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

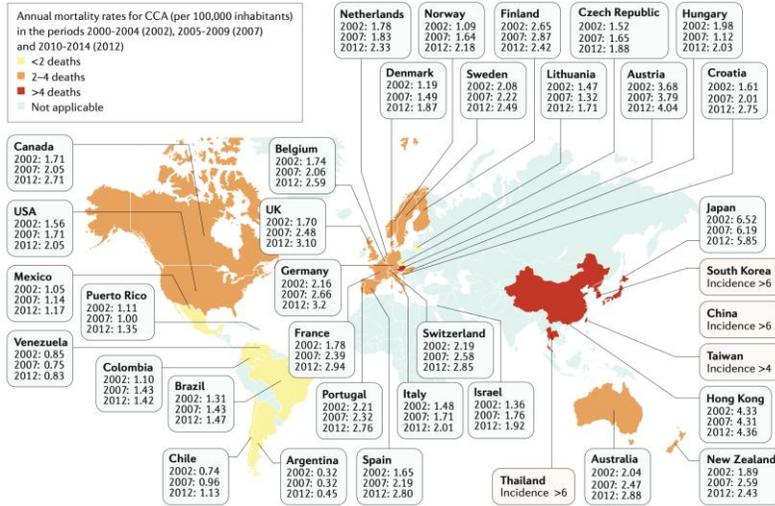Speaker: **Prof. Somchai Pinlaor**

Institute : Khon Kaen University (KKU), Khon Kaen 40002, Thailand
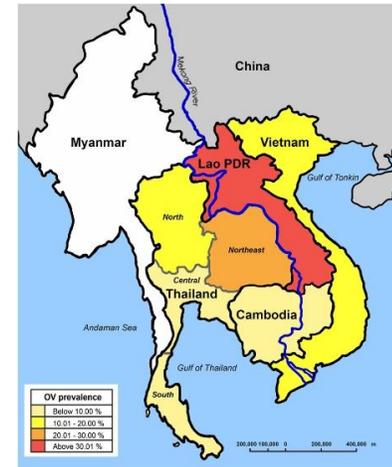
November 19, 2025; Singapore

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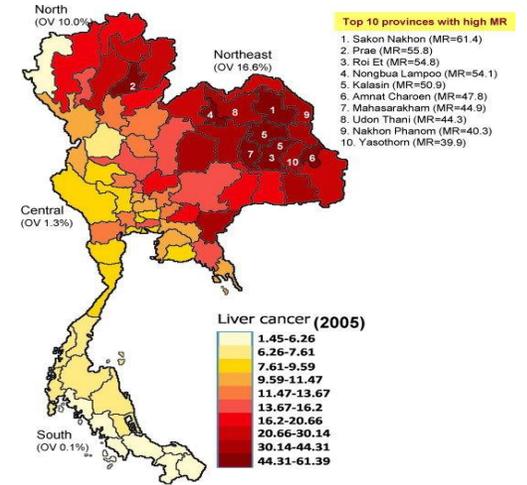
Background

Banales. J.M. et al, 2020

Khuntikeo N *et al*. 2018

Sripa B *et al*. 2011

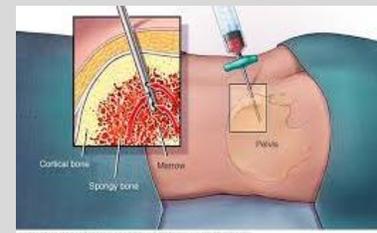## Current diagnostic techniques

Tumor screening
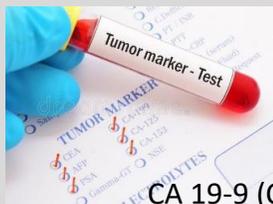

Ultrasound

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

Our hypothesis

Background :

Previous finding

CCA development is cell-type specific.

O. viverrini infection contributes to CCA development
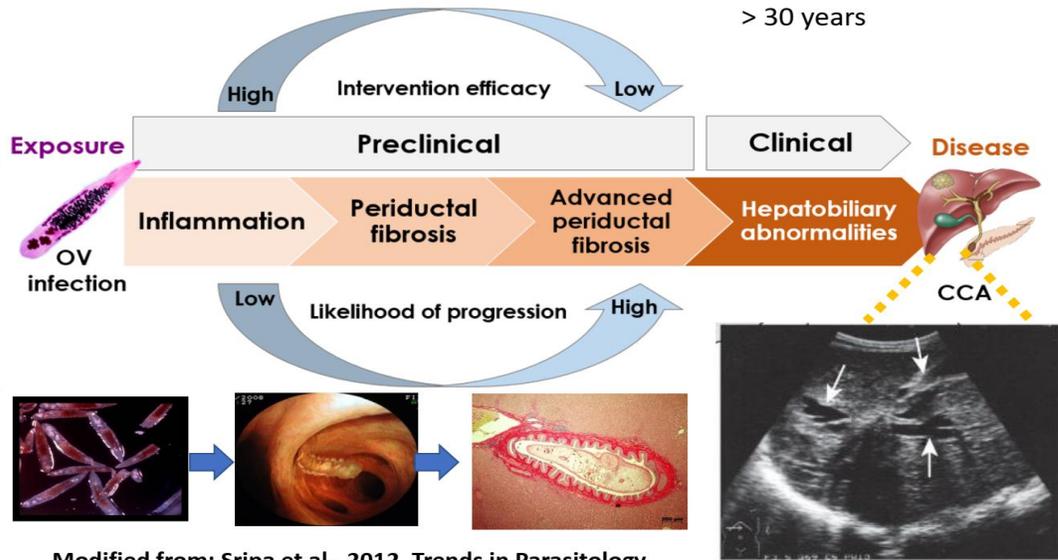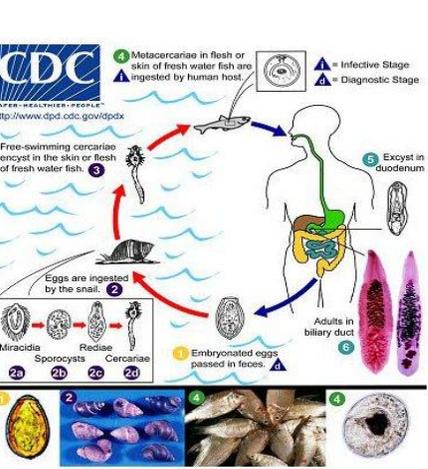
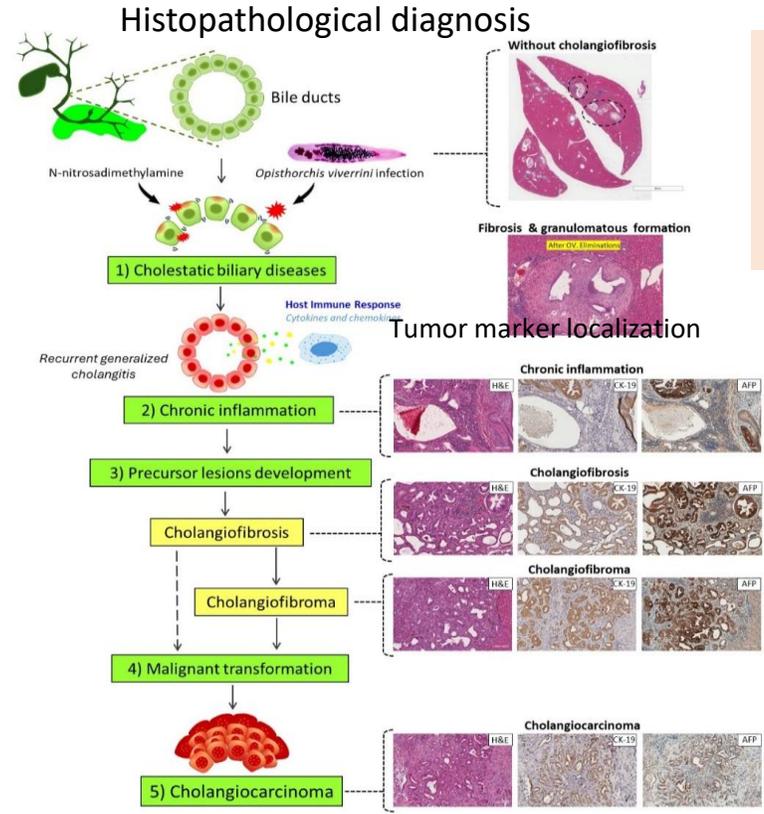Modified from: Sripa et al., 2012, Trends in Parasitology

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

Histopathological diagnosis

Tumor marker localization

OV-induced chronic inflammation

Precancerous lesion   CCA lesions

Target

To develop and validate innovative optical and electrochemical biosensing platforms for the early diagnosis of cholangiocarcinoma.

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

Proposed methods

NECTEC SERS Chips

DNA, RNA, protein, peptide, lipid, etc.,
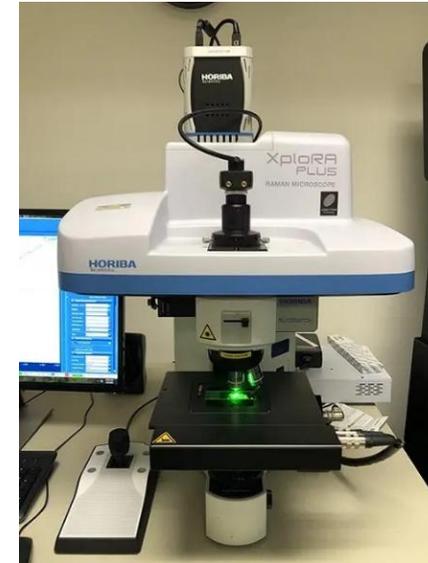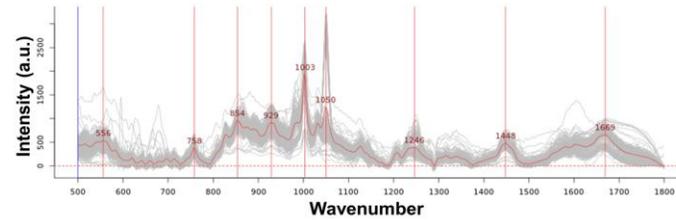
Raman spectroscopy
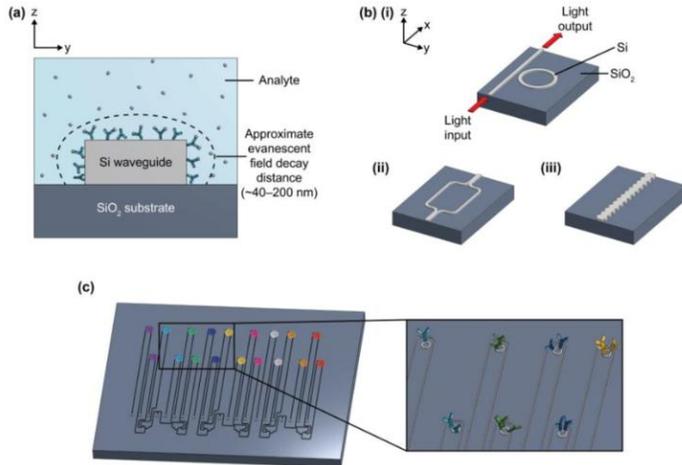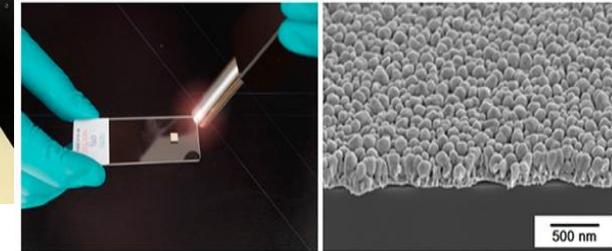
Silicon photonic sensor

LC-MS/MS
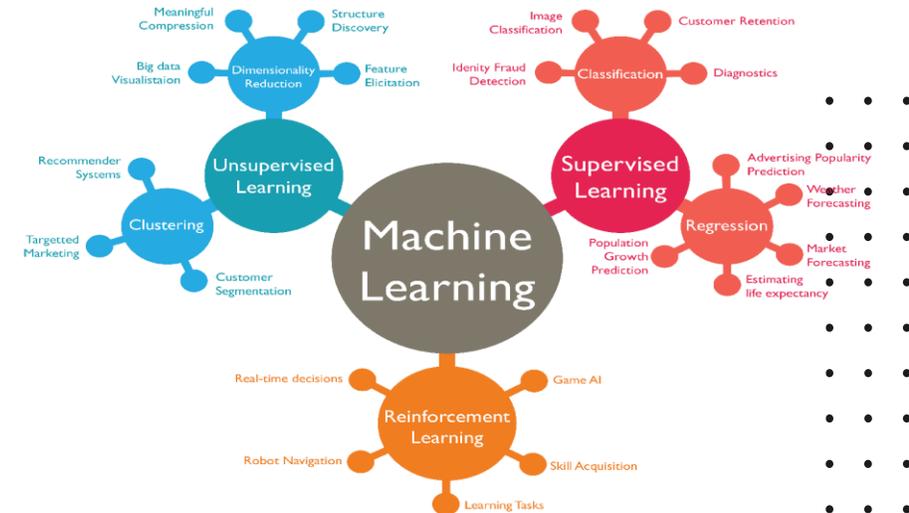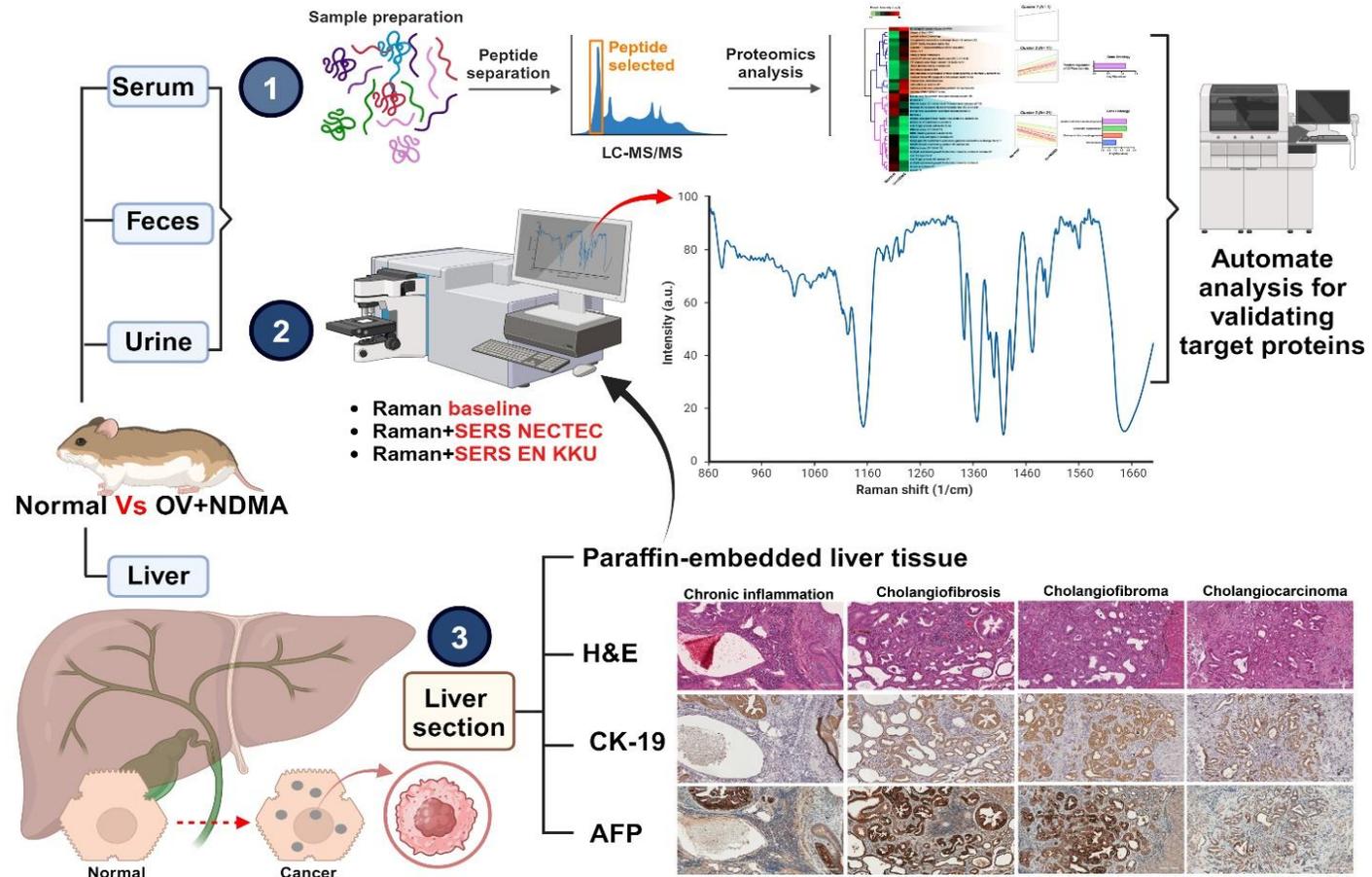
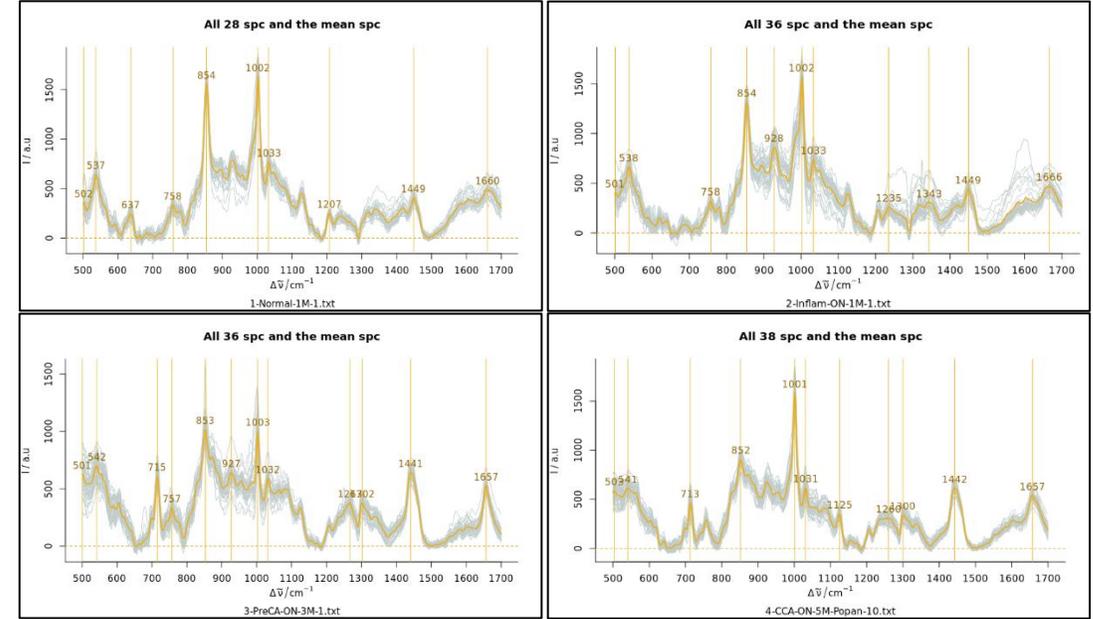**Schematic diagram illustration of the scope of the study in animal model.** The step include 1) proteomic analysis for protein identification, 2) Raman spectroscopy based on based line peak, SERS from NECTEC and SERS from EN-KKU, and 3) histopathological study by staining with hematoxylin-eosin (H&E), cytokeratin-19 (CK-19) and alpha-fetoprotein (AFP).

Project activities: MDKKU-NECTEC

SERS & AI (PCA) distinguish CCA staging in animal
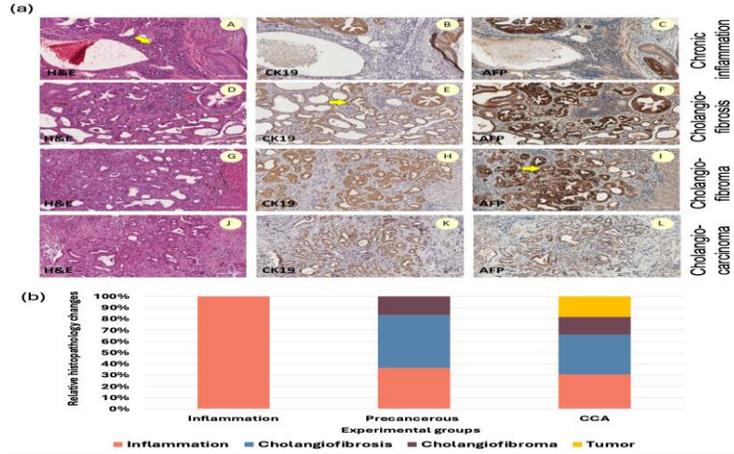
CCA development is classified into inflammation, pre-cancerous lesion and tumor lesion according to histopathological changes

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%.

PCA was used to compare Raman spectra of four classes (Normal, Inflammation, Pre-cancer, Tumor). However, classical ML algorithms using Raman intensity **could not distinguish between the Pre-cancerous and CCA (Tumor) groups.**

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**

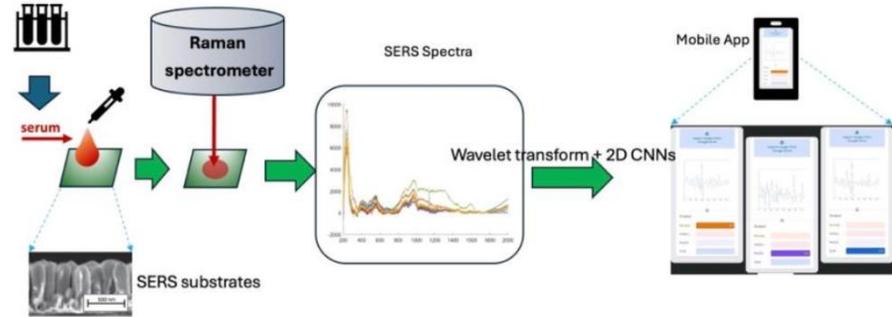SERS + 2D CNN: A New Tool for Early CCA Diagnosis

Different staging of CCA development was established.
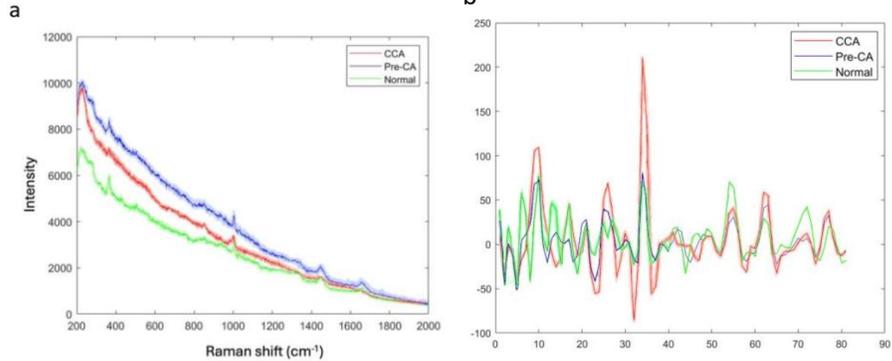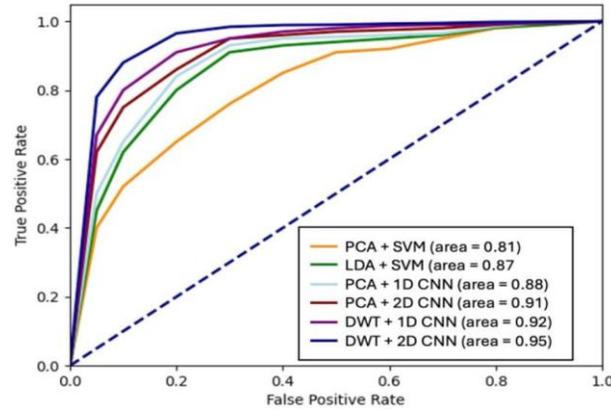

Illustration of study design for SERS+AI marks early stage of CCA

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

---

SERS + 2D CNN: A New Tool for Early CCA Diagnosis in animal

- ROC curve analysis was used to compare the performance of all classification models.
- The **DWT + 2D CNN** model demonstated the highest accuracy for CCA diagnosis.
- **Result: AUC = 0.95**
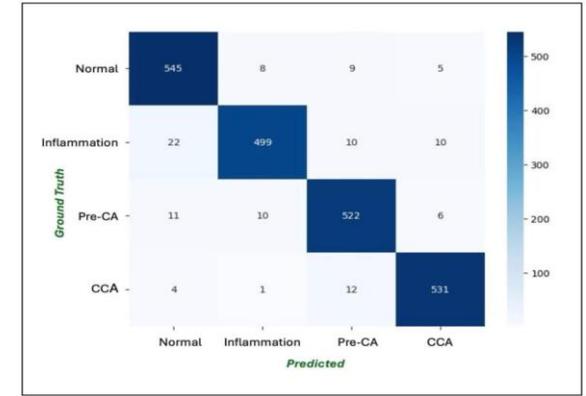
Confusion matrices of each signal processing and machine learning methods by 2D CNN.

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%**

- **Breakthrough:** Our 2D CNN model can differentiate all stages of CCA (Normal, Inflammation, Pre-Cancer, Tumor), something classical ML cannot do.
- **Accuracy:** This SERS-based method shows high accuracy for *early* detection.
- **Future:** This technology is suitable for development into a point-of-care mobile application.

Project activities: ENKKU: Biosensing platform based on SERS

**Surface Enhance Raman Spectroscopic (SERS) chip development**

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

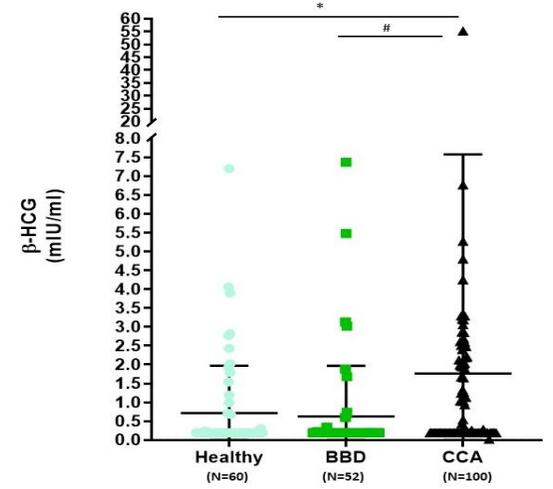Part I: Synthesis of AuNPs and novel techniques to pair nanoparticles to yield greatest enhancement
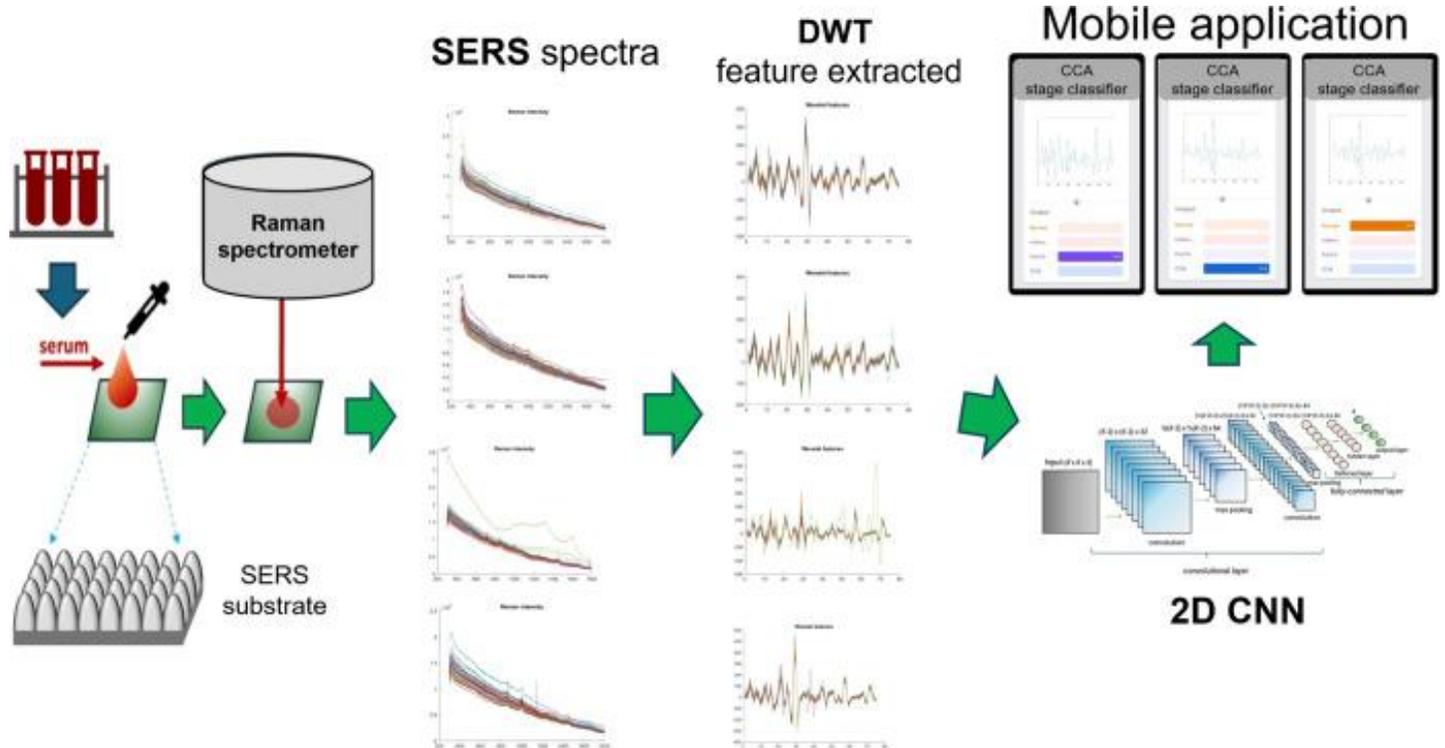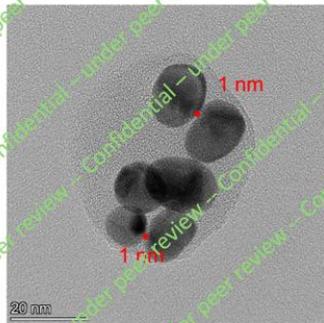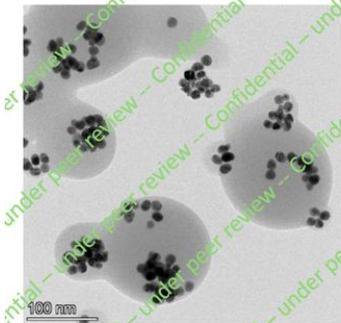
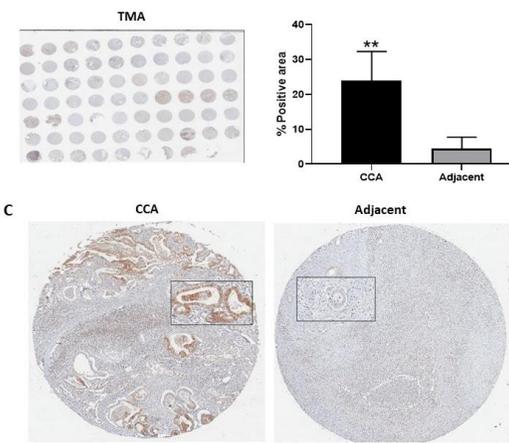+ carried out characterization on TEMs, SEM, processing technique, etc. (Figures will be licensed under publisher)

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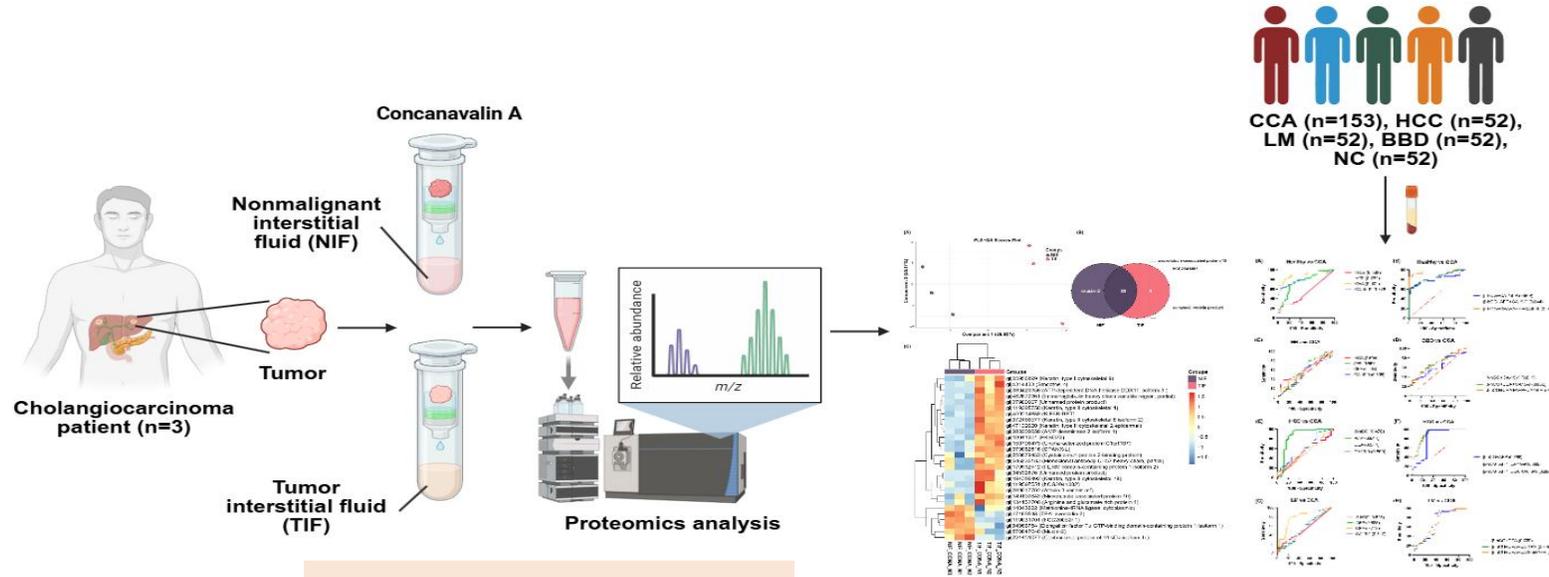
Flow chart of the study

Determine beta HCG level by automate

Verify beta-HCG expression in CCA array by IHC technique
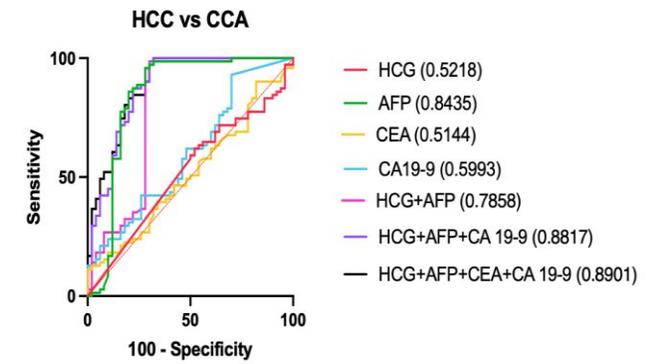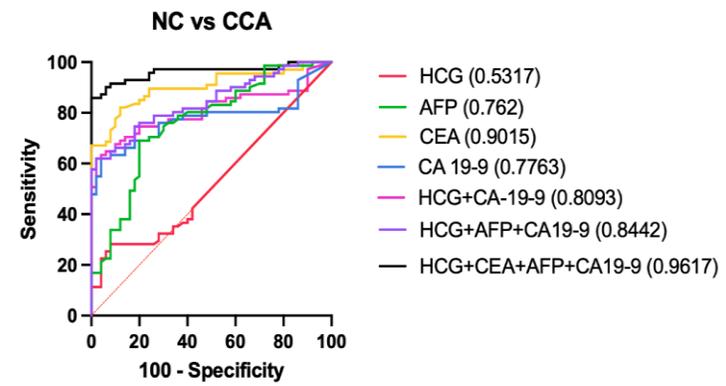
ROC analysis for the efficacy of its performance by compare/combine with routine biomarkers.

NC vs CCA
- HCG (0.5317)
- AFP (0.762)
- CEA (0.9015)
- CA 19-9 (0.7763)
- HCG+CA-19-9 (0.8093)
- HCG+AFP+CA19-9 (0.8442)
- HCG+CEA+AFP+CA19-9 (0.9617)

HCC vs CCA
- HCG (0.5218)
- AFP (0.8435)
- CEA (0.5144)
- CA19-9 (0.5993)
- HCG+AFP (0.7858)
- HCG+AFP+CA 19-9 (0.8817)
- HCG+AFP+CEA+CA 19-9 (0.8901)

Combination of Label-Free SERS-Based Nanosensors and Machine Learning for Diagnosis of Cholangiocarcinoma

SERS+LDA Model: High-Accuracy CCA Differentiation
81% accuracy and 0.97 of AUC values.

Human samples analysis (n=221): CCA = 71, HCC = 50, LM = 50, HA = 50
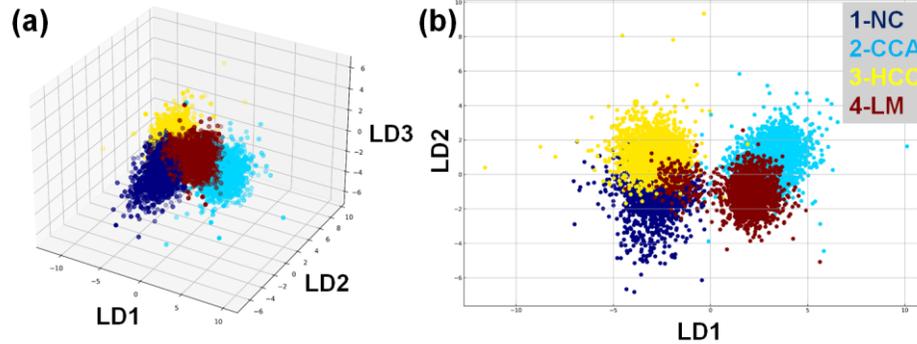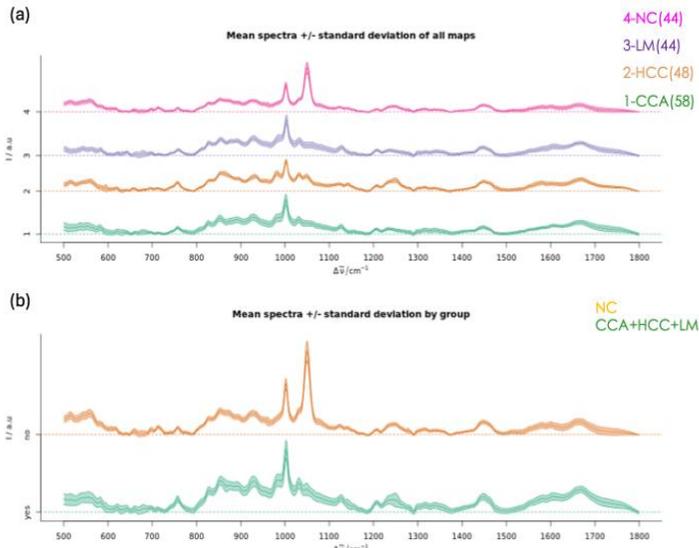
LDA Achieves Superior Separation of Liver diseases

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

SERS Distinguishes Healthy vs. Malignant Liver Tissue

(a) Group Comparison: Unique mean spectra for Normal, HCC, CCA, and LM.
(b) Cancer vs. Normal: Significant spectral shifts identified between healthy (NC) and combined cancer (HCC+CCA+LM) samples.

Key Raman shifts can be used to distinguish healthy from malignant liver tissue.

(a) PCA (3D Plot):
•Shows some natural clustering, but with significant overlap between groups.
•Visualizes spectral data for all four groups (NC, HCC, CCA, LM).
(b) LDA (2D Plot):
•Applied specifically to maximize class separability.
•Achieves **distinct and clear clustering**, effectively distinguishing all four groups.

LDA is a more powerful tool than PCA for classifying these spectral data.

LDA Model Achieves High Classification Accuracy

•**ROC Curves:** Evaluate the LDA model's ability to differentiate the four groups (NC, HCC, CCA, LM).
•**High AUC Values:** The Area Under the Curve (AUC) for each class is high, indicating excellent separation.
•**Strong Performance:** The model significantly outperforms a random classifier (diagonal line).

The SERS with LDA model is highly effective for accurate liver cancer detection.

2024 PROGRESS
CMU: Biosensing platform based on silicon photonic development

Fabrication of silicon photonic sensors

Development of New sensor measurement system prototype

Proof of concept In Laboratory → broadband source → MEMS filter → Power Meter
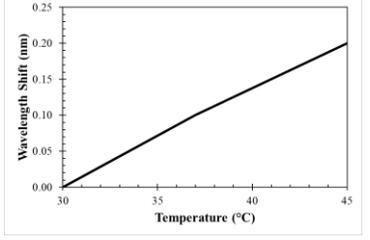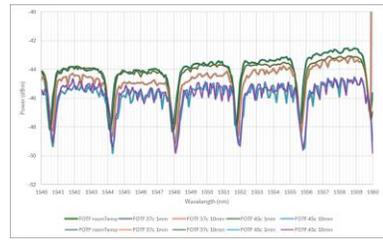
September 2024 ✓

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

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

Fabricated sensors expected by **March 2025** ✓

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

Total cancer incidence in Laos between 2010 and 2024

Distribution of dat.laos$year
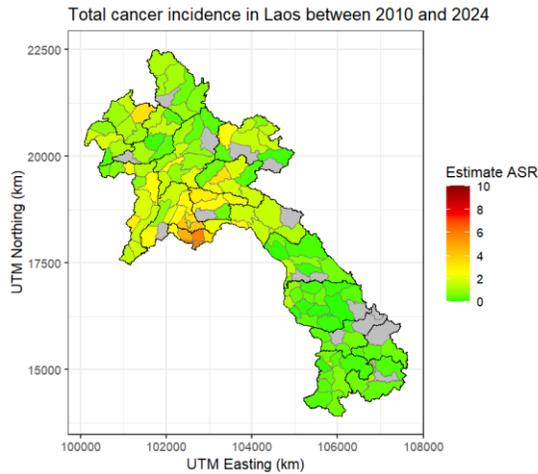
5. Transfer technology of SERS and AI for CCA diagnosis to Laos' team

Transportation Laos' samples to KKU

Detection of Serum Using Raman Spectroscopy
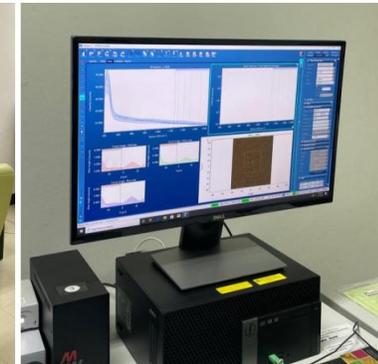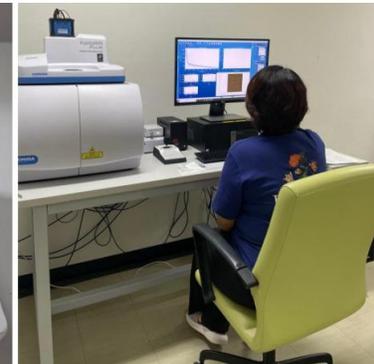
The raw Raman data and an example of the Raman peak results.
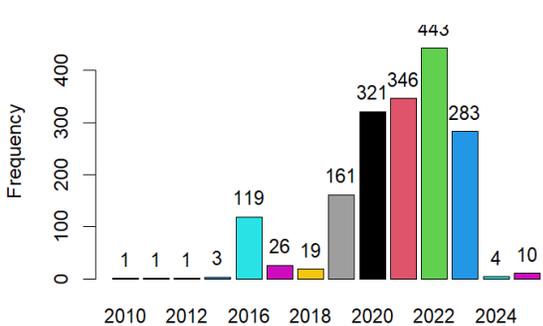
Phase II

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

Biomedical Engineering Institute, Chiang Mai University

Project activities: ASEAN IVO project review & report 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

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

Three 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)

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 Pongkng b,f, Suppakrit Kongsintaweesuk b,f , Mati Horprathumg, Pitak Eiamchaig, Saksorn Limwicheang, Noppadon Nuntawongg	aDepartment of Parasitology, Faculty of Medicine, and bCholangiocarcinoma Research Institute, cFaculty of Engineering, dCollege of Computing, eDepartment of Pathology, Faculty of Medicine, fBiomedical Sciences Program, Khon Kaen University, Khon Kaen 40002, Thailand. gNational 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
2	Early-Stage Cholangiocarcinoma Detection Using Surface-Enhanced Raman Spectroscopy and 1D CNN with Discrete Wavelet Transform	Pobporn Danvirutaia, Chawalit Pairojkulb,e, Sartra Wongthanavasua, Chavis Srichanc, Somchai Pinlaor d,e	aCollege of Computing, Khon Kaen University, Khon Kaen 40002, Thailand ; bDepartment of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand; cDepartment of Computer Engineering, Faculty of Engineering, Khon Kaen University, Khon Kaen 40002, Thailand.; dDepartment of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand.; eCholangiocarcinoma Research Institute, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand	16th Biomedical Engineering International Conference, BMEiCON 2024	21 November 2024 - 24	Pattaya, Thailand

Output/Outcome: Innovation of photonic and electrochemical biosensors for cholangiocarcinoma diagnosis

ENKKU: SERS chip development

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)

Future works:

Greater Mekong subregion

Thailand

Lao PDR

Phase II
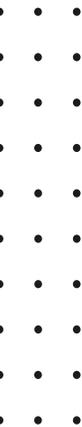
Thailand---Lao PDR---China

Conclusion: **Innovation of photonic and electrochemical biosensors for cholangiocarcinoma diagnosis**

1. Target: This study aims to develop and validate innovative optical and electrochemical biosensing platforms for the early diagnosis of cholangiocarcinoma.
2. Methods: We used SERS and AI, proteomic, silicon photonics platform.
3. Scientific papers: 3 international published, 2 international under revised/review
4. Societal impact: Our data Raw data are available to publish access. This technology is ongoing to implement in Lao PDR in Phase II project.
5. New partners: We have new collaborative partner including NECTECT, BIOTECH from Thailand, Philippines, and China.
6. Collaborative: We have expanded of the larger cohort to study in Thailand, Lao, and China.

Thank you