Project Title: **Innovation of photonic and electrochemical biosensors for cholangiocarcinoma diagnosis**

Speaker: **Prof. Somchai Pinlaor,** et al.,

Project Duration : 24 months

First Year : May 1st , 2023 to April 30th , 2024
Second Year: May 1st , 2024 to April 30th , 2025

Project Budget: 80,000 USD

First Year : 40,000 USD
Second Year: 40,000 USD

25 Sep 2025, BBK, Thailand

Project Title: Innovation of photonic and electrochemical biosensors for cholangiocarcinoma diagnosis

Project members

7 institutes, 3 countries

KKU (THA) : **Somchai Pinlaor**, Chavis Srichan, Pobporn Danvirutai, Kitti Intuyod, Apisit Chaidee, Sirinapha Klungsaeng

CMU (THA) : Ukrit Mankong, Suruk Udomsom

TMEC : Nithi Atthi

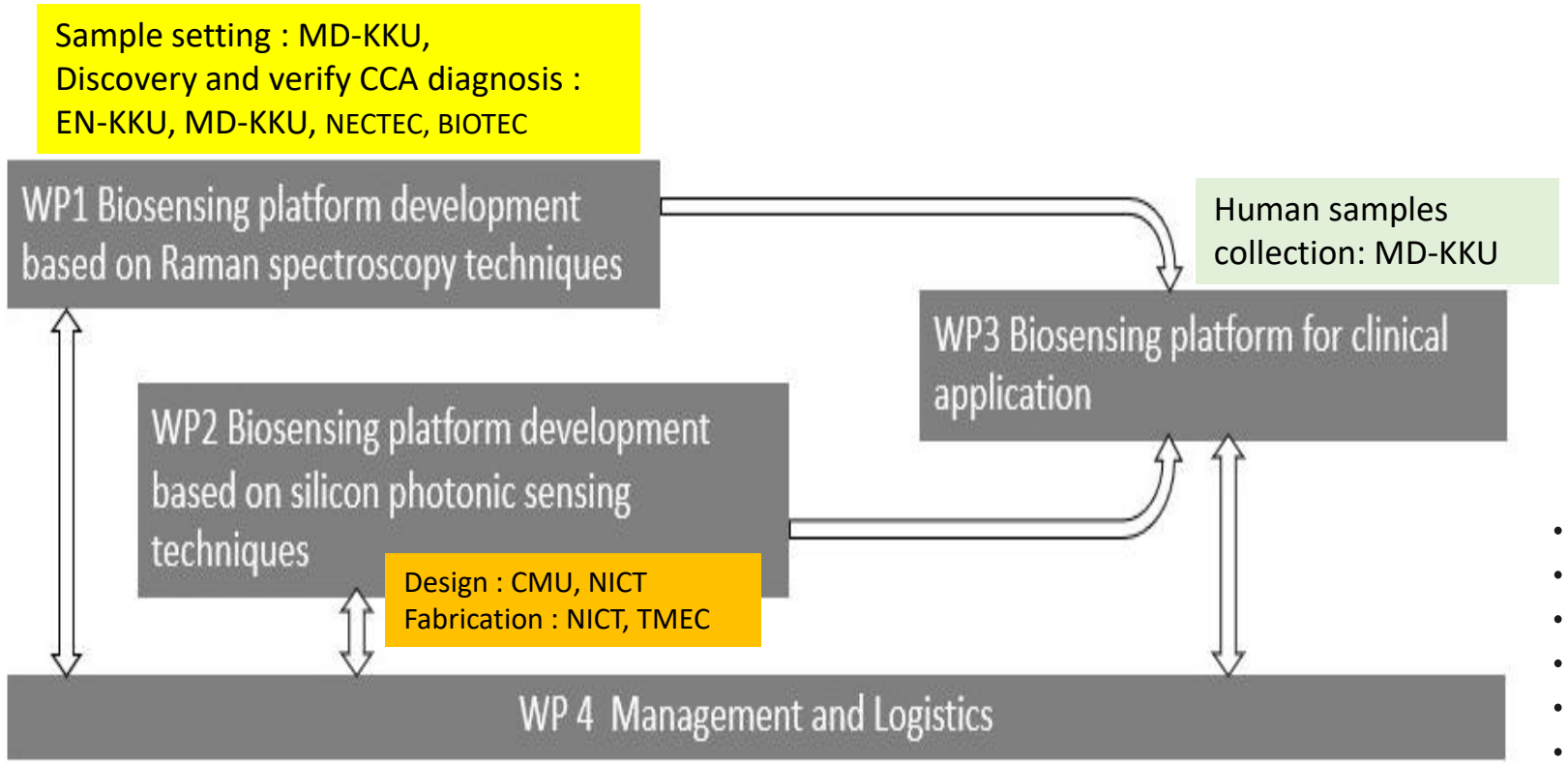
BIOTEC : Sittiruk Roytrakul, Janthima Jaresitthikunchai, Narumon Phaonakrop

NECTEC : Noppadon Nuntawong, Mati Horprathum, Pitak Eiamchai, Saksorn Limwichean

Mittaphab Hospital (LAO) : Champadeng Vongdala, Keooudone Thammavong

NICT (JPN) : Toshimasa Umezawa, Atsushi Matsumoto, Kouichi Akahane

Project management

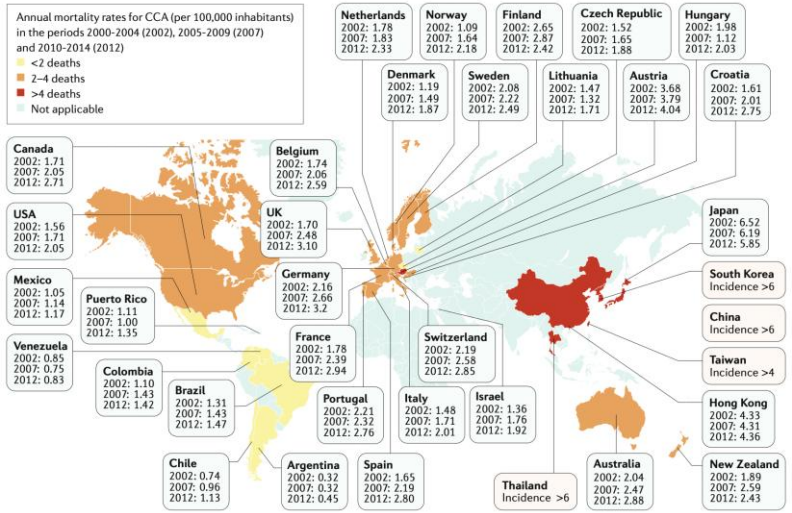


LAOS

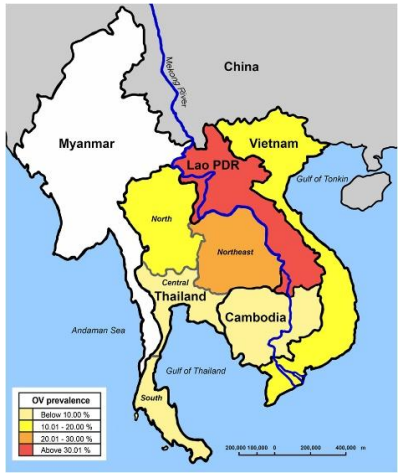
Workspace of project management

Project title: Innovation of photonic and electrochemical biosensors for cholangiocarcinoma diagnosis

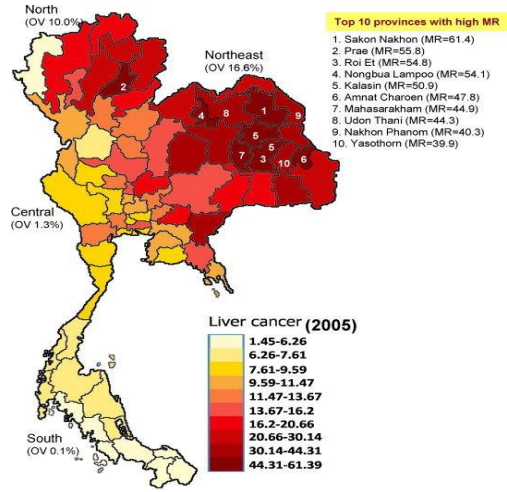
Background



Banales. J.M. et al, 2020



Khuntikeo N *et al*. 2018



Sripa B *et al*. 2011

Current diagnostic techniques

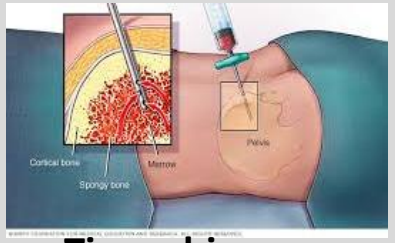
Tumor screening

Ultrasound

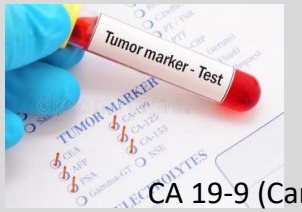
Confirmation

MRI

Tumor type identifying & staging



Tissue biopsy



Tumor markers: CA19-9, CEA, AFP

CA 19-9 (Carbohydrate Antigen 19-9), CEA (Carcinoembryonic Antigen), and AFP (Alpha-fetoprotein)

Project title: Innovation of photonic and electrochemical biosensors for cholangiocarcinoma diagnosis

Previous finding

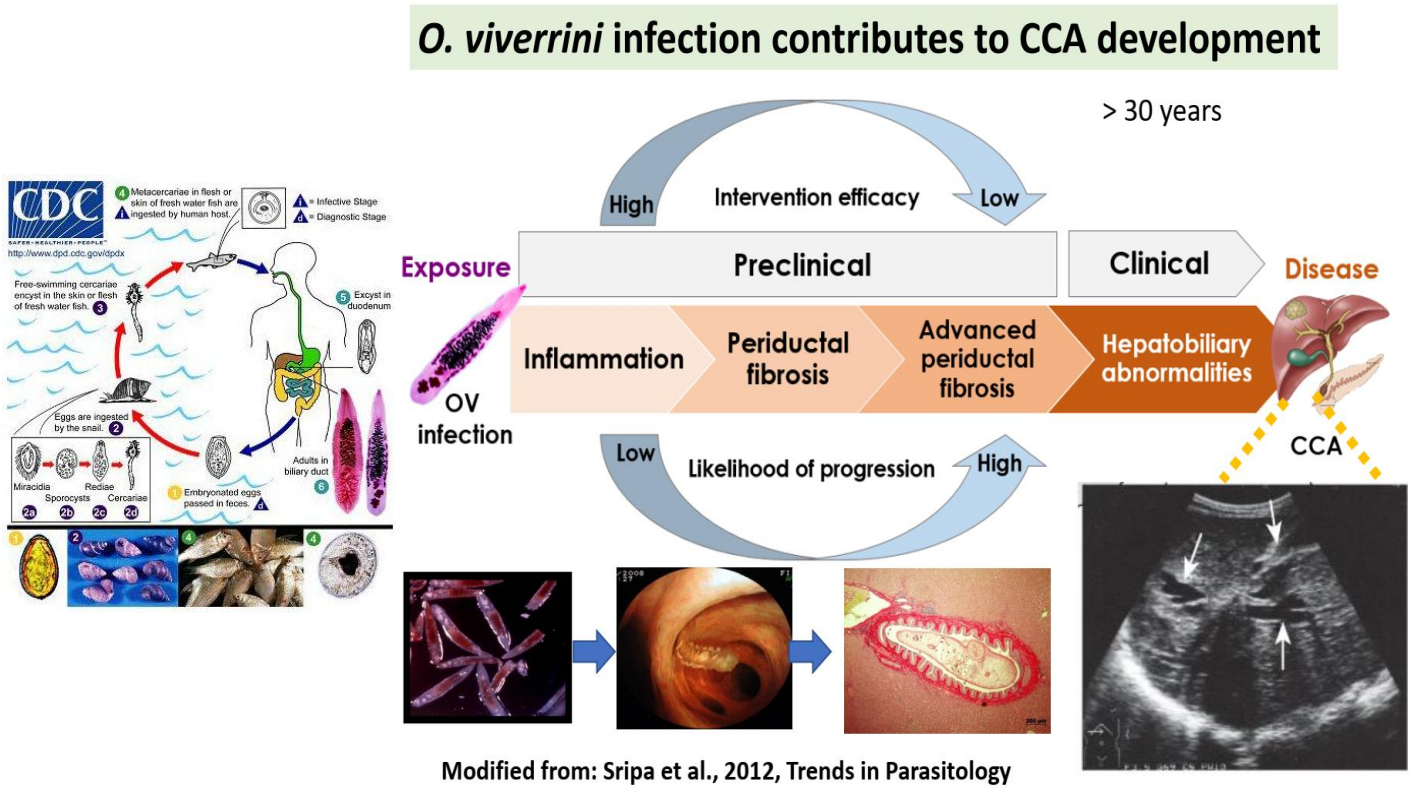


Fig.1 OV-induced inflammation, fibrosis, advanced fibrosis, and contribution risk to CCA. Thus, radiology such as MRI & Ultrasound are used for CCA screening.

Our hypothesis

CCA development is cell-types specifics.

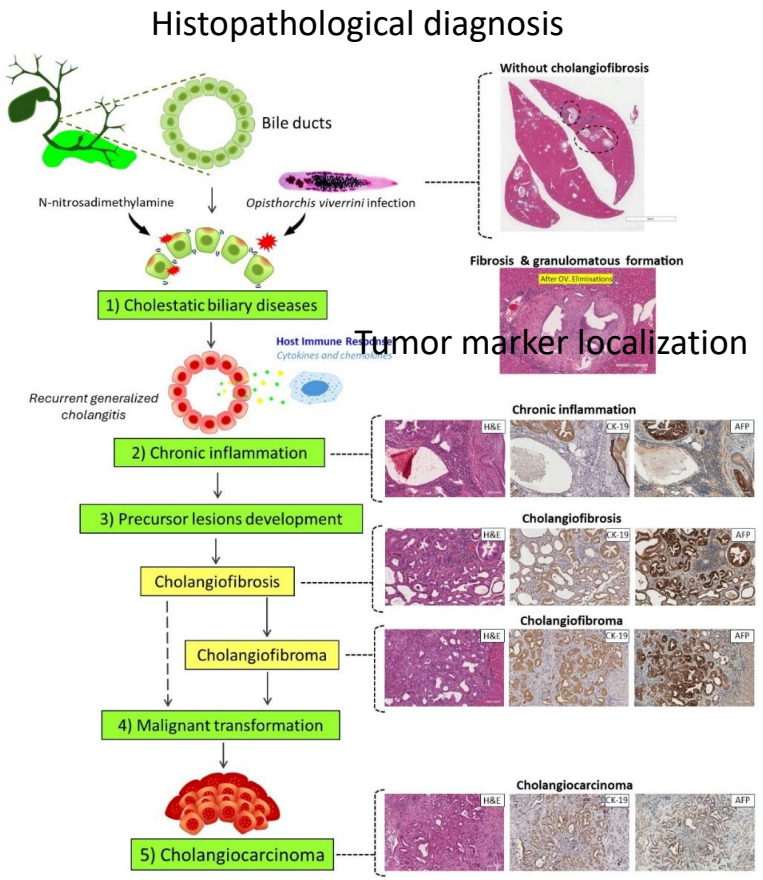
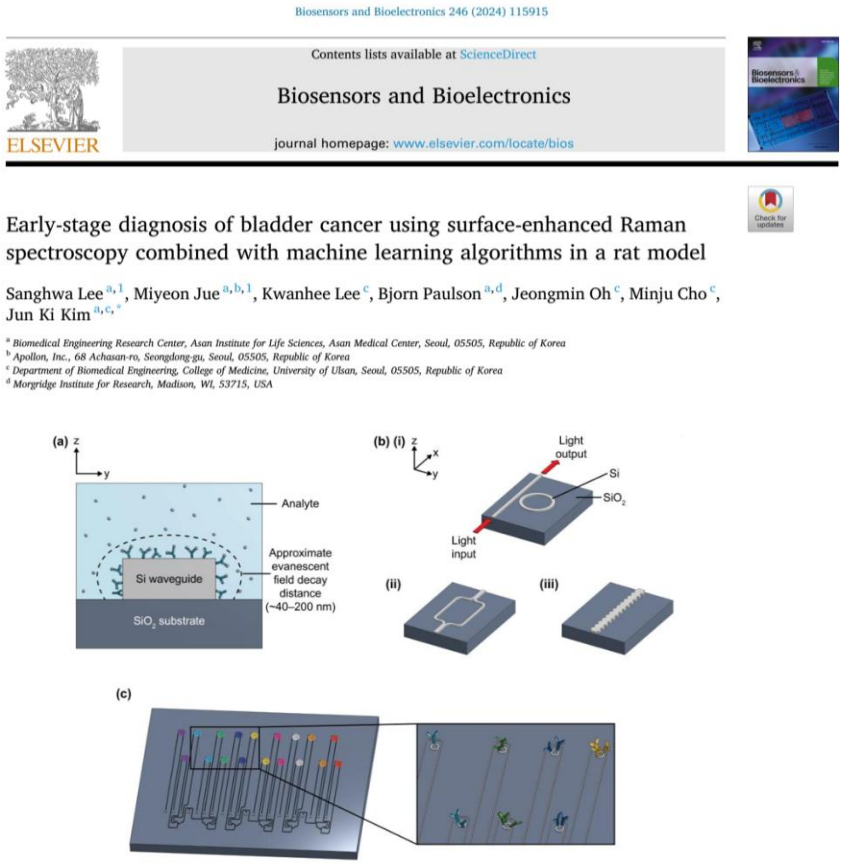


Fig.2 OV-induced chronic inflammation, precancerous lesion and CCA development

Project title: Innovation of photonic and electrochemical biosensors for cholangiocarcinoma diagnosis

Challenge



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Early-stage diagnosis of bladder cancer using surface-enhanced Raman spectroscopy combined with machine learning algorithms in a rat model

Sanghwa Lee [a,1], Miyeon Jue [a,b,1], Kwanhee Lee [c], Bjorn Paulson [a,d], Jeongmin Oh [c], Minju Cho [c], Jun Ki Kim [a,c,*]

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[b] Apollon, Inc., 68 Achasan-ro, Seongdong-gu, Seoul, 05505, Republic of Korea
[c] Department of Biomedical Engineering, College of Medicine, University of Ulsan, Seoul, 05505, Republic of Korea
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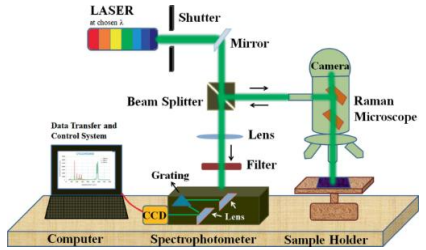
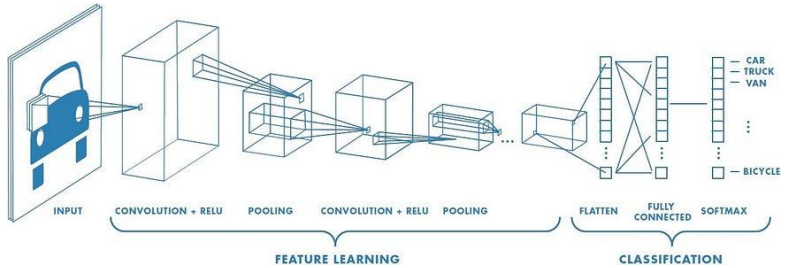
Silicon photonic sensor

Raman spectroscopy



NECTEC SERs Chips

+



Convolutional neuron network, CNN

Target

To develop photonic and electrochemical biosensors for cholangiocarcinoma diagnosis

Hamster's CCA establishment for hypothesis testing

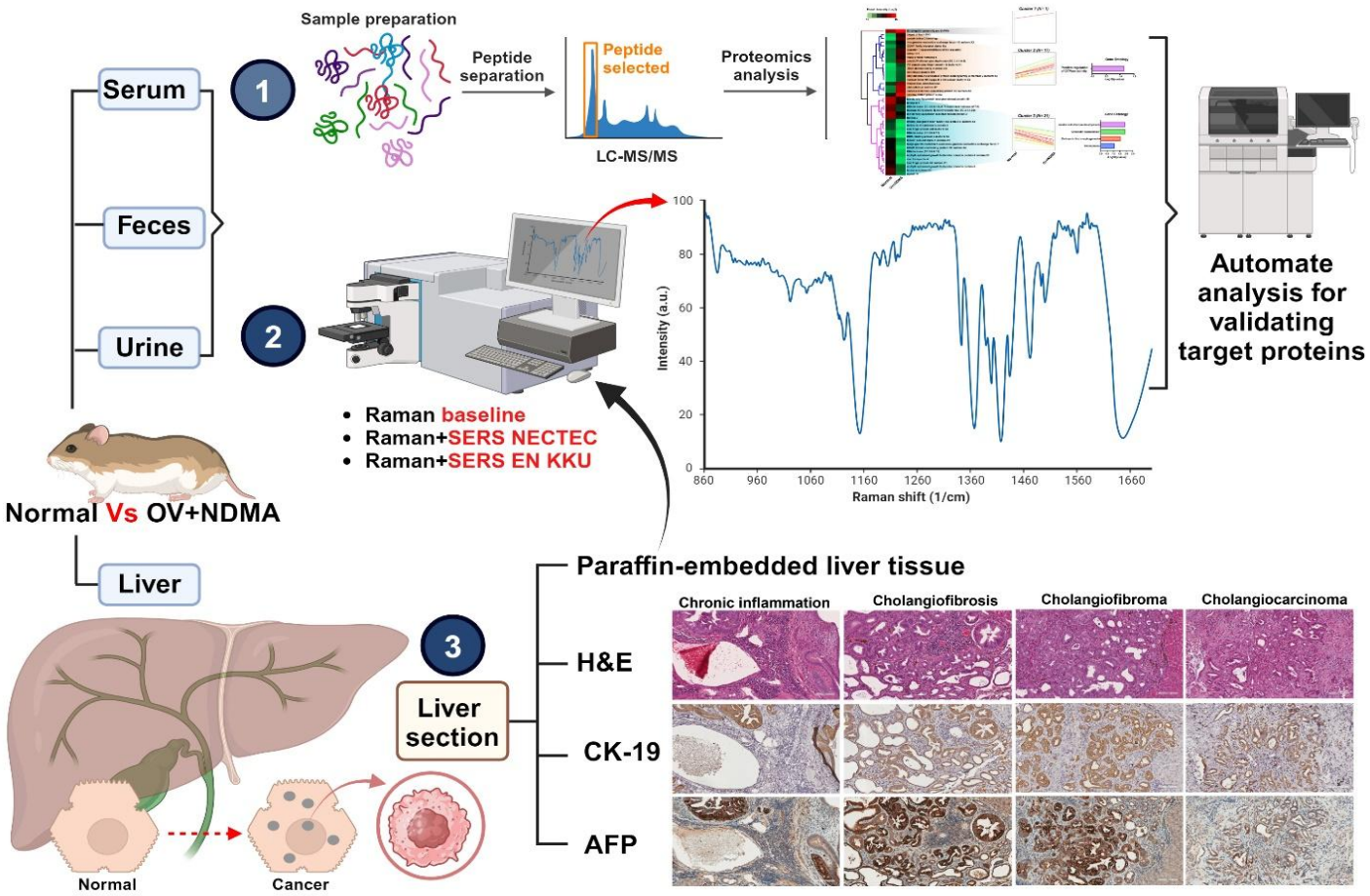


Fig.3 Schematic diagram illustration of the scope of the study in animal model. The early marker for CCA diagnosis was discovered in hamster model and apply in the patients. The step include 1) proteomic analysis for protein identification, 2) Raman spectroscopy based on based line peak, SERS from NECTEC and SERS from EN-KKU, and 3) histopathological study by staining with hematoxylin-eosin (H&E), cytokeratin-19 (CK-19) and alpha-fetoprotein (AFP).

Project activities: MDKKU-NECTEC

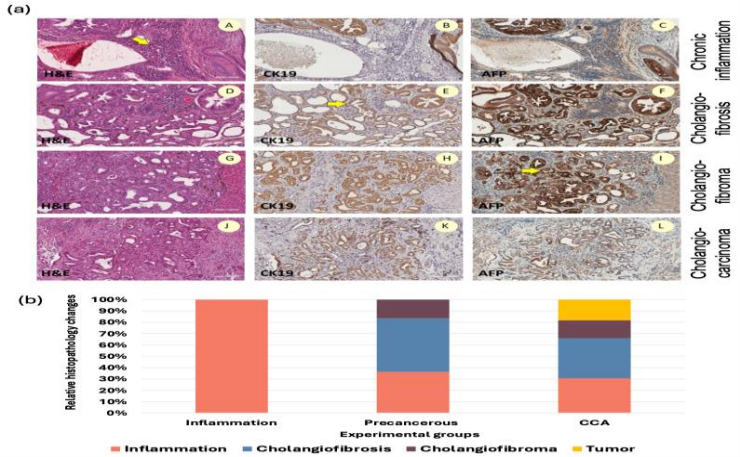


Fig.4 CCA development is classified into inflammation, pre-cancerous lesion and tumor lesion according to histopathological changes

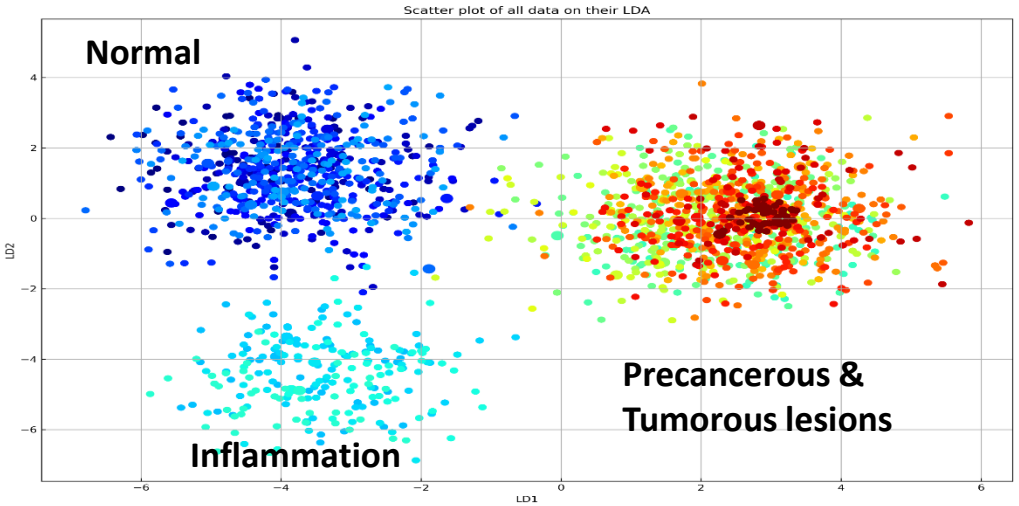


Fig.5 Comparison among principle components analysis (PCA) results of Raman spectra of four classes of normal, inflammation, pre-cancerous and tumor. However, Raman intensities couldn't distinguish between pre-cancerous and CCA groups based on classical machine (ML) algorithm.

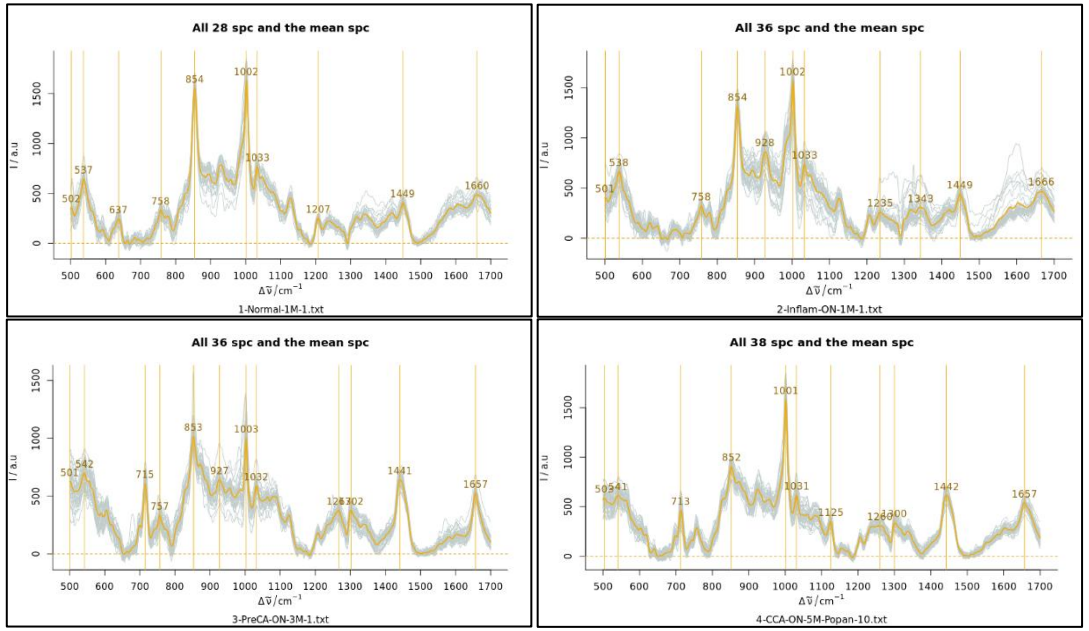


Fig.6 Comparison among Raman peak based on classical machine (ML) algorithm of four classes based on normal, inflammation, pre-cancerous and tumor features.

	precision	recall	f1-score	support
Group 1-N	0.61	0.84	0.71	231
Group 2-I	0.40	0.07	0.11	61
Group 3-P	0.61	0.51	0.56	210
Group 4-C	0.65	0.65	0.65	220
accuracy			0.62	722
macro avg	0.57	0.52	0.51	722
weighted avg	0.61	0.62	0.60	722

SERS couple with PCA couldn't distinguish precancerous-tumor lesion and yield accuracy of test 62%.

The integration of SERS and machine learning achieved a diagnostic sensitivity of 93%, specificity of 95%, and accuracy of ≥ 67% for precancerous lesions and CCA

Project activities: MDKKU-ENKKU

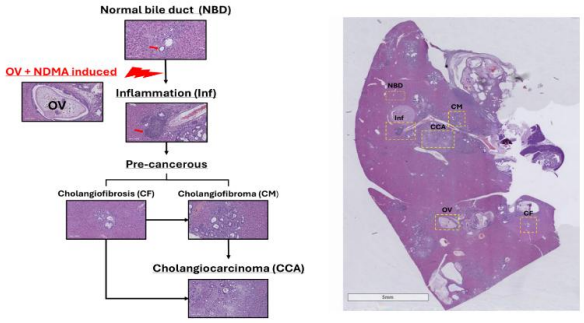


Fig.7 Different staging of CCA development was established.

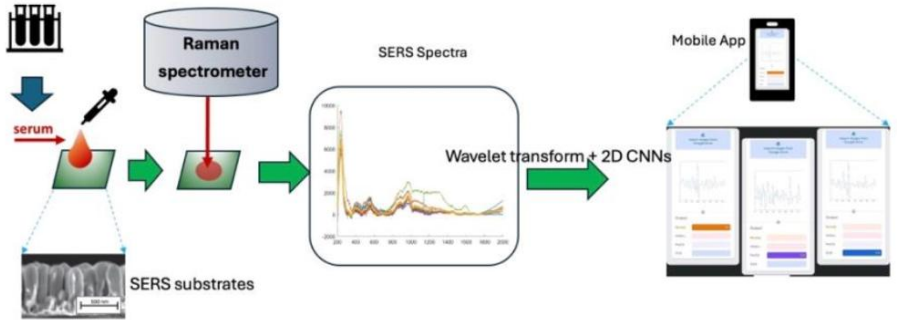


Fig.8 Illustration of study design for SERS+AI marks early stage of CCA

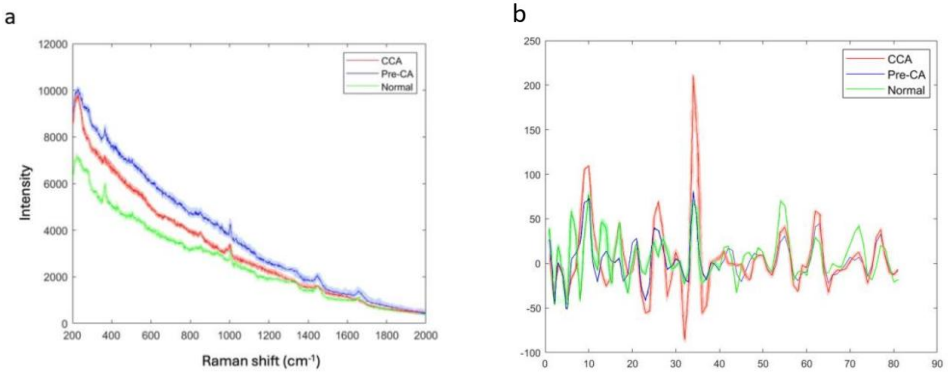


Fig.9 Raman peak identifies the different staging of inflammation, precancerous (Pre-CA) and CCA lesions

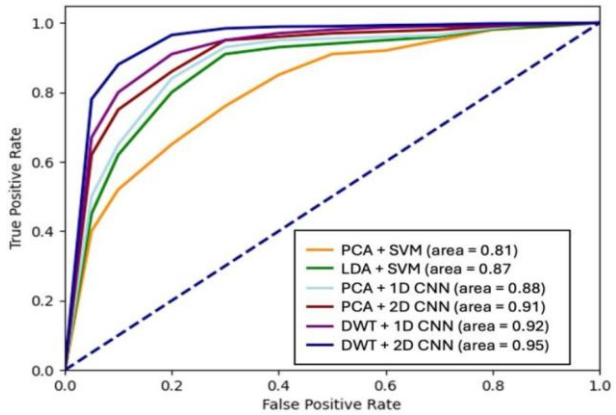


Fig.10 ROC curves analysis the performance of various classification models. The DWT + 2D CNN model achieved an AUC of 0.95, which gave the highest accuracy performance for CCA diagnosis compared to the other models.

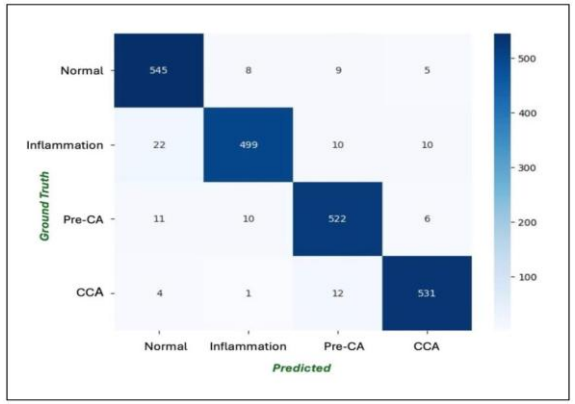


Fig.11 Confusion matrices of each signal processing and machine learning methods by 2D CNN.

DWT = discrete wavelet transform

Method	Accuracy (%)	Sensitivity (Recall) (%)	Specificity (%)	F1 Score (%)
DWT + 2D CNN	95.10	95.08	98.36	95.10
DWT + 1D CNN	92.00	91.50	96.00	91.75
PCA + 1D CNN	88.00	87.50	94.00	87.75
LDA + SVM	84.50	84.00	90.50	84.25
PCA + SVM	82.40	82.00	88.40	82.20

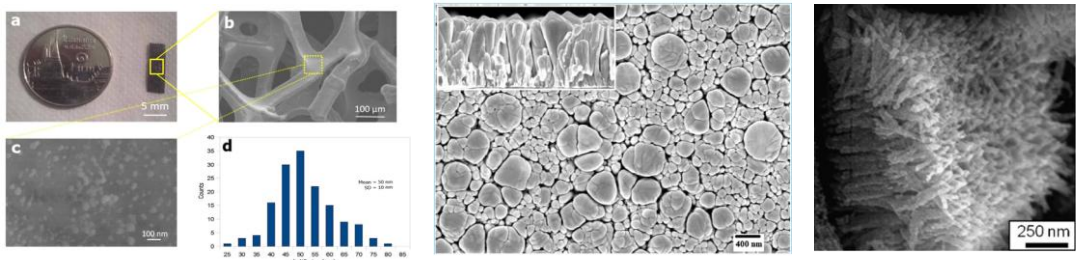
Accuracy 95%, sensitivity 95%. Specificity 96%

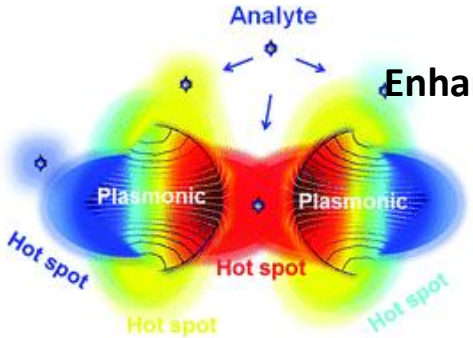
Raman spectrometry coupled with machine learning, especially, 2D CNN, could distinguish stage-specific for CCA development for normal, inflammation, pre-cancerous and tumor, but doesn't by classical machine learning of Raman intensity. Integrative SERS with 2D CNN explores high accuracy performance for early CCA diagnosis, which can apply for a mobile application use.

Project activities: ENKKU: Biosensing platform based on SERS

Biosensing platform development based on Raman spectroscopy techniques

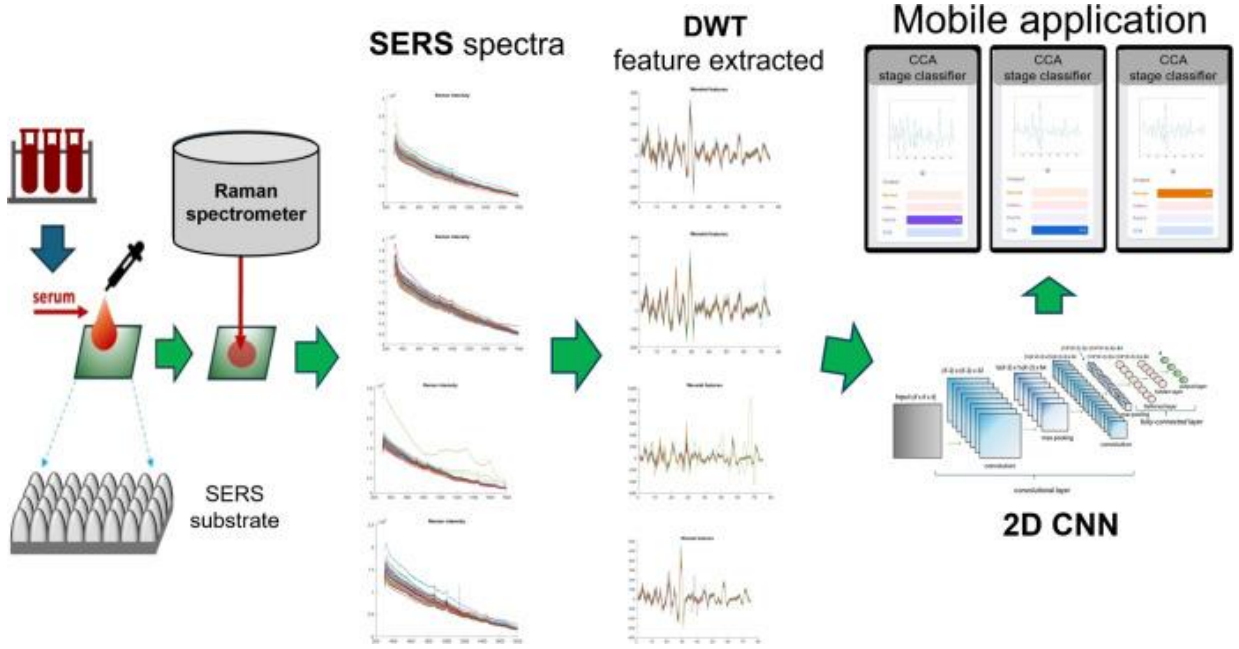
Surface Enhance Raman Spectroscopic (SERS) chip development





Enhanced hotspot with linkers and receptors for biomarker

SERS chip using (a) 3D microporous graphene (Srichan *et al.*, 2016) (b) Silver nanorods fabricated by glancing angle method (Botta *et al.*, 2018) (c) electrochemically synthesized silver nanowires (Fang *et al.*, 2012) (d) Hot spot model as one of the mechanisms behind Raman signal enhancement (Radziuk & Moehwald, 2015).



(a) Raman peak selection for a specific analyte (Srichan *et al.*, 2016), (b) designed portable SERS-based sensor for early CCA detection (this proposal).

Project activities: ENKKU: Biosensing platform based on SERS

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Article

Highly Accurate and Robust Early Stage Detection of Cholangiocarcinoma Using Near-Lossless SERS Signal Processing with Machine Learning and 2D CNN for Point-of-care Mobile Application

Pobporn Danvirutai, Thatsanapong Pongking, Suppakrit Kongsintaweesuk, Somchai Pinlaor, Sartra Wongthanavasu, and Chavis Srichan*

Cite This: *ACS Omega* 2025, 10, 11296−11311

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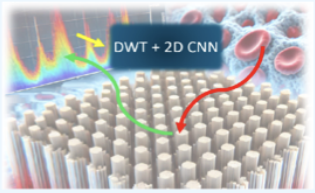
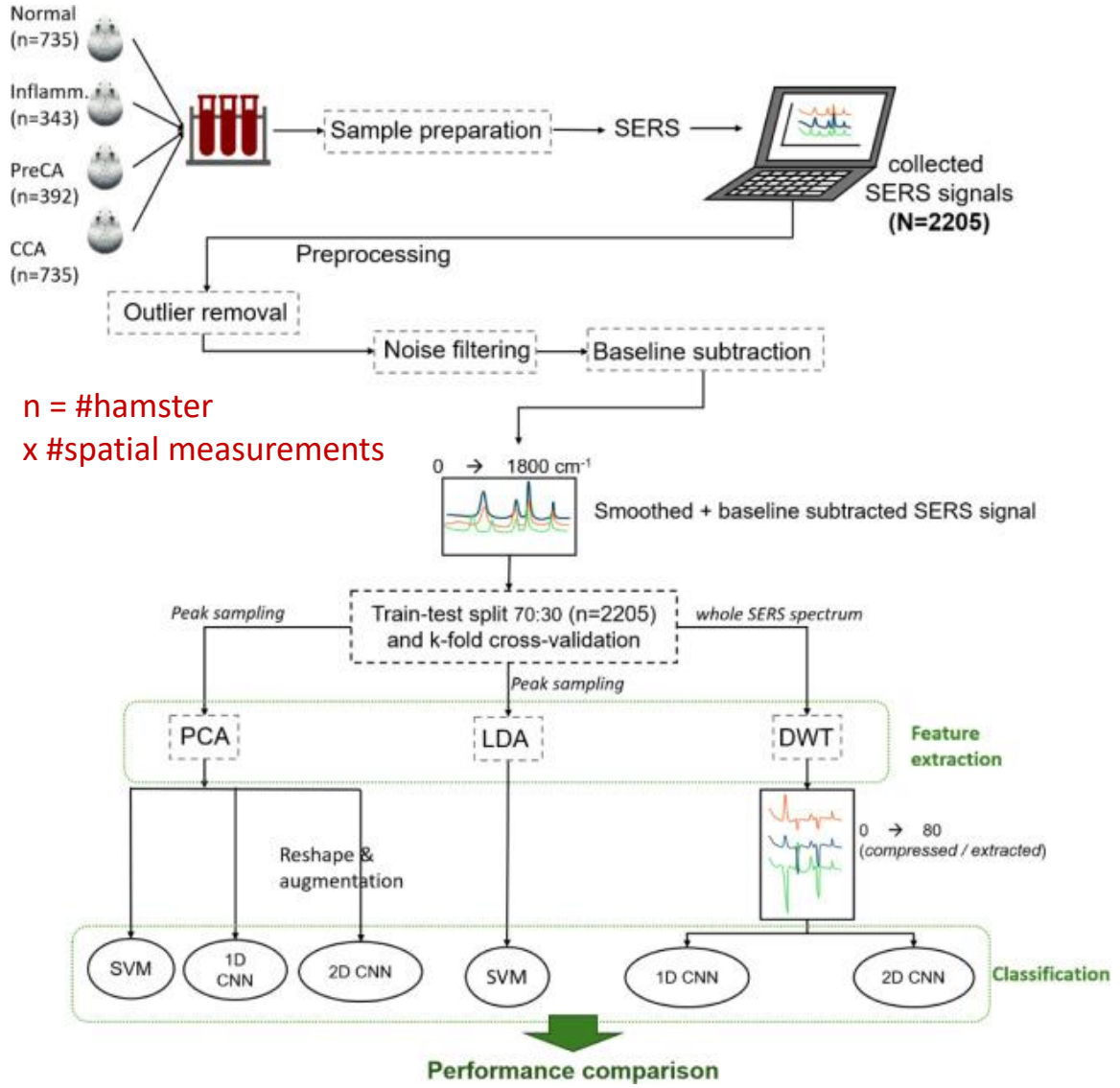
ABSTRACT: Introduction: Cholangiocarcinoma (CCA), a malignancy of the bile ducts, presents a significant health burden with a notably high prevalence in Northeast Thailand, where its incidence ratio is 85 per 100,000 population per year. The prognosis for CCA patients remains poor, particularly for proximal tumors, with a dismal 5-year survival rate of just 10%. The challenge in managing CCA is exacerbated by its typically late detection, contributing to a high mortality rate. Current screening methods, such as ultrasound, are insufficient, as many CCA patients do not exhibit prior symptoms or detectable liver fluke (*Opisthorchis viverrini:* OV) infections, underscoring the urgent need for alternative early detection methods. Methods: In this study, we introduce a novel approach utilizing surface-enhanced Raman spectroscopy (SERS) combined with near-lossless signal compression via discrete wavelet transform (DWT) together with 2D CNN for the first time. Hamster serums of different stages were collected as the data set. DWT was employed for feature extraction, enabling the capture of the entire SERS spectrum, unlike traditional methods like PCA and LDA, which focus only on specific peaks. These features were used to train a 2D convolutional neural network (2D CNN), which is particularly robust against translation, rotation, and scaling, thus effectively addressing the SERS peak shifting issues. We validated our approach using gold-standard histology, and notably, our method could detect CCA at an early stage. The ability to identify CCA at the early stage significantly improves the chances of successful intervention and patient outcomes. Results and conclusion: Our results demonstrate that our method, combining SERS with extremely compact wavelet feature extraction and 2D CNN, outperformed other approaches (PCA + SVM, PCA + 1D CNN, PCA + 2D CNN, LDA + SVM, and DWT + 1D CNN), achieving performance of 95.1% accuracy, 95.08% sensitivity, 98.4% specificity, and an area under the curve (AUC) of 95%. The trained model was further deployed on a server and mobile application interface, paving the way for future field experiments in rural areas and home-use potential point-of-care services.



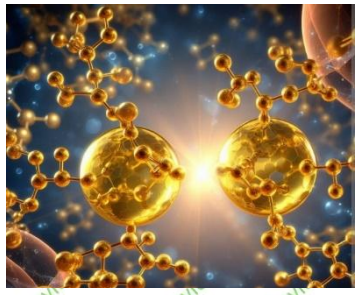


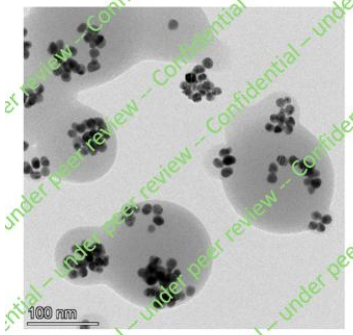
Project activities: ENKKU: Biosensing platform based on SERS

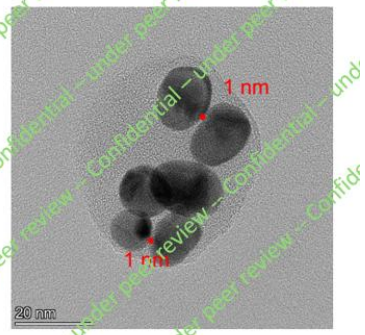
1. Software license
2. (Q1) ACS OMEGA
3. (Scopus-index) Conference paper to appear in IEEEXplore

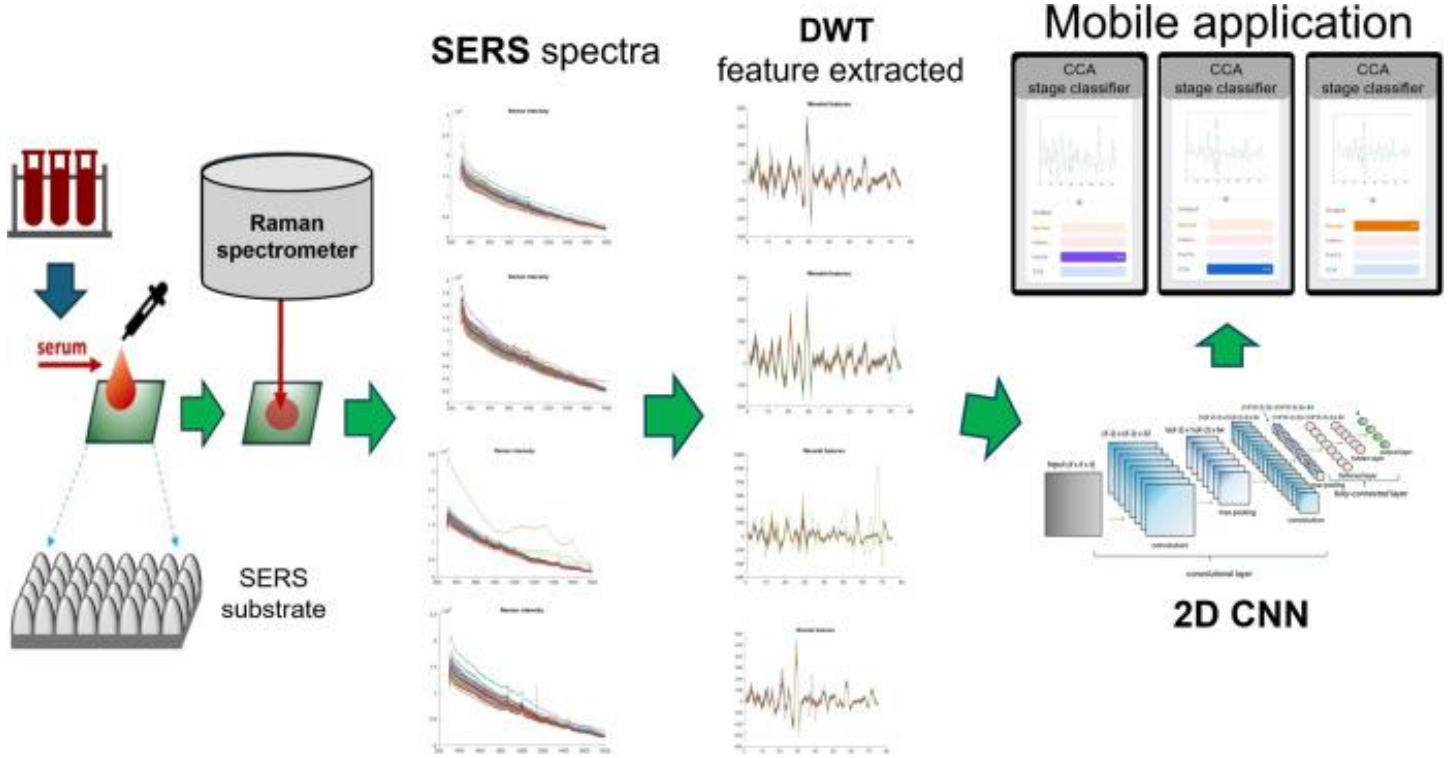
Surface Enhance Raman Spectroscopic (SERS) chip development

Part I: Synthesis of AuNPs and novel techniques to pair nanoparticles to yield greatest enhancement



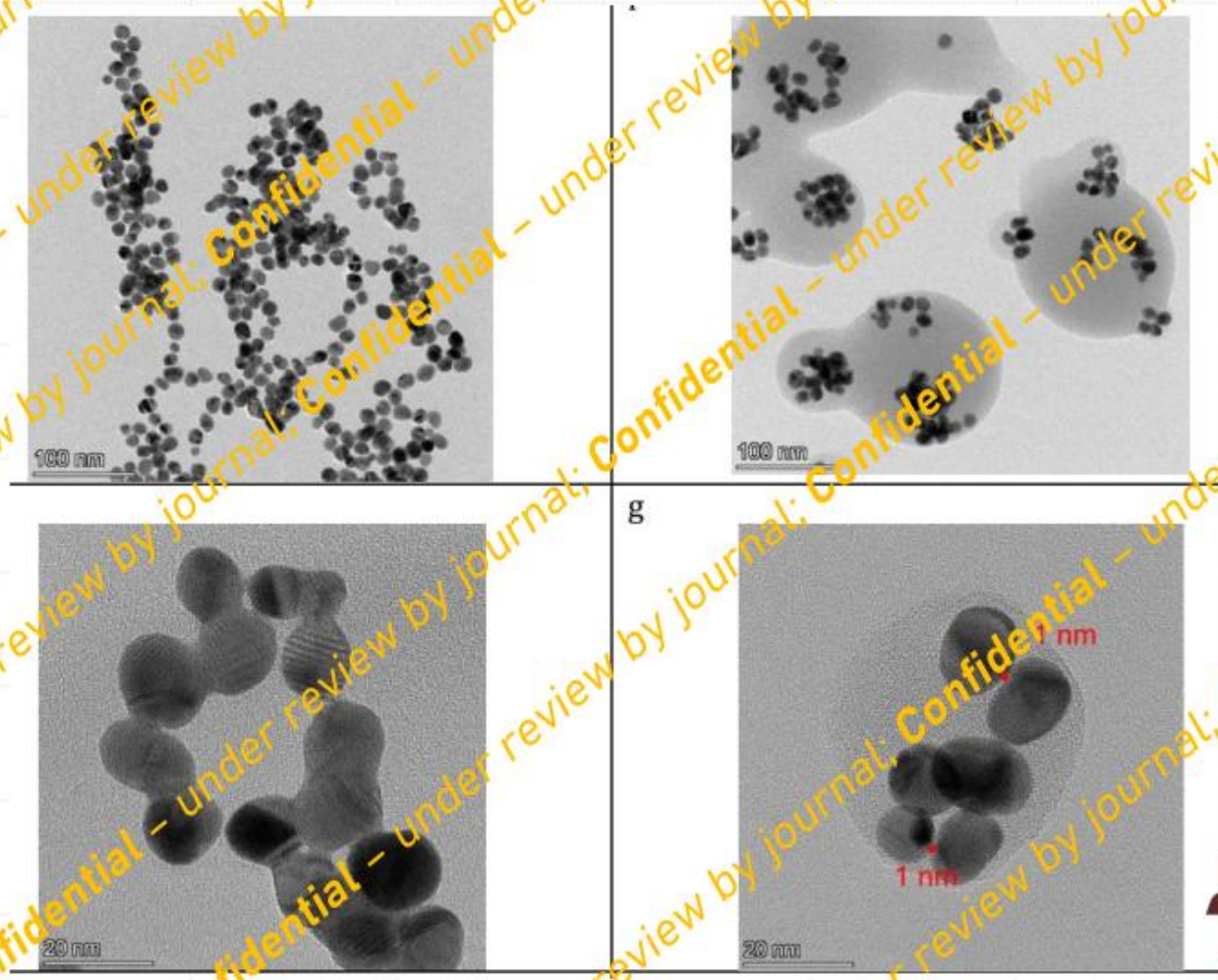






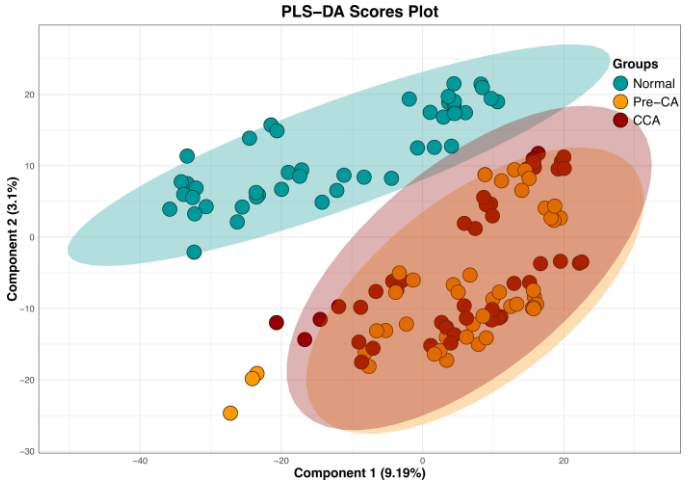
+ carried out characterization on TEMs, SEM, processing technique, etc. (Figures will be licensed under publisher)

Novel Gold Nanoparticles with 1nm gap for optimized SERS Enhancement

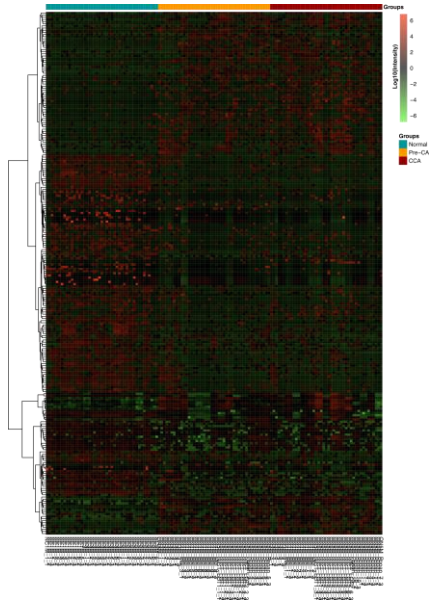


Project activities: MDKKU-BIOTEC

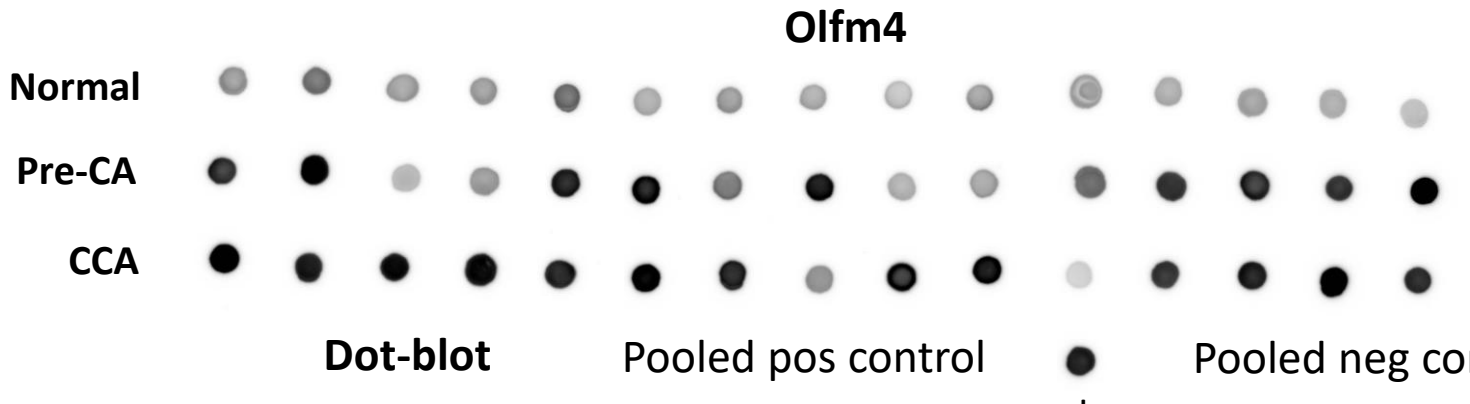
Proteomics discovery Olf4, SMC2, B4GALT4 for CCA diagnosis

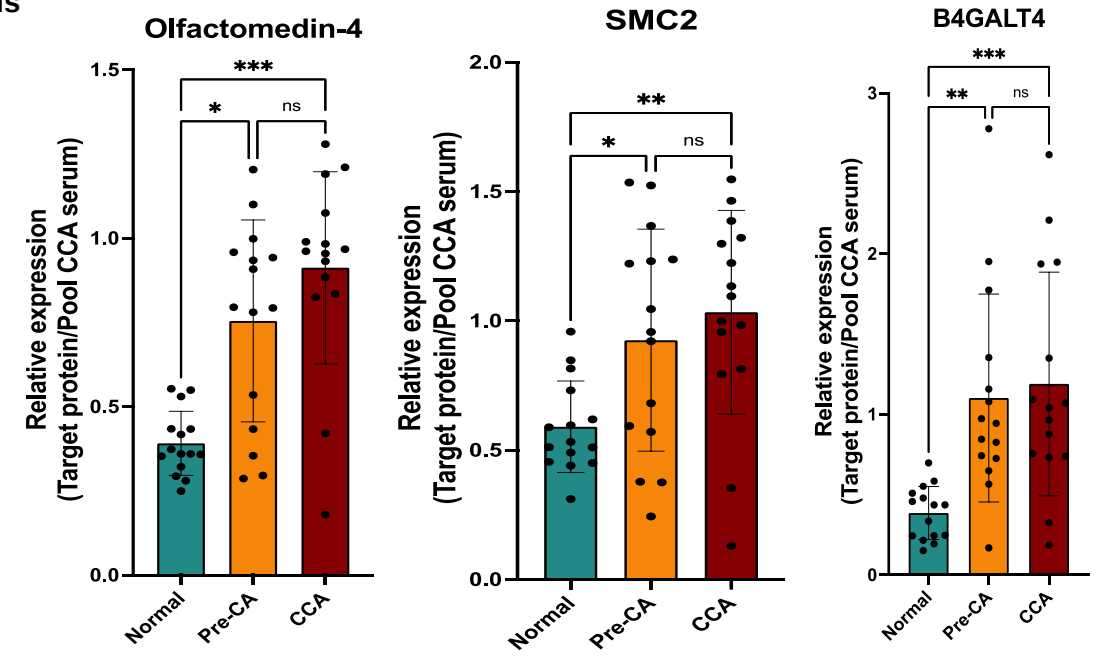


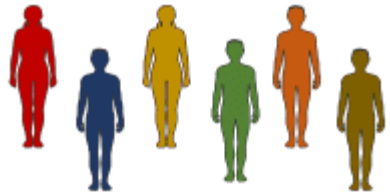
Partial Least Squares Discriminant Analysis (PLS-DA) analysis



Heatmap

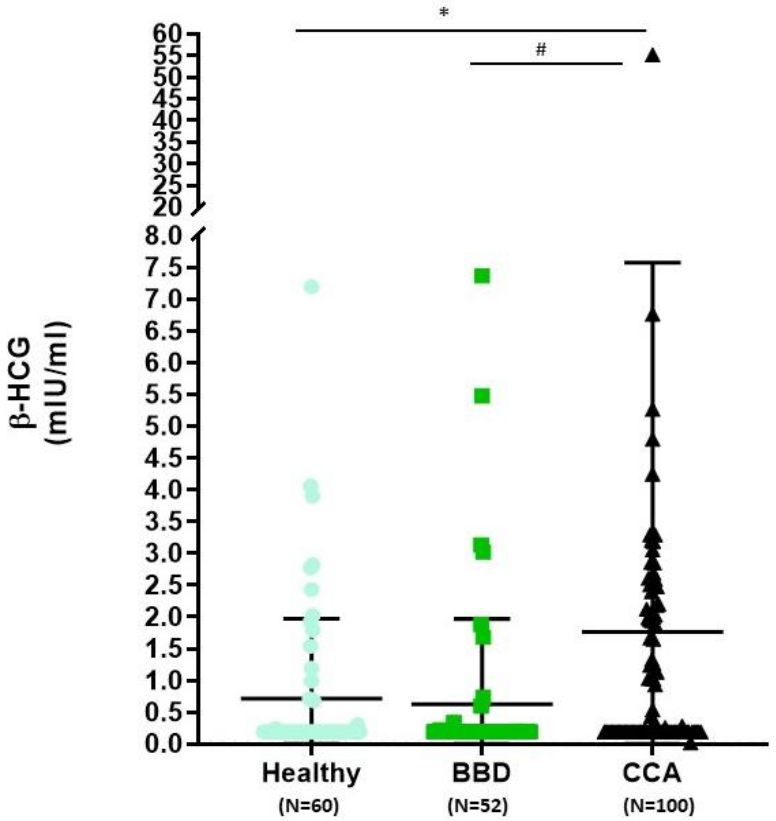




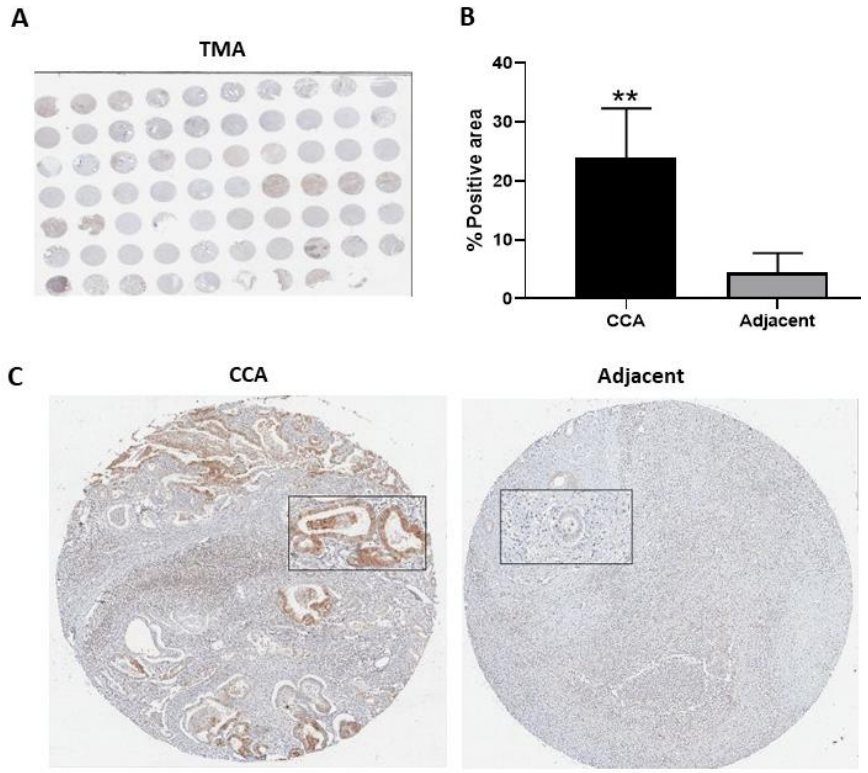


In progress to verify in human samples

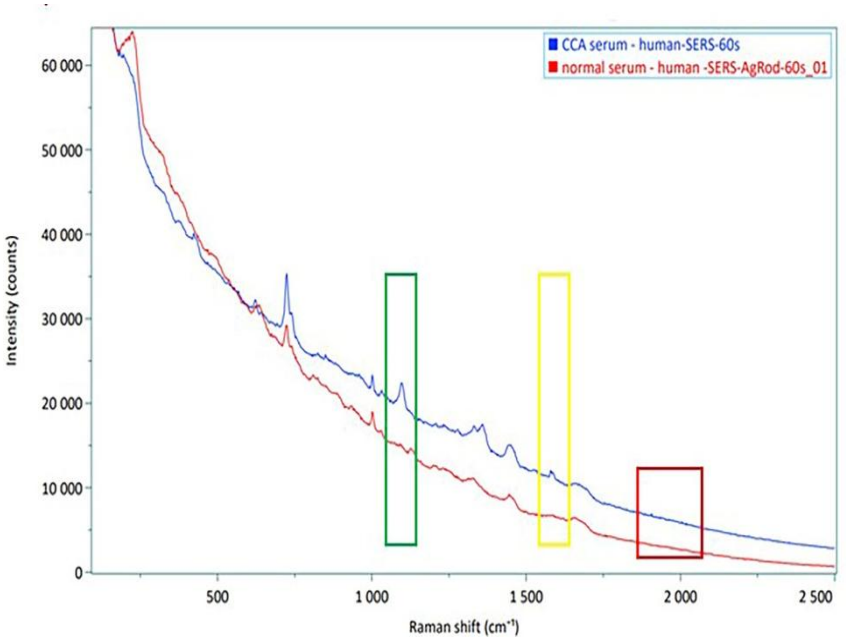
Project activities: MDKKU-BIOTECH; Sample collection, identification and evaluation of biomarker in CCA patients



Determination of β-HCG levels in human serum. Serum β-HCG levels in healthy (N=60), Bening biliary disease (N=52), and CCA patient (N=100) were determined using electrochemiluminescence immunoassay. * P<0.05 compared with healthy group. #P<0.05 compared with BBD group.

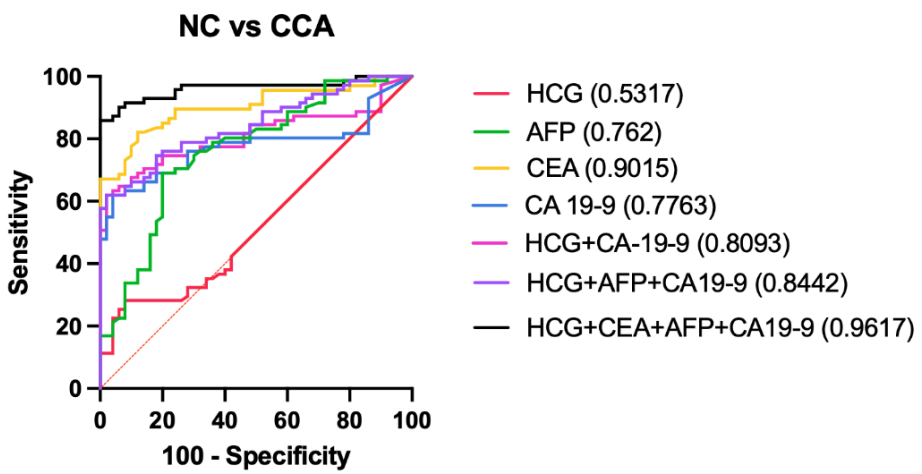


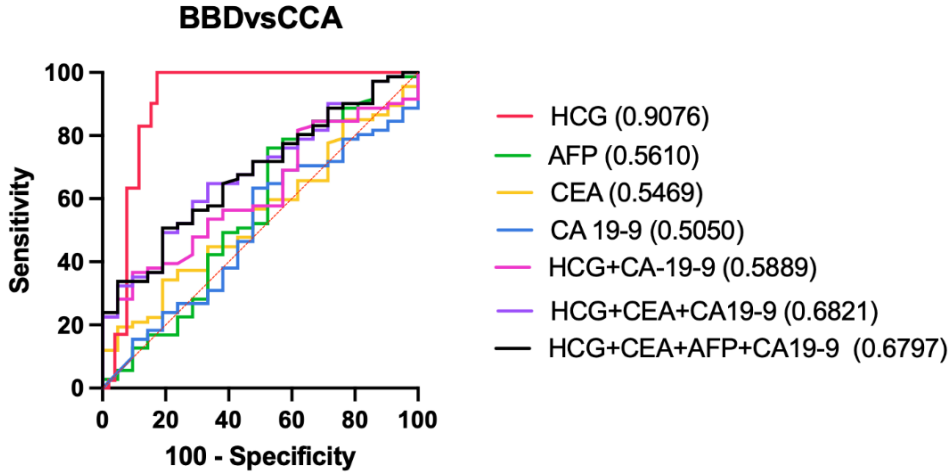
Immunohistochemical staining for β-hCG in cholangiocarcinoma and adjacent tissues. Staining pattern of the TMA section (A). Immunoreactive positive area (%) in cancerous tissues was higher than in adjacent tissues (B). Images showing β-hCG staining in CCA (C), and adjacent tissues (D). ** P<0.001 compared with adjacent tissue.

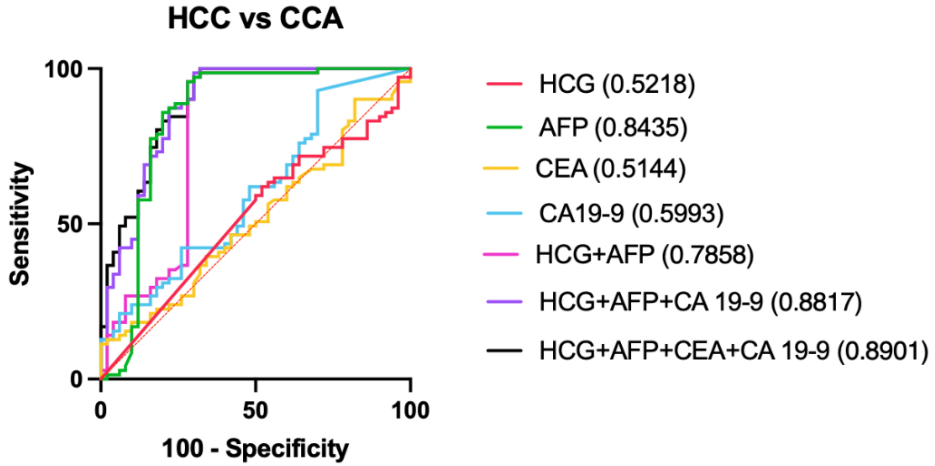


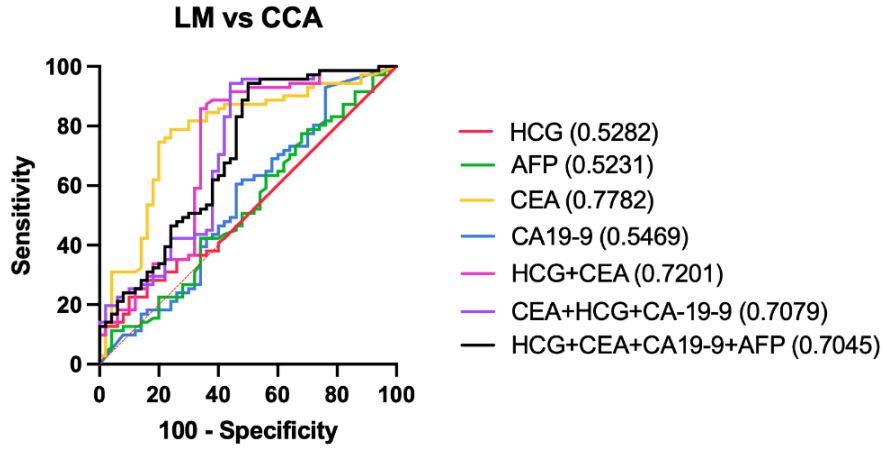
Raman peak separates serum CCA from the patients from normal serum by Raman spectroscopy. Upper line is CCA, and under line is normal.

Project activities: MDKKU-BIOTECH, Identification and evaluation of biomarker in CCA patients







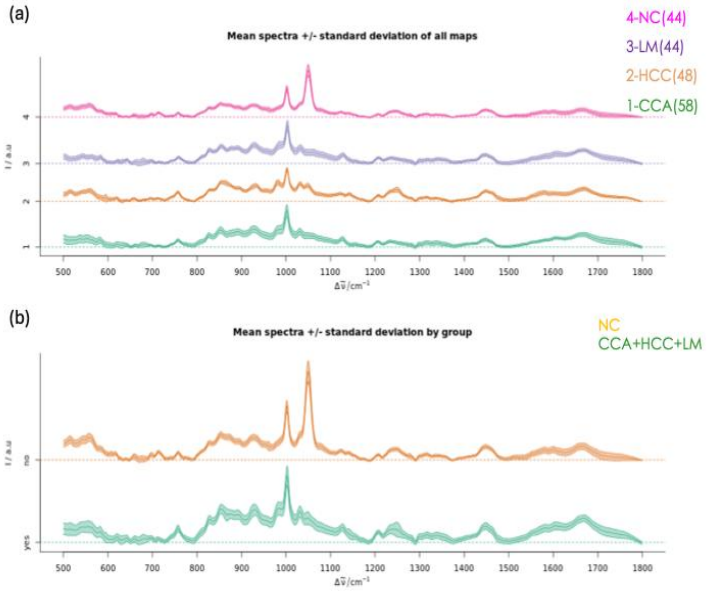


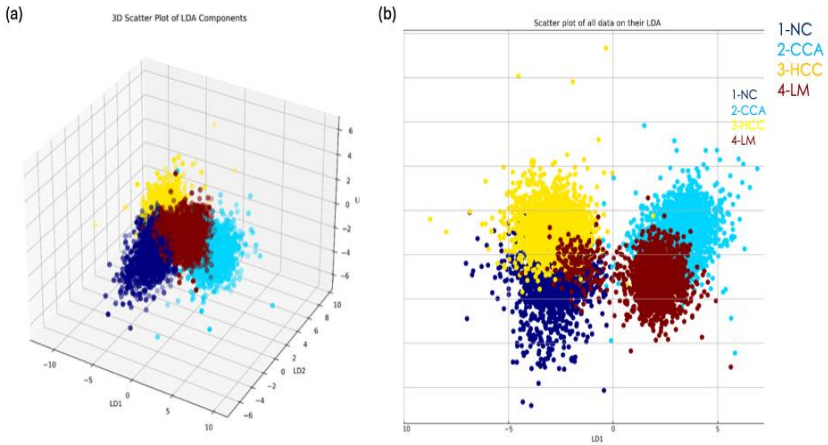
ROC analysis results when combined HCG, AFP, CEA and CA19-9

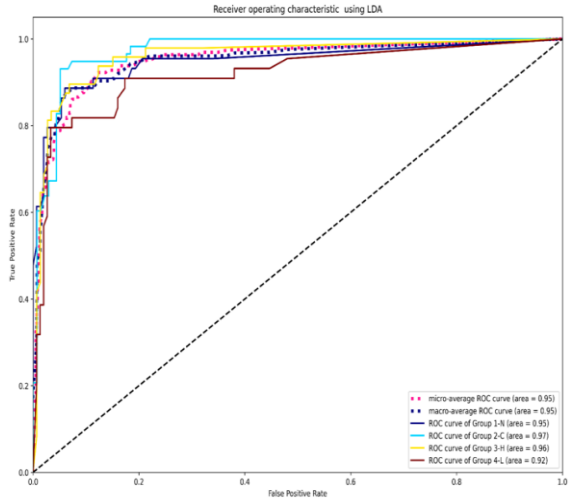
Project activities: MDKKU/NECTEC; Raman spectrum detection and Machine learning training

Human samples analysis (n=221): CCA = 71, HCC = 50, LM = 50, HA = 50

Combination of SERS and silver nanorod substrate with LDA could effectively identify and differentiate CCA from healthy subjects and patients with other liver malignancies with **81% accuracy and 0.97 of AUC values**.







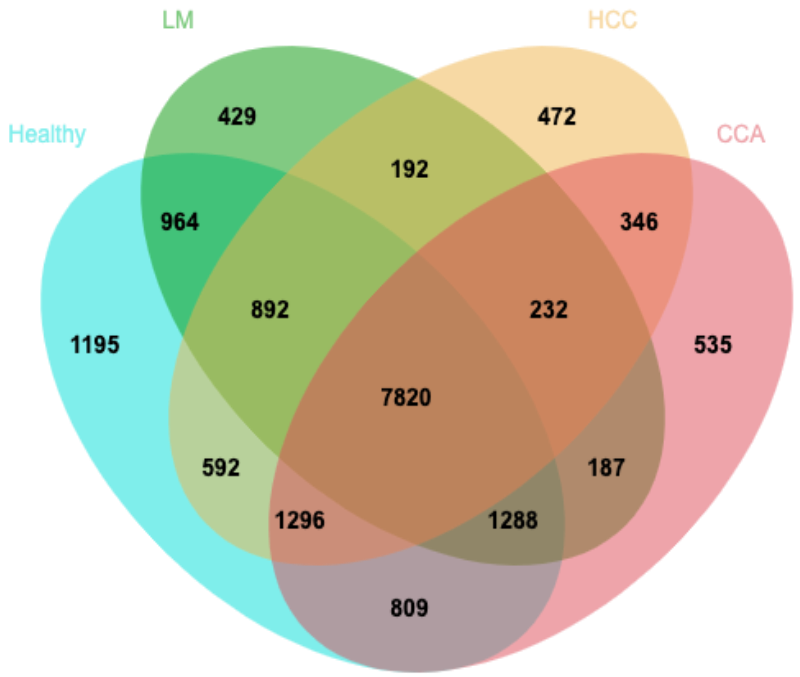
Valid data (n=194) : CCA = 58, HCC = 48, LM = 44, HA = 44

(a) Mean Raman spectra ± standard deviation for all four groups: normal control (NC, n = 44, pink), liver metastases (LM, n = 44, purple), hepatocellular carcinoma (HCC, n = 48, orange), and cholangiocarcinoma (CCA, n = 58, green). Each spectrum represents the averaged SERS signal for the respective group.
(b) Mean Raman spectra ± standard deviation grouped into two categories: normal control (NC, orange) and combined liver cancer samples (CCA + HCC + LM, green). The spectral differences between the normal and cancer groups highlight key Raman shifts relevant for distinguishing healthy and malignant samples.

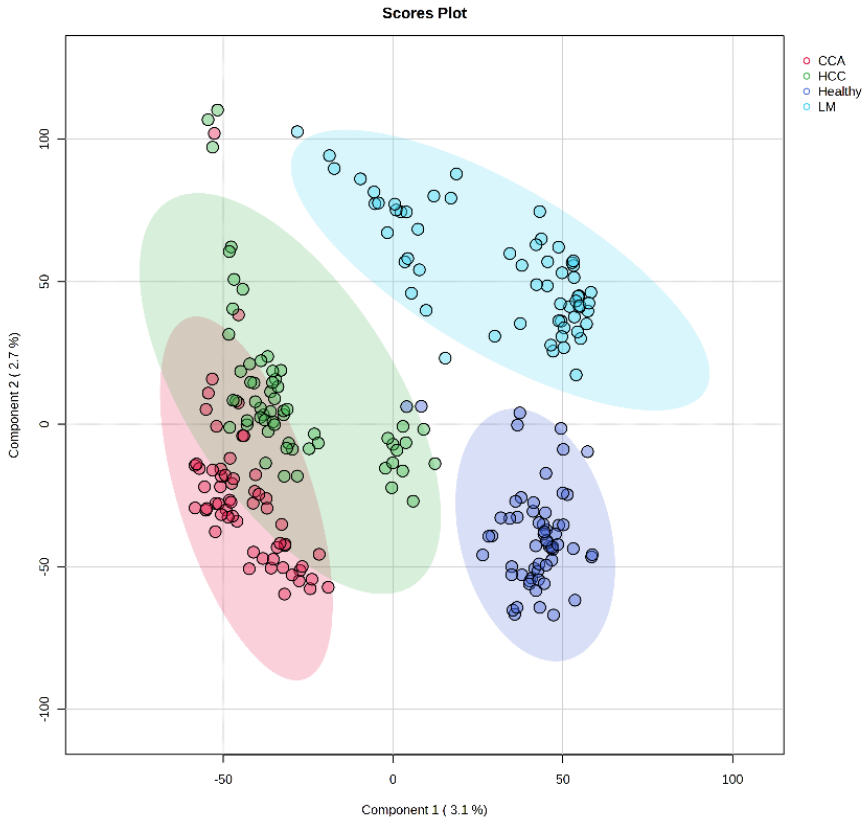
(a) 3D Scatter Plot of Principal Component Analysis (PCA) Components: Visualization of Raman spectral data projected onto three principal components, showing the distribution of different groups: normal control (NC, blue), cholangiocarcinoma (CCA, light blue), hepatocellular carcinoma (HCC, yellow), and liver metastases (LM, red). The clustering pattern suggests some separation between the groups.
(b) 2D Scatter Plot of Linear Discriminant Analysis (LDA) Components: LDA was applied to maximize class separability in the spectral data, resulting in distinct clustering of the four groups. The improved separation compared to PCA indicates the effectiveness of LDA in distinguishing between normal and cancerous samples.

The ROC curves represent the classification performance of the Linear Discriminant Analysis (LDA) model in differentiating between the four groups: normal control (NC), cholangiocarcinoma (CCA), hepatocellular carcinoma (HCC), and liver metastases (LM). The area under the ROC curve (AUC) values for each class indicate the model's ability to distinguish between groups, with higher AUC values reflecting better classification performance. The diagonal dashed line represents a random classifier (AUC = 0.5) for reference. The LDA model demonstrates high classification accuracy, particularly for distinguishing normal and cancerous samples, supporting the effectiveness of SERS-based machine learning for liver cancer detection.

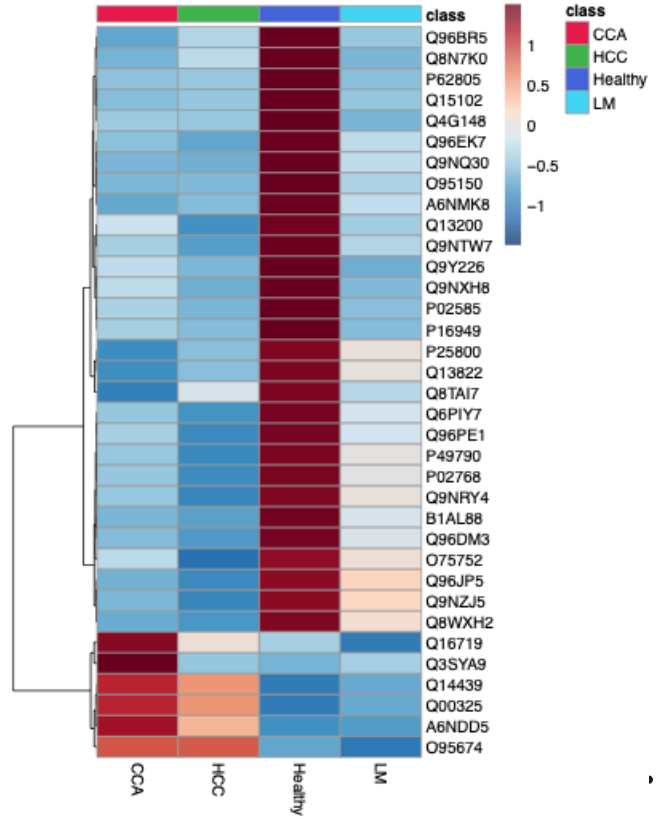
Project activities: MDKKU/BIOTEC; Protein identification in participants serum



The Venn diagram illustrates the overlap of differentially expressed proteins among the four groups: **cholangiocarcinoma (CCA, red), hepatocellular carcinoma (HCC, yellow), liver metastases (LM, green), and healthy controls (cyan)**. The numbers within each section represent the count of unique or shared proteins between the groups. The central region (7820) indicates the core set of proteins common across all groups, while peripheral numbers highlight proteins uniquely expressed in each condition. This analysis provides insights into shared and distinct molecular features among liver cancer subtypes and healthy individuals, aiding in biomarker discovery for disease classification.



The PCA score plot visualizes the clustering patterns of different sample groups based on their principal component scores. Each point represents an individual sample, color-coded by group: **cholangiocarcinoma (CCA, red), hepatocellular carcinoma (HCC, green), liver metastases (LM, blue), and healthy control (cyan)**. The ellipses represent the 95% confidence intervals for each group. The clear separation between groups suggests distinct molecular or spectral differences, supporting the potential of PCA in distinguishing liver cancer subtypes from normal samples.



The heatmap represents the expression patterns of selected proteins across four groups: **cholangiocarcinoma (CCA, red), hepatocellular carcinoma (HCC, green), liver metastases (LM, blue), and healthy controls (cyan)**. The color scale indicates normalized protein expression levels, with red representing **upregulated proteins**, blue representing **downregulated proteins**, and white indicating **intermediate expression levels**. The hierarchical clustering on the left groups proteins based on similarity in expression patterns. The observed differences in protein expression suggest potential biomarkers for distinguishing between liver cancer subtypes and normal samples.

Project activities: MDKKU, ENKKU, NECTEC, BIOTEC

Budget usage for the 1st year ✓ COMPLETED

Activities	Budget
Hamsters' CCA setting, samples collection and histopathological confirmation	4,000 USD
Discovery of Raman peak for CCA diagnosis	3,000 USD
Patients sample collection	2,500 USD
Identification & evaluation of biomarker in CCA patients: training set	3,500 USD
Verification of electrochemical sensor for CCA diagnosis: verification set	3,000 USD
SERS development: Synthesis	4,000 USD
SERS development: Characterization	1,000 USD
Total	**21,000 USD**

Budget usage for the 2nd year ✓ COMPLETED

Activities	Budget
Sample collection and providing standard protein & antibody for CMUs' team	4,000 USD
Proteins panel discovery and identification	7,500 USD
Raman spectrum identification	5,000 USD
Construct AI & Machine learning model	2,000 USD
Transfer technology of SERS and AI for CCA diagnosis to Laos' team	2,500 USD
KKU facilities services	2,100 USD
Total	**21,000 USD**

Overview of research objectives and activities of CMU

CMU: Biosensing platform based on silicon photonic development

Activities in ASEAN IVO 2023

1. Sensor device design investigation specifically for the detection of CCA biomarker (s)
2. Simulation of silicon nitride biosensor devices

Activities in ASEAN IVO 2024

1. Prototype sensor system development
2. Sensing experiment using samples provided by KKU or Laos
   - Detection of new CCA biomarkers from KKU
   - Comparison with conventional method

(Year 1) Key elements demonstrated in laboratory

Budget usage	Budget
Survey report of the photonic sensor technologies that are applicable to cholangiocarcinoma detection.	$3000 ✓
investigation of new photonic sensor design and simulation results using numerical based simulation.	$3000 ✓
Computer-aided design (CAD) drawing of silicon photonic sensor mask design	$1500 ✓

✓ COMPLETED

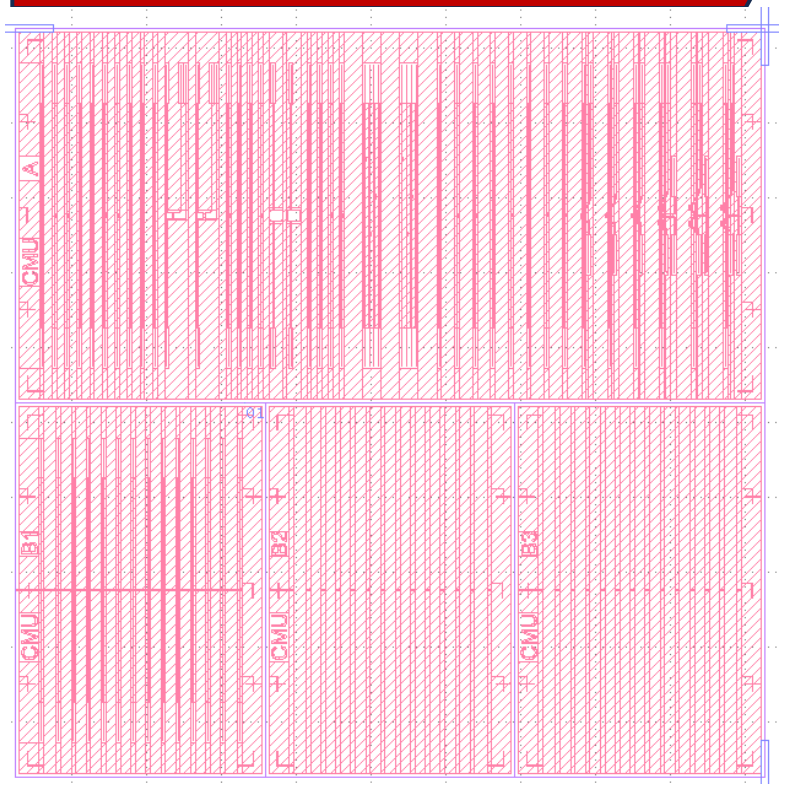
(Year 2) Device manufacturing & performance assessment using standard testing method

Budget usage	Budget
Photonic (resonator) sensor devices and a prototype of the measuring system	$7000 ✓
Project workshop and meeting	$3500 ✓

✓ COMPLETED

2024 PROGRESS
CMU: Biosensing platform based on silicon photonic development

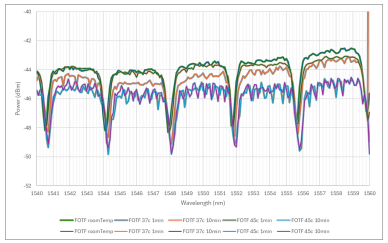
Fabrication of silicon photonic sensors

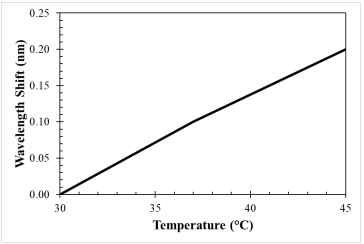


Photomask designs submitted to manufacturer (TMEC) **August 2024**

Fabricated sensors expected by **March 2025**

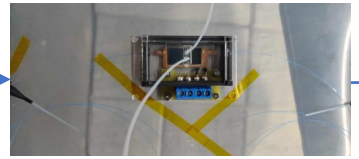
Development of New sensor measurement system prototype





Proof of concept In Laboratory

broadband source



MEMS filter

Power Meter

September 2024

Measurement system prototype and sensors purchase order

October 2024 – March 2025

The project will order a developer to created a system based on the laboratory concept with software control

Tech transfer (workshop in CMU, demo of the prototype)

CCA detection test (Pending samples provided by KKU and Laos Team

February 2025

COMPLETED

Project activities: LAOS ; Biosensing platform for clinical application

Biosensing potable platform for clinical sample of Laos CCA patients in various specimens

COMPLETED

Budget plan for year 1st

Budget usage	Budget
Freezer	$6,000
Total	$6000

Budget plan for year 2nd

Budget usage	Budget
Collection of various samples from the patients: healthy, CCA, HCC, and other cancers; materials, volunteer compensation, physician, surgeon, etc.,	$2500
Identification & evaluation of biomarker in CCA patients: training set	$1500
Total	$4000

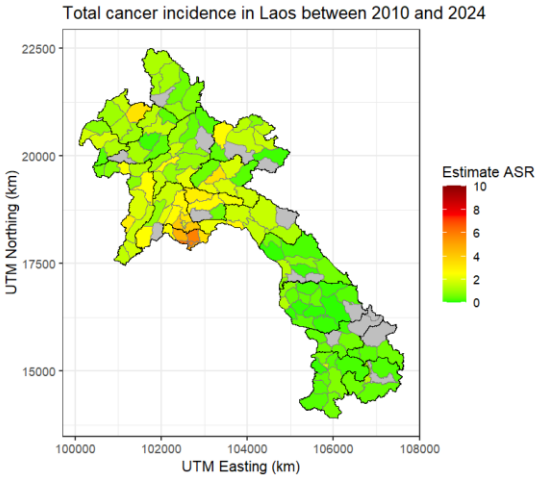
Collect and verify CCA samples: urine, serum

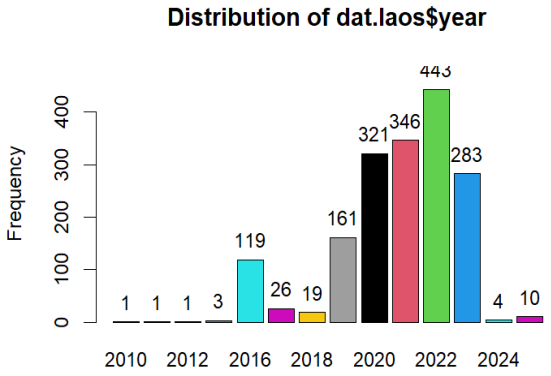
COMPLETED

Project activities: LAOS ; Biosensing platform for clinical application

Biosensing potable platform for clinical sample of Laos CCA patients in various specimens

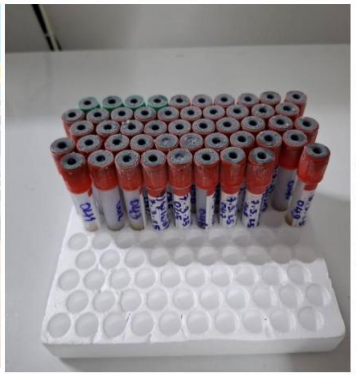
Spatial Distribution of Cancer Cases in Hospital registries in Laos PRD



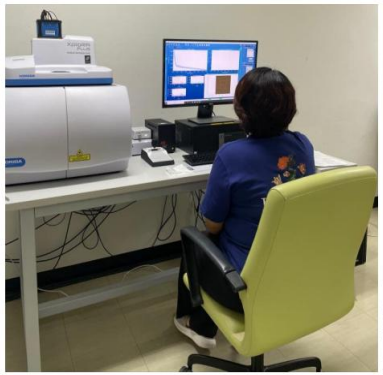


5. Transfer technology of SERS and AI for CCA diagnosis to Laos' team

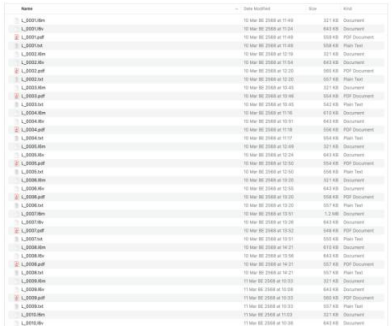
Transportation Laos' samples to KKU

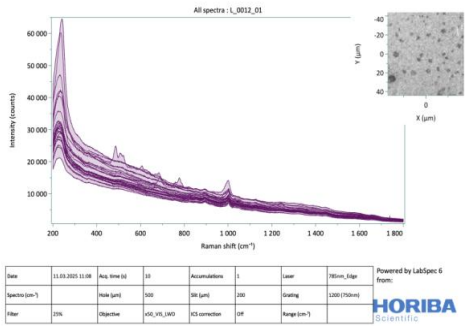


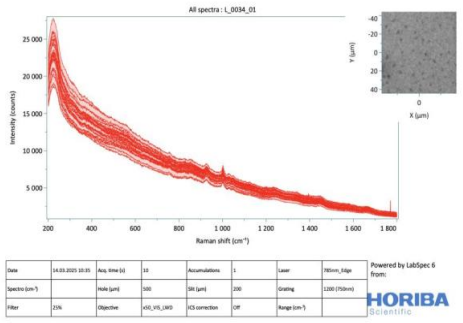
Detection of Serum Using Raman Spectroscopy



The raw Raman data and an example of the Raman peak results.



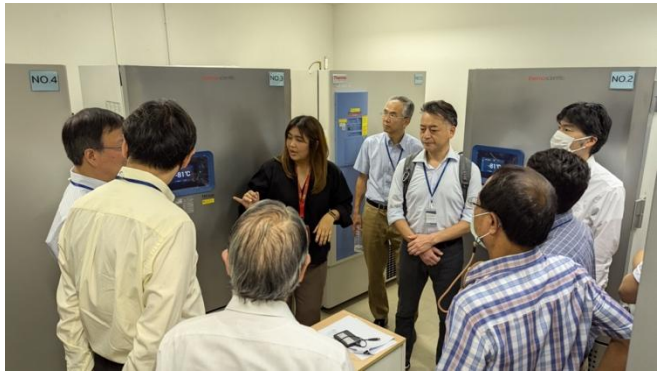


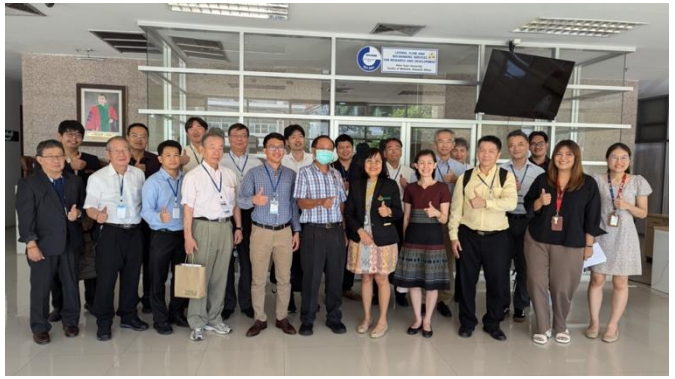


Phase II

Project activities: Workshop on Nov 13-16, 2023 at Vientiane, LAOS

Project Activities: Workshop on June 20-21, 2024 at KKU, Thailand





Project activities: Workshop on Feb 18, 2025 at CMU, Thailand

Project activities: Progress review at CMU, Thailand

Dr.Emoto Hiroshi (NICT)
Yoshihiro Sakuda (NICT Asia center, BBK, Thailand)
Assoc.Prof.Sansanee Auephunviriyakul (CMU)
Assoc.Prof.Ukrit Mankong (CMU)
Dr.Suruk Udomsom (CMU)
Prof.Somchai Pinlaor (KKU)

Group discussion at CMU, 27 Dec 2024

Project activities: ASEAN IVO project review at LAOS and Cambodia

ASEAN IVO Forum 2023, 15-16 Nov 2023

6 months Review & Report, 15-16 Nov 2023, LAO

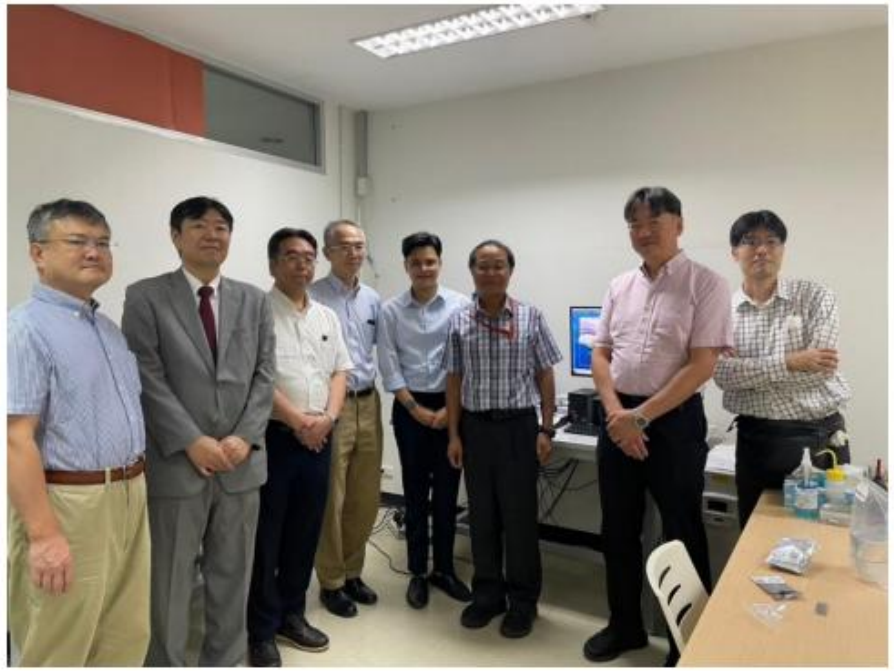
ASEAN IVO Forum 2024, 7-9 Nov 2024, Camdodia

1st Year Review & Report, 7-9 Nov 2024, at Phnom Penh

4 Directors of NICT have visited and discussed at MD-KKU for next project activity

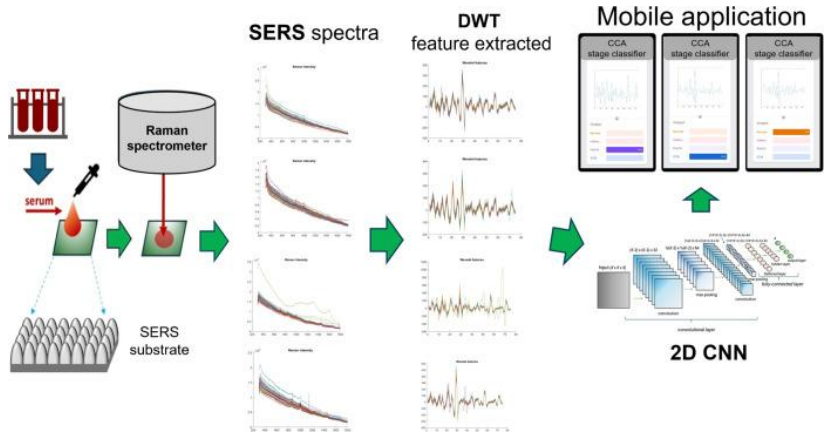
NICT 20 November 2024

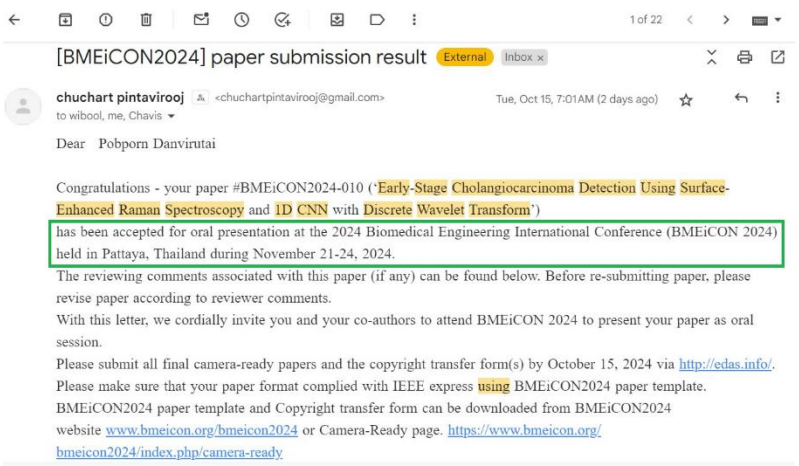
1. **Hiroaki Harai** (Director General of Network Research Institute)
2. **Tetsuya Kawanishi** (Research Executive Director, Prof. of Waseda University)
3. **Yoshinari Awaji** (Director General of Photonic ICT Research Center)
4. **Kouichi Akahane** (Director of Optical Access Technology Laboratory)
5. **Toshimasa Umezawa** (Senior Researcher of Optical Access Technology Laboratory)
6. **Atsushi Matsumoto** (Senior Researcher of Optical Access Technology Laboratory)



R&D results: ENKKU-MDKKU; Biosensing platform development based on Raman spectroscopy techniques.

ENKKU: Surface Enhance Raman Spectroscopic (SERS) chip development.



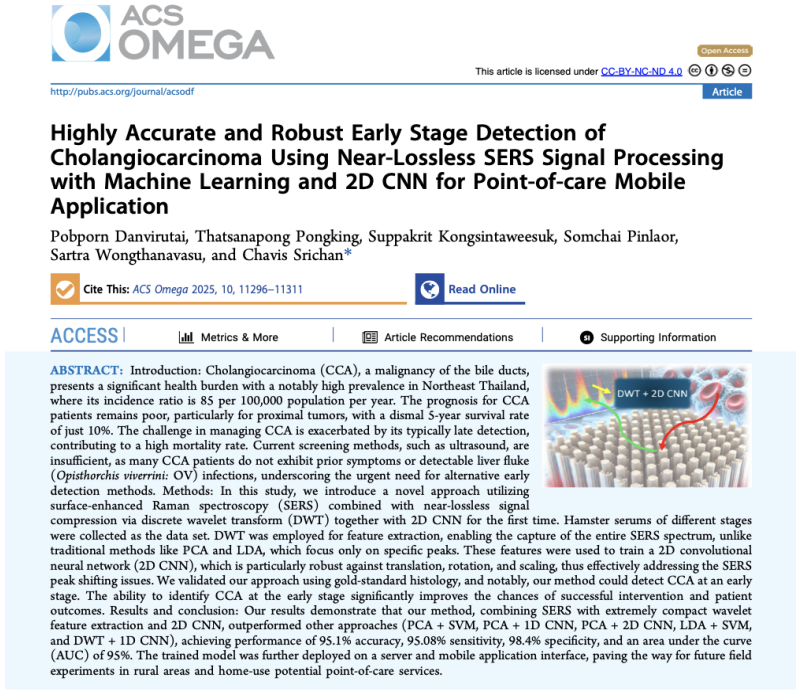


[BMEiCON2024] paper submission result External Inbox

chuchart pintavirooj <chuchartpintavirooj@gmail.com> Tue, Oct 15, 7:01AM (2 days ago)
to wibool, me, Chavis

Dear Pobporn Danvirutai

Congratulations - your paper #BMEiCON2024-010 ('Early-Stage Cholangiocarcinoma Detection Using Surface-Enhanced Raman Spectroscopy and 1D CNN with Discrete Wavelet Transform')
has been accepted for oral presentation at the 2024 Biomedical Engineering International Conference (BMEiCON 2024) held in Pattaya, Thailand during November 21-24, 2024.
The reviewing comments associated with this paper (if any) can be found below. Before re-submitting paper, please revise paper according to reviewer comments.
With this letter, we cordially invite you and your co-authors to attend BMEiCON 2024 to present your paper as oral session.
Please submit all final camera-ready papers and the copyright transfer form(s) by October 15, 2024 via http://edas.info/.
Please make sure that your paper format complied with IEEE express using BMEiCON2024 paper template.
BMEiCON2024 paper template and Copyright transfer form can be downloaded from BMEiCON2024 website www.bmeicon.org/bmeicon2024 or Camera-Ready page. https://www.bmeicon.org/bmeicon2024/index.php/camera-ready



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http://pubs.acs.org/journal/acsodf

Article

Highly Accurate and Robust Early Stage Detection of Cholangiocarcinoma Using Near-Lossless SERS Signal Processing with Machine Learning and 2D CNN for Point-of-care Mobile Application

Pobporn Danvirutai, Thatsanapong Pongking, Suppakrit Kongsintaweesuk, Somchai Pinlaor, Sartra Wongthanavasu, and Chavis Srichan*

Cite This: *ACS Omega* 2025, 10, 11296−11311 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Introduction: Cholangiocarcinoma (CCA), a malignancy of the bile ducts, presents a significant health burden with a notably high prevalence in Northeast Thailand, where its incidence ratio is 85 per 100,000 population per year. The prognosis for CCA patients remains poor, particularly for proximal tumors, with a dismal 5-year survival rate of just 10%. The challenge in managing CCA is exacerbated by its typically late detection, contributing to a high mortality rate. Current screening methods, such as ultrasound, are insufficient, as many CCA patients do not exhibit prior symptoms or detectable liver fluke (*Opisthorchis viverrini*: OV) infections, underscoring the urgent need for alternative early detection methods. Methods: In this study, we introduce a novel approach utilizing surface-enhanced Raman spectroscopy (SERS) combined with near-lossless signal compression via discrete wavelet transform (DWT) together with 2D CNN for the first time. Hamster serums of different stages were collected as the data set. DWT was employed for feature extraction, enabling the capture of the entire SERS spectrum, unlike traditional methods like PCA and LDA, which focus only on specific peaks. These features were used to train a 2D convolutional neural network (2D CNN), which is particularly robust against translation, rotation, and scaling, thus effectively addressing the SERS peak shifting issues. We validated our approach using gold-standard histology, and notably, our method could detect CCA at an early stage. The ability to identify CCA at the early stage significantly improves the chances of successful intervention and patient outcomes. Results and conclusion: Our results demonstrate that our method, combining SERS with extremely compact wavelet feature extraction and 2D CNN, outperformed other approaches (PCA + SVM, PCA + 1D CNN, PCA + 2D CNN, LDA + SVM, and DWT + 1D CNN), achieving performance of 95.1% accuracy, 95.08% sensitivity, 98.4% specificity, and an area under the curve (AUC) of 95%. The trained model was further deployed on a server and mobile application interface, paving the way for future field experiments in rural areas and home-use potential point-of-care services.

ACKNOWLEDGMENT

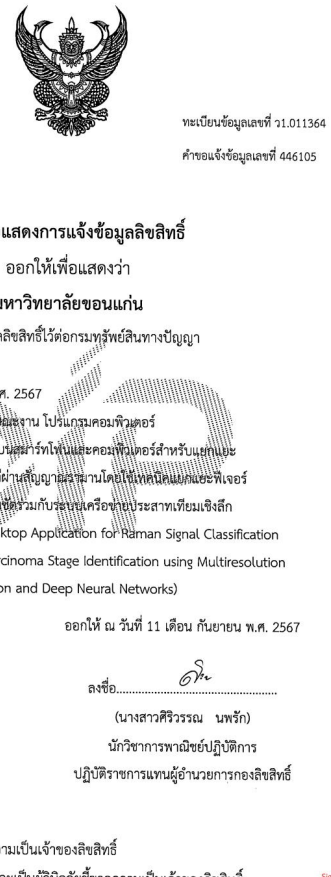
We acknowledge the funding support from NICT, Japan, under the ASEAN IVO 2023 Project, "Innovation of Photonic and Electrochemical Biosensors for Cholangiocarcinoma Diagnosis." Special thanks are extended to Dr. Sirinapa Klungsaeng for her contributions during the initial phase of this work. Collaborations with NECTEC team through Dr. Pitak Eiamchai and Dr. Noppadon Nuntawong are greatly appreciated.

Received

1. Software license

2. (Q1) Article published on ACS OMEGA]

3. (Scopus-index) Conference paper to appear in IEEEXplore



ทะเบียนข้อมูลเลขที่ ว1.011364

คำขอแจ้งข้อมูลเลขที่ 446105

หนังสือแสดงการแจ้งข้อมูลลิขสิทธิ์

ออกให้เพื่อแสดงว่า

มหาวิทยาลัยขอนแก่น

ได้แจ้งข้อมูลลิขสิทธิ์ไว้ต่อกรมทรัพย์สินทางปัญญา

เมื่อวันที่ 5 เดือน สิงหาคม พ.ศ. 2567

ประเภทงาน วรรณกรรม สิ่งบันทึกงาน โปรแกรมคอมพิวเตอร์

ชื่อผลงาน โปรแกรมประยุกต์บนสมาร์ทโฟนและคอมพิวเตอร์สำหรับแยกแยะ
ความละเอียดสูงสัญญาณรามานโดยใช้เทคนิคแยกและฟีเจอร์
แบบหลายความคมชัดร่วมกับระบบเครือข่ายประสาทเทียมเชิงลึก
(Mobile and Desktop Application for Raman Signal Classification
for Cholangiocarcinoma Stage Identification using Multiresolution
Feature Extraction and Deep Neural Networks)

ออกให้ ณ วันที่ 11 เดือน กันยายน พ.ศ. 2567

ลงชื่อ.....

(นางสาวศิริวรรณ นพรัก)

นักวิชาการพาณิชย์ปฏิบัติการ

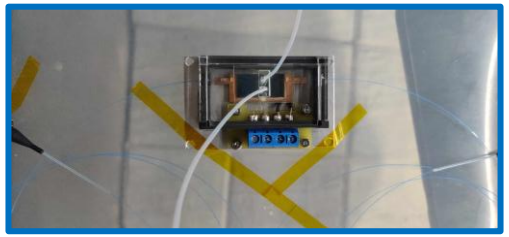
ปฏิบัติราชการแทนผู้อำนวยการกองลิขสิทธิ์

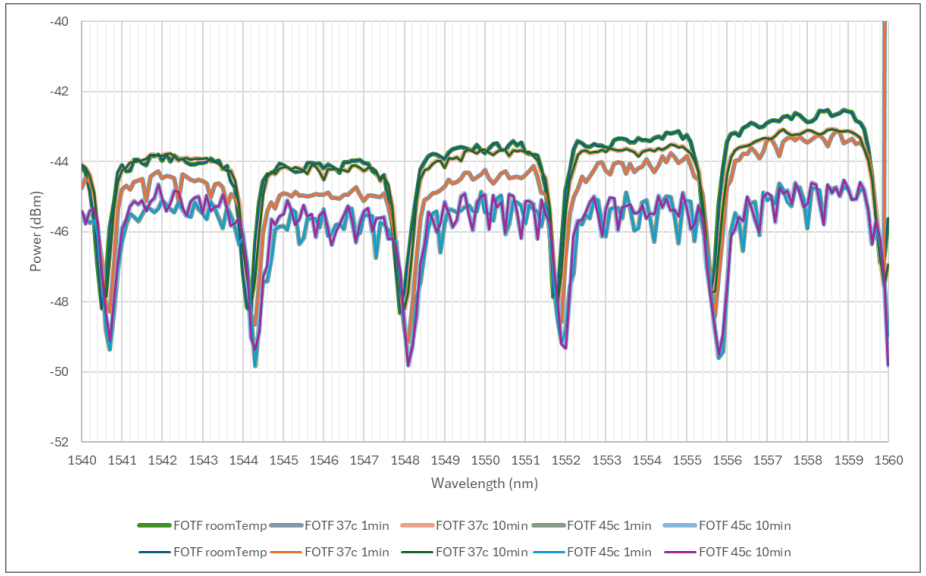
หมายเหตุ เอกสารนี้มิได้รับรองความเป็นเจ้าของลิขสิทธิ์
ในกรณีมีข้อพิพาทศาลจะเป็นผู้วินิจฉัยชี้ขาดความเป็นเจ้าของลิขสิทธิ์

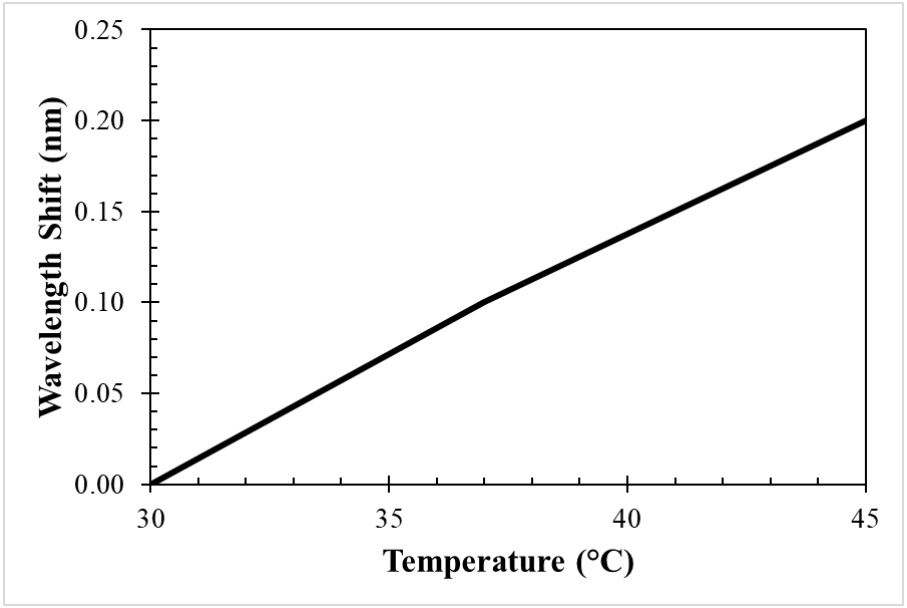
Signed by DIP-CA

R&D results: CMU

CMU: Designed and developed photonic sensor device for CCA diagnosis.

Broadband source →  → MEMS filter → Power meter





Sensing results: Laboratory proof of concept of the prototype

Next step CCA biomarker test

Scientific contribution:

Presentations at International Conferences:

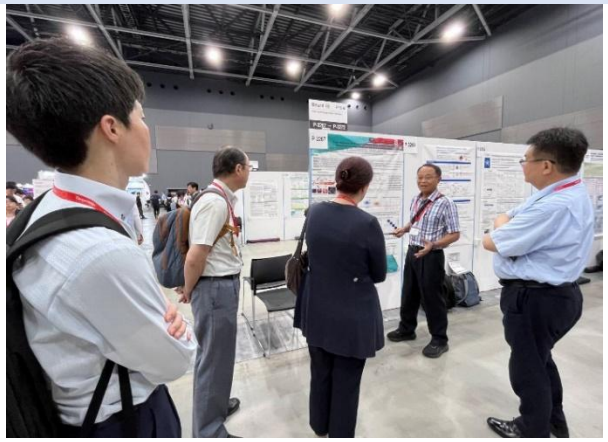
No:	Paper title:	Author names	Affiliation	Conference	Date	Venue
1	Early diagnosis of cholangiocarcinoma using surface-enhanced Raman spectroscopy combined with AI in a hamster model	Somchai Pinlaor a,b, Chavis Srichanc, Pobporn Danvirutaid, Kitti Intuyodb,e, Apisit Chaideea,b, Chawalit Pairojkuleb,e, Thatsanapong Pongking b,f, Suppakrit Kongsintaweesuk b,f , Mati Horprathumg, Pitak Eiamchaig, Saksorn Limwicheang, Noppadon Nuntawongg	[a]Department of Parasitology, Faculty of Medicine, and [b]Cholangiocarcinoma Research Institute, [c]Faculty of Engineering, [d]College of Computing, [e]Department of Pathology, Faculty of Medicine, [f]Biomedical Sciences Program, Khon Kaen University, Khon Kaen 40002, Thailand. [g]National Electronics and Computer Technology Center (NECTEC), National Science and Technology Development Agency (NSTDA), Pathum Thani, Thailand	The 83rd Annual Meeting of the Japanese Cancer Association (JCA2024)	September 19 (Thu.) - 21 (Sat.), 2024	Fukuoka, Japan

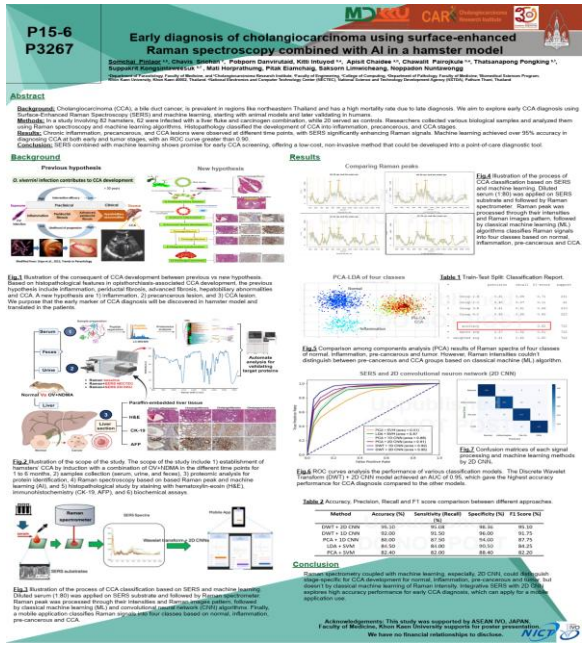
Published Journal Papers:

No:	Paper title:	Author names	Affiliation	Journal name:	Publisher	Volume number and Pages
1	**Highly Accurate and Robust Early Stage Detection of Cholangiocarcinoma Using Near-Lossless SERS Signal Processing with Machine Learning and 2D CNN for Point-of-care Mobile Application**	Pobporn Danvirutai, Thatsanapong Pongking, Suppakrit Kongsintaweesuk, Somchai Pinlaor, Sartra Wongthanavasu, Chavis Srichan*	1College of Computing, Khon Kaen University, Khon Kaen 40002, Thailand 2Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand. 3Cholangiocarcinoma Research Institute, Faculty of Medicine, Khon Kaen University, Thailand 4Biomedical Sciences Program, Graduate School, Khon Kaen University, Khon Kaen 40002, Thailand 5Department of Computer Engineering, Faculty of Engineering, Khon Kaen University, Khon Kaen 40002, Thailand. 6Department of Biomedical Engineering, Faculty of Engineering, Khon Kaen University, Khon Kaen 40002, Thailand..	ACS OMEGA	ACS Publisher	Vol 10, Issue 11,March, 2025

Scientific contribution:

Poster presentation on JCA 2024





Proceeding

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The 2024 Biomedical Engineering International Conference (BMEiCON-2024)

Early-Stage Cholangiocarcinoma Detection Using Surface-Enhanced Raman Spectroscopy and 1D CNN with Discrete Wavelet Transform

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***Abstract*—This Early detection of cholangiocarcinoma (CCA) is critical for improving patient prognosis and survival rates. Surface-Enhanced Raman Spectroscopy (SERS) offers a promising non-invasive diagnostic tool due to its high sensitivity and specificity. In this study, we propose a novel approach combining Discrete Wavelet Transform (DWT) and a one-dimensional Convolutional Neural Network (1D CNN) for the detection and differentiation of first stage CCA from precancerous, inflammation, and healthy states using SERS data. Our method is compared with a traditional Principal Component Analysis (PCA) followed by Support Vector Machine (SVM) classification. In contrast, the PCA + SVM method could only differentiate late-stage CCA and healthy states due to the nonlinearity of the SERS dataset. Receiver Operating Characteristic (ROC) curve analysis further validates the superior performance of our proposed method. We studied on hamster serum and the concept can be extended to human serum in the near future work.

***Index Terms*—Cholangiocarcinoma detection, Early stage CCA, SERS, Wavelet transform, 1D CNNs**

I. INTRODUCTION *(HEADING 1)*

Cholangiocarcinoma (CCA), a malignant tumor of the biliary tract, poses significant diagnostic challenges due to its asymptomatic nature in early stages and aggressive progression, most detectable cases are in late-stage yielding high mortality rate. Early detection is crucial for effective treatment and improved patient outcomes [1]. Surface-Enhanced Raman Spectroscopy (SERS) has emerged as a powerful tool for biomedical diagnostics, offering molecular-level information with high sensitivity [2-7]. However, differentiating early-stage CCA from precancerous, inflammatory, and healthy conditions remains challenging due to the subtle spectral differences and nonlinear characteristics of the SERS data.

Machine learning techniques have been applied to SERS data for cancer detection, with methods such as Principal Component Analysis (PCA) combined with Support Vector Machines (SVM) showing promising results [8]. Nevertheless, PCA + SVM approaches often struggle with nonlinear datasets, limiting their ability to distinguish between early-stage CCA and other conditions. Deep learning, particularly Convolutional Neural Networks (CNNs), has demonstrated superior performance in handling complex, nonlinear data in various pattern recognition tasks.

II. RELATED WORK

The application of Raman spectroscopy in cancer diagnostics has been extensively studied over the past decades. Raman spectroscopy provides a molecular fingerprint of biological samples, making it a valuable tool for disease detection. Surface-Enhanced Raman Spectroscopy (SERS), in particular, enhances the Raman signal by several orders of magnitude, allowing for the detection of low-concentration biomarkers [9-12]. Report on SERS in CCA detection could be rarely found in literature. Here are the list of work related to this study.

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ทะเบียนข้อมูลเลขที่ ว1.011364

คำขอแจ้งข้อมูลเลขที่ 446105

หนังสือแสดงการแจ้งข้อมูลลิขสิทธิ์

ออกให้เพื่อแสดงว่า

มหาวิทยาลัยขอนแก่น

ได้แจ้งข้อมูลลิขสิทธิ์ไว้ต่อกรมทรัพย์สินทางปัญญา

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ประเภทงานวรรณกรรม สิ่งบันทึกเสียง งาน โปรแกรมคอมพิวเตอร์

ชื่อโปรแกรมประยุกต์บนสมาร์ทโฟนและคอมพิวเตอร์สำหรับแยกแยะ

สัญญาณสเปกตรัมรามานจากน้ำดีผ่านสัญญาณรามานโดยใช้เทคนิคแยกแยะฟีเจอร์

และหลายความคมชัดร่วมกับระบบเครือข่ายประสาทเทียมเชิงลึก

Mobile and Desktop Application for Raman Signal Classification

or Cholangiocarcinoma Stage Identification using Multiresolution

Feature Extraction and Deep Neural Networks)

ออกให้ ณ วันที่ 11 เดือน กันยายน พ.ศ. 2567

ลงชื่อ.....

(นางสาวศิริวรรณ นพรัก)

นักวิชาการพาณิชย์ปฏิบัติการ

ปฏิบัติราชการแทนผู้อำนวยการกองลิขสิทธิ์

หมายเหตุ เอกสารนี้มิได้รับรองความเป็นเจ้าของลิขสิทธิ์

ในกรณีมีข้อพิพาทศาลจะเป็นผู้วินิจฉัยชี้ขาดความเป็นเจ้าของลิขสิทธิ์

Signed by DIP-CA

Submitted & revised manuscripts

MDKKU

Animal Raman

PLOS ONE

Minimally invasive detection for an early stage of opisthorchiasis-associated cholangiocarcinoma using label-free surface-enhanced Raman spectroscopy (SERS)

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Article
Full Title:	Minimally invasive detection for an early stage of opisthorchiasis-associated cholangiocarcinoma using label-free surface-enhanced Raman spectroscopy (SERS)
Short Title:	Label-Free SERS Detects Early Opisthorchiasis-Associated CCA
Corresponding Author:	Somchai Pinlaor, PhD Khon Kaen University Amphoe Mueang, Khon Kaen THAILAND
Keywords:	Bile-duct cancer; biosensor; Raman spectroscopy; Artificial intelligence; cancer staging
Abstract:	Cholangiocarcinoma (CCA) is a deadly cancer often detected late. Current diagnostic methods, such as ultrasound and invasive biopsies, have limitations; there is a critical need for a rapid, minimally invasive, and effective strategy for the early diagnosis and staging of CCA. We aimed to address this need using serum samples and label-free surface-enhanced Raman spectroscopy (SERS) combined with machine learning. CCA development was induced in hamsters using a combination of Opisthorchis viverrini infection and administration of N-nitrosodimethylamine with induction time courses spanning 1–5 months (s). Normal and pathological stages (inflammation, precancerous lesion, and CCA) were assigned based on histopathological features, as well as the expression of cytokeratin 19 and alpha-fetoprotein. Raman spectra were subjected to dimensionality reduction using principal component analysis, and diagnostic clusters were acquired using partial least-squares discriminant analysis. For pathological staging, the integration of SERS and machine learning achieved a diagnostic sensitivity of 93%, specificity of 95%, and accuracy of ≥ 67% for precancerous lesions and CCA, with an area under the receiver operating characteristic curve exceeding 0.67. These results have significant potential for accurately detecting the precancerous and cancerous stages of bile-duct neoplasms in the hamster model. Further development of this cost-effective, label-free SERS approach is likely to be ideal for community-based CCA screening.
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	Keerapach Tunbenjasiri, MSc
	Aye Myat Mon, Msc.
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	Kesara Na-Bangchang, PhD
	David Blair, PhD
	Naruechar Charoenram, Bsc.
	Somchai Pinlaor, PhD
Opposed Reviewers:	
Additional Information:	
Question	Response

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Human Raman

Surface-enhanced Raman spectroscopy and machine learning differentiate specific liver cancers

Journal:	*ACS Applied Nano Materials*
Manuscript ID	Draft
Manuscript Type:	Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Intuyod, Kitti; Khon Kaen University Faculty of Medicine Kongsintaweesuk, Suppakrit; Khon Kaen University, Biomedical sciences program, Graduate school Eiamchai, Pitak; National Electronics and Computer Technology Center, Luvira, Vor; Khon Kaen University, Department of Surgery, Faculty of Medicine Chaidee, Apisit; Khon Kaen University, Department of Parasitology, Faculty of Medicine Techasen, Anchalee ; Khon Kaen University, Centre for Research and Development of Medical Diagnostic Laboratories, Faculty of Associated Medical Sciences, Pinlaor, Porntip; Khon Kaen University Pairojkul, Chawalit; Khon Kaen University, Department of Pathology, Faculty of Medicine Blair, David; James Cook University Umezawa, Toshimasa; National Institute of Information and Communications Technology (NICT) Matsumoto, Atsushi; Kyushu University Akahane, Kouichi; National Institute of Information and Communications Technology horprathum, mati; National Electronics and Computer Technology Center, Opto-Electrochemical Sensing Research Team Limwichean, Saksorn ; National Electronics and Computer Technology Center Nuntawong, Noppadon; National Electronics and Computer Technology Center, Pinlaor, Somchai; Khon Kaen University, Department of Parasitology

SCHOLARONE™
Manuscripts

Biomarker in human serum

Advances in Medical Sciences

Development and clinical validation of a β-hCG-based panel for cholangiocarcinoma screening: from proteomics to clinical validation

--Manuscript Draft--

Manuscript Number:	
Article Type:	Original Article / Research
Keywords:	Bile-duct cancer, Biomarker, Liver cancer, Tumor interstitial fluid
Corresponding Author:	Porntip Pinlaor, PhD Khon Kaen University THAILAND
First Author:	Suppakrit Kongsintaweesuk
Order of Authors:	Suppakrit Kongsintaweesuk
	Thatsanapong Pongking
	Keerapach Tunbenjasiri
	Pakornkiat Tanasuka
	Sitiruk Roytrakul
	Sudarat Onsurathum
	Chawalit Pairojkul
	Kitti Intuyod
	Vor Luvira
	David Blair
	Somchai Pinlaor
	Porntip Pinlaor, PhD
Abstract:	Purpose: Opisthorchis viverrini-associated cholangiocarcinoma (CCA) in Northeast Thailand is marked by late diagnosis and poor prognosis, creating a critical need for effective early-detection biomarkers. This study aimed to identify a novel glycoprotein biomarker panel from tumor interstitial fluid (TIF) to improve CCA screening. Materials/Methods: Glycoprotein-enriched TIF and paired normal interstitial fluid samples from three CCA patients were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Given its increase in various cancers, the protein expression of beta-human chorionic gonadotropin (β-hCG) was assessed using immunohistochemistry (IHC) in a tissue array of 100 CCA samples. Serum levels of β-hCG, carbohydrate antigen 19-9 (CA 19-9), alpha-fetoprotein (AFP), and carcinoembryonic antigen (CEA) were quantified in 405 individuals, including patients with CCA (n=153), other hepatobiliary diseases, and healthy controls. Performance of β-hCG in screening for CCA was evaluated . Results: Proteomic analysis identified elevated β-hCG specifically in TIF from CCA patients. IHC confirmed significantly higher β-hCG expression in tumor tissues compared to adjacent non-tumor areas (p<0.0001). Serum β-hCG levels were significantly increased in CCA patients and correlated with tumor volume and reduced survival (p<0.05). While β-hCG alone had limited diagnostic performance, a combined panel adding CA 19-9, CEA, and AFP yielded excellent diagnostic accuracy (AUC: 0.962; sensitivity: 86%; specificity: 100%). Conclusions: β-hCG is a valuable prognostic marker for CCA and in a multi-marker panel significantly enhances diagnostic performance. This panel shows strong potential for improving the early detection and prognostic evaluation of CCA, warranting large-scale validation for clinical application.
Opposed Reviewers:	

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Societal impact:

1. One license for SERS signal processing using multiresolution compression and convolutional neural networks
2. Sharing proteomics data: The accession numbers are PXD057256 for ProteomeXchange and JPST003438 for jPOST.
3. **Sharing Raman data** : Mendeley: DOI: 10.17632/8gs6tc6vgr.1 (Animal)
   Mendeley: DOI:10.17632/hzg43f63k9.1. (Human)
4. Two international publications
   - SERS with DWT and CNN for CCA diagnosis (ACS Omega, 10;11,2025)—Q1
   - Proceeding on international conference on BMiCON 2024 (online)
5. Four manuscripts have been submitted & revised on Q1 journal
   - SERS diagnosis in hamsters' serum (PLOS ONE, revised)
   - SERS diagnosis in patients' serum (ACS Applied Nano Materials, revised)
   - Colloidal SERS for CCA diagnosis (ACS Omega, under review)
   - beta-HCG marks for CCA diagnosis (Advances in Medical Sciences, under review)
6. Two international conferences:
   - The 83rd Annual Meeting of the Japanese Cancer Association (JCA) on September 19-21, 2024 at Fukuoka, Japan (poster presentation)
   - International conference on BMCiCON 2024, 21-24 Nov 2024, Pattaya, Thailand (Oral presentation)
7. Manuscript preparation: INPROGRESS
   - Incidence of cancer in Lao PDR (The Lancet Regional Health - Southeast Asia, will be submitted)
   - Proteomics identified candidate markers for CCA diagnosis (Manuscript preparation)

Activity and budget planning in the 2nd year

No	Activities	Budget (US)	Responsibility	Out put
1	1. Sample collection and Std protein & ab for CMUs' team 2. Proteins panel discovery 3. Raman spectrum identification 4. Construct AI & Machine learning 5. Transfer technology of SERS and AI for CCA diagnosis to Laos' team	21,000	KKU (MD & EN) BIOTECH NECTEC	- Samples of CCA, HCC, metastasis liver and healthy subjects collection, -At least 1 publication -1 patent/pretty patent
2	Optical sensing development: equipment, materials, research exchange at NICT	7,000	CMU	-1 publication
3	Human sample & clinical data collection: Surgery team & pathology team for samples collection, biobank, histopathological study, freezer, equipment, etc.,	4,000	Laos	Team initiation, CCA sample & other cancer sample collection
4	Project meeting or workshop at the end of the 2nd year	3,000	KKU for all team (CMU-KKU-Laos)	Meeting & conference: 1 session for biosensor for CCA diagnosis
5	Project meeting or workshop at the end of the 2nd year	3,500	CMU for all team (CMU-KKU-Laos)	Meeting & conference: 1 session for biosensor for CCA diagnosis
6	**Project meeting and report (estimate)**	1,500	KKU team	Attend on Campodia, 6-7 Nov 2024
	Total	**40,000**		

Two years timeline schedule

Institution	Research activity	2023												2024											
		4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3
MD-KKU, NECTEC, BIOTECH	Supplies & Materials: Animal model setting, equipment, samples collection, Raman spectrometer finger print																								
EN-KKU,	SERS chip development : equipment & materials, SERS substrate synthesis and design of portable CCA specific SERS-based sensor, training with NICT																								
CMU	Optical sensing development: equipment, materials, training with NICT																								
MD-KKU	Surgery team & pathology team for samples collection, biobank, histopathological study, standard method confirmation by ELISA & WB																								
Lao PDR	Surgery & pathology team management, workshop, training, samples collection, prototype evaluation, team meeting and conference																								
MD-KKU, EN-KKU, CMU,BIOTECH, NECTEC, TMEC, Laos, NICT	Meeting and conference, transport, report, publication, biosensing prototype																								

Future works:

สถาบันวิจัยระบบสาธารณสุข

Phase II

Lancang–Mekong Cooperation

瀾沧江–湄公河合作

Greater Mekong subregion

Thank you

CARI Cholangiocarcinoma Research Institute

ASEAN IVO

Cancer Center Lao PDR
Est. 2017

CMU
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