for biomolecule immobilization. The observed frequency shift demonstrates the AuNS-modified QCM biosensor's ability to detect PSA at various concentrations, with a remarkable limit of detection (LOD) of 24 pg mL^{-1} and a linear range of $0.1\text{--}100 \text{ ng mL}^{-1}$, displaying remarkable sensitivity. These approaches set the stage for the development of customized AuNS-QCM biosensors for specific biosensing applications in future research. Further investigations into the size of the AuNS structure and fine-tuning the relationship between the Au structure on the developed QCM electrode surface and the size of biomolecule immobilization are essential to enhance QCM efficiency for biomarker detection.

Statements and declarations

We, the undersigned, declare that this manuscript is original, has not been published before, and is not currently being considered for publication elsewhere.

CRediT authorship contribution statement

Thita Sonklin: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. **Suparat Tongpeng:** Conceptualization, Data curation, Investigation, Methodology. **Dhananjaya Munthala:** Conceptualization, Methodology, Writing – review & editing. **Sanong Suksaweang:** Supervision, Visualization, Writing – review

Table 3

A comparison of QCM biosensors with different signal amplification approaches reported for PCa detection. Gold nanoparticle: AuNP Gold nanospikes: AuNS.

Signal amplification	Biomarker	Linear range (ng mL ⁻¹)	Detection limit (ng mL ⁻¹)	Response time	Ref
Anti-PSA antibody -AuNP	PSA	–	0.054	1 hour (including drying)	[20]
Anti-PSA antibody -AuNP	PSA and PSA- α 1-antichymotrypsin complex (in 75 % serum)	0.29–150	0.29	8 min reaction time	[42]
Bare QCM	PSA	0.39–200	0.39	1 h	This work
AuNS (20 % duty cycle)	PSA	0.1–10	0.17	1 h	This work
AuNS (80 % duty cycle)	PSA	0.1–100	0.024	1 h	This work
		0.1–100	0.045	1 h	This work

& editing. **Pattanaiphong Janphuang**: Investigation, Methodology, Writing – review & editing. **Anu Bharti**: Investigation, Methodology, Writing – review & editing. **Ashish Mathur**: Investigation, Methodology, Writing – review & editing. **Devesh Kumar Avasthi**: Investigation, Methodology, Writing – review & editing. **Sukanda Jiansirisomboon**: Investigation, Methodology, Writing – review & editing. **Soodkhet Pojprapai**: Funding acquisition, Project administration, Supervision, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Data availability statements

This manuscript has no associated data. All data generated or analyzed during this study are included in this published article.

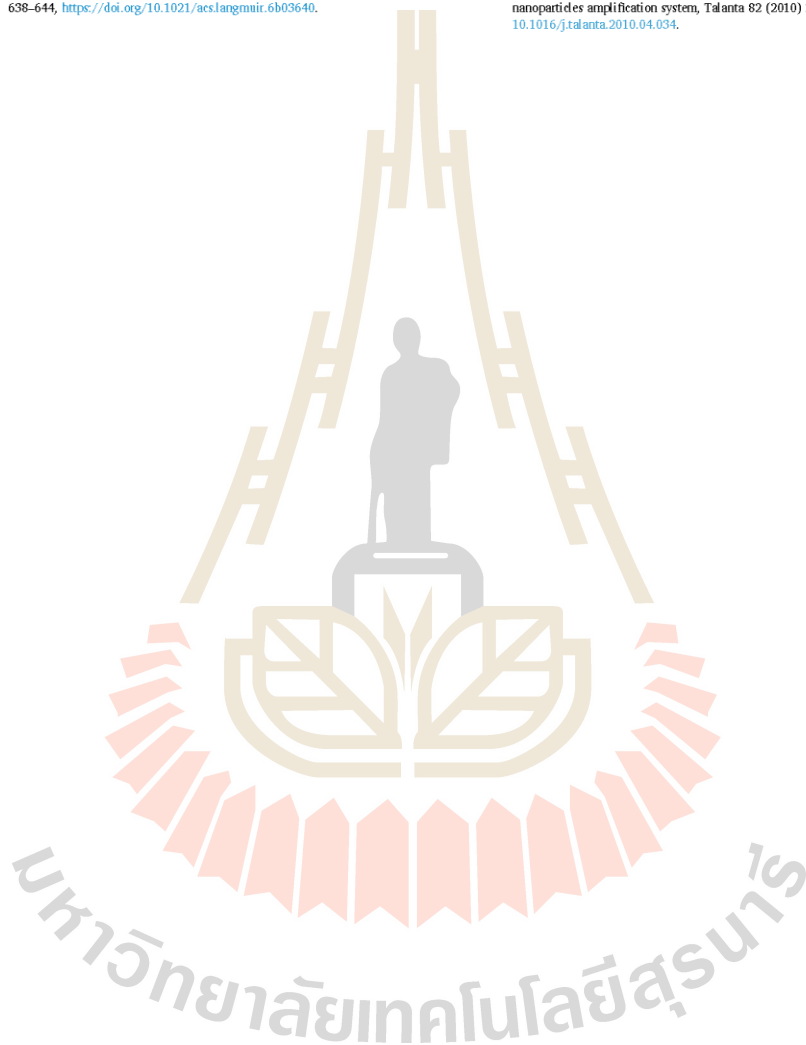
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BIOGRAPHY

Thita Sonklin was born in Surin, Thailand, on 1 MAY 1995. She graduated with a Bachelor's degree in Engineering of Ceramic with First Class Honours from Suranaree University of Technology in 2017. After graduation, she received a grant from the Royal Golden Jubilee (RGJ) Ph.D. Program through the National Research Council of Thailand (NRCT), Thailand Research Fund (TRF), and Synchrotron Light Research Institute (SLRI) Public Organization Thailand for studying for a philosophy degree. She is a Ph.D. candidate in the Materials Engineering Program at the Institute of Engineering, Suranaree University of Technology since 2017. While studying, she participated in an international research exchange program and attended international conferences. She has attended conferences and workshops more than 10 times. She has 12 publications, with 3 as the first author. She also participated in a research internship at University of Hyderabad, India. She is also the recipient of an AISTDF Fellowship for research training at University of Petroleum and Energy Studies (UPES), Dehradun, India. Additionally, she conducted research at University of New South Wales (UNSW), Australia. Thita completed her Doctoral degree in 2024. Her research expertise lies in piezoelectric materials and their properties for biosensor applications, with a focus on:

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2. Biosensors and applications for biomarker detection.
3. Surface acoustic wave (SAW), film bulk acoustic resonator (FBAR) device fabrication, simulation, and characterization.
4. Fabrication of colloidal nanoparticles and embedded nanoparticles for immunoassay detection.