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

P-022

**Background :**

Cholangiocarcinoma (CCA) is common in the Greater Mekong Subregion, particularly in northeastern Thailand and Lao PDR, where the liver fluke *Opisthorchis viverrini* is endemic. CCA patients are usually diagnosed at an advanced stage and have a short survival time after surgery because no biomarker is available. CCA diagnosis is based on radiological and histopathological confirmation, which is very costly and time-consuming. We have discovered many biomarkers for CCA in animal models and successfully transferred them to patients for clinical application. Recently, the biosensor platform, which uses photonic and electrochemical detector technology, allows for a high degree of integration, enabling the devices and sensors to be moved from research laboratories into the hands of analytical chemistry and clinical medicine for large-scale detection in various samples such as urine, feces, and sera. We hypothesize that the use of biosensor platforms to detect trace amounts of CCA biomarkers in animal models could be translated into clinical applications in patients.

**Targets:**

To develop photonic and electrochemical biosensors for cholangiocarcinoma diagnosis.

**Speaker:**

**Dr. Somchai Pinlaor**
& Dr.Chavis Srichan

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

Project Members :

**Project members include four institutes from three countries, Thailand, Lao and Japan as following.**


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

**CMU (THA)** : Ukrit Mankong, Suruk Udomsom

**Mittaphab Hospital (LAO)** : Champadeng Vongdala, Keooudone Thammavong

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


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

# Project management: Team working among 4 institutes, KKU-CMU-LAO-NICT

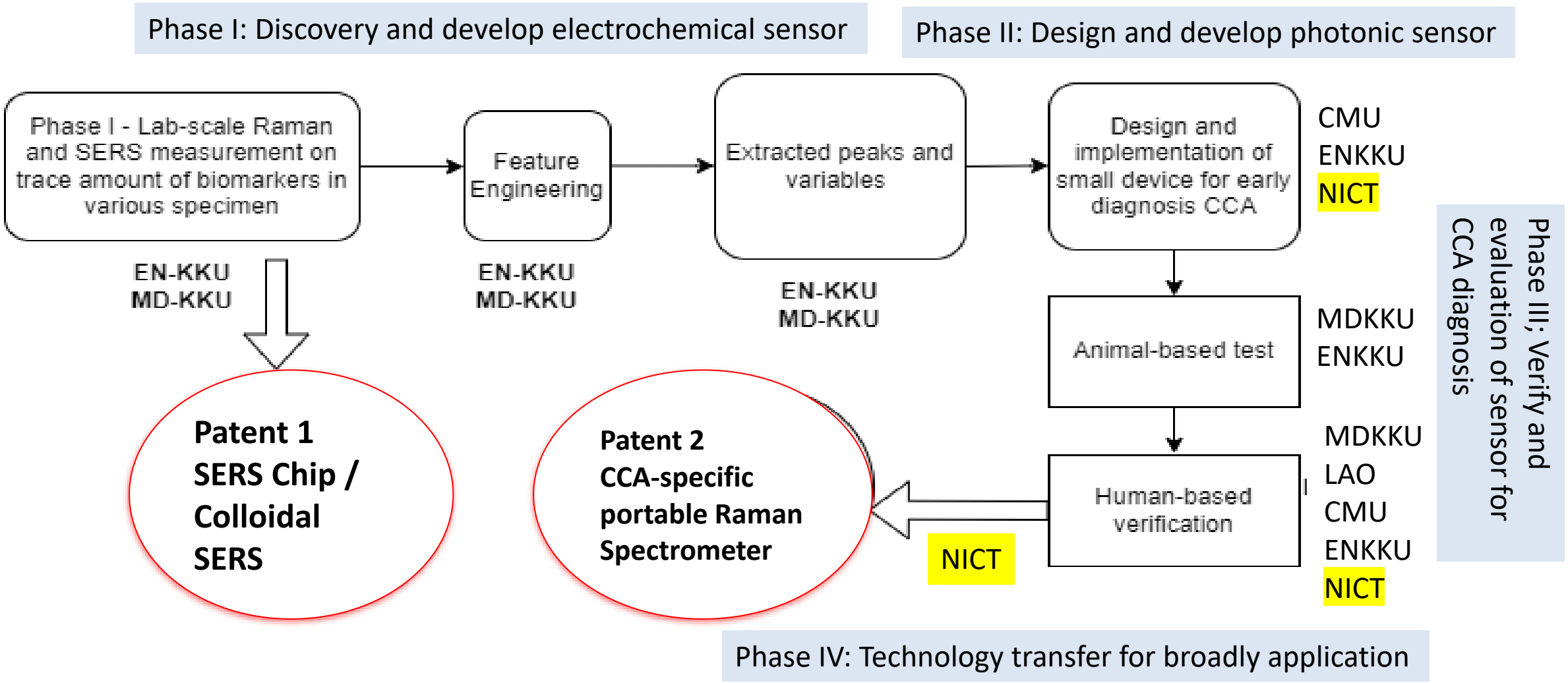


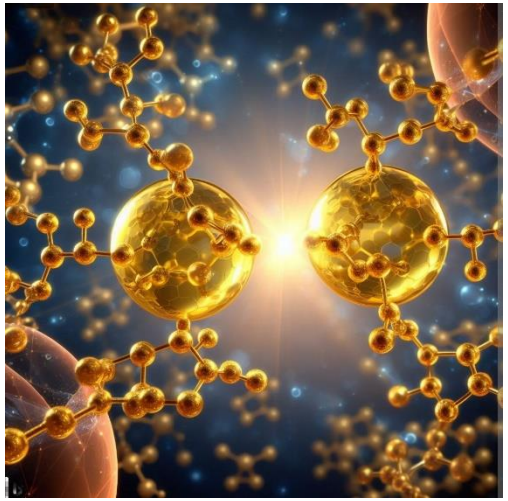

Figure 1 Project management

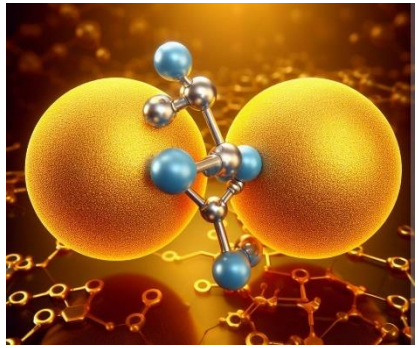
## Biosensing platform development based on Raman spectroscopy techniques

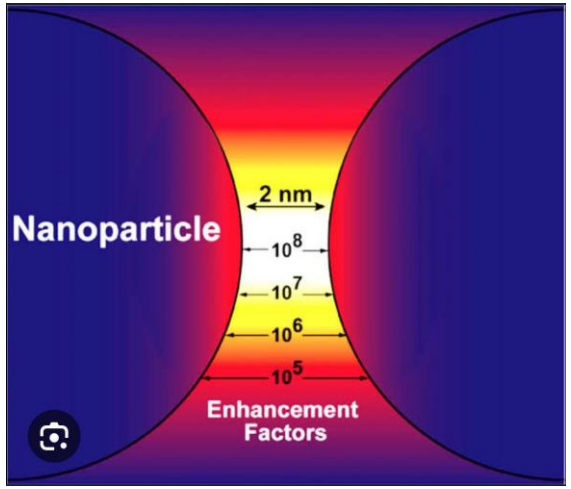
1 Patent/pretty patent +
1 publication

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

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



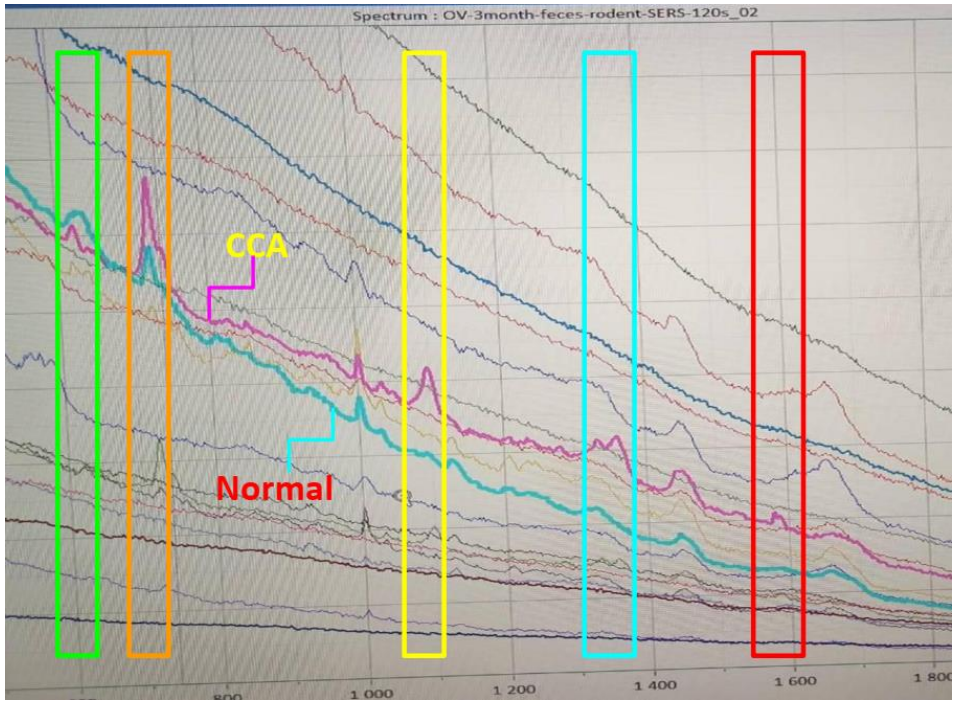



Petryayeva & Krull (2011)

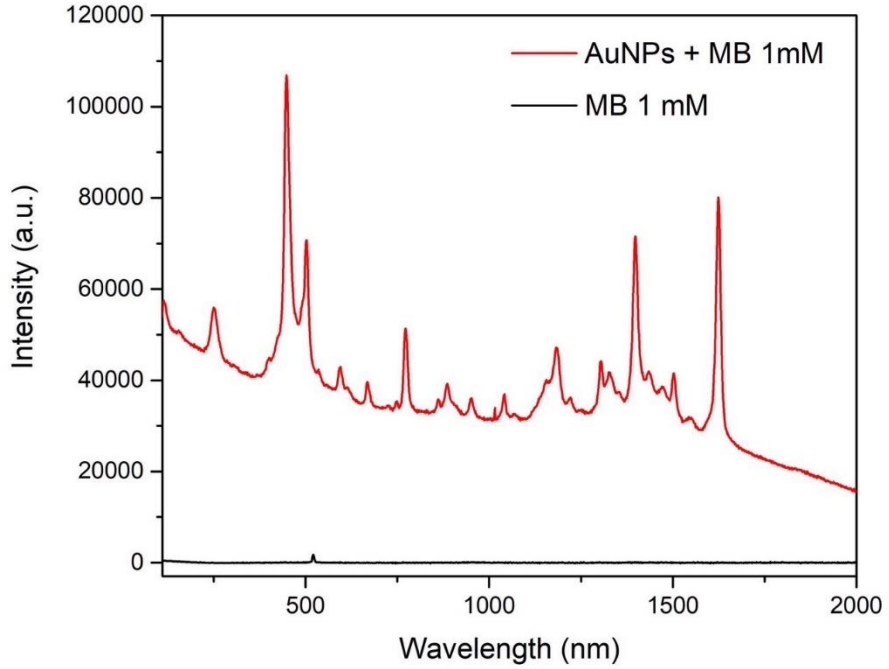
+ carried out characterization on TEMs, SEM, etc. (Figures will be licensed under publisher)

# Project Activities: **ENKKU: Biosensing platform based on SERS**

CCA distinguishable peaks using SERS



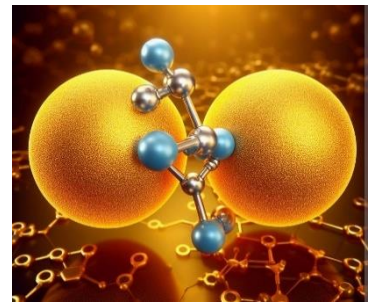

SERS of our new method on standard methylene blue
- Significant enhancement on Raman signal





**Future work**

1) add technique to bind two nanoparticles to gain greater enhancement
2) Verify enhancement performance



## Establishment of OV associated CCA in hamster for early biomarker of CCA detection, which can use to apply for screening of CCA in the patients

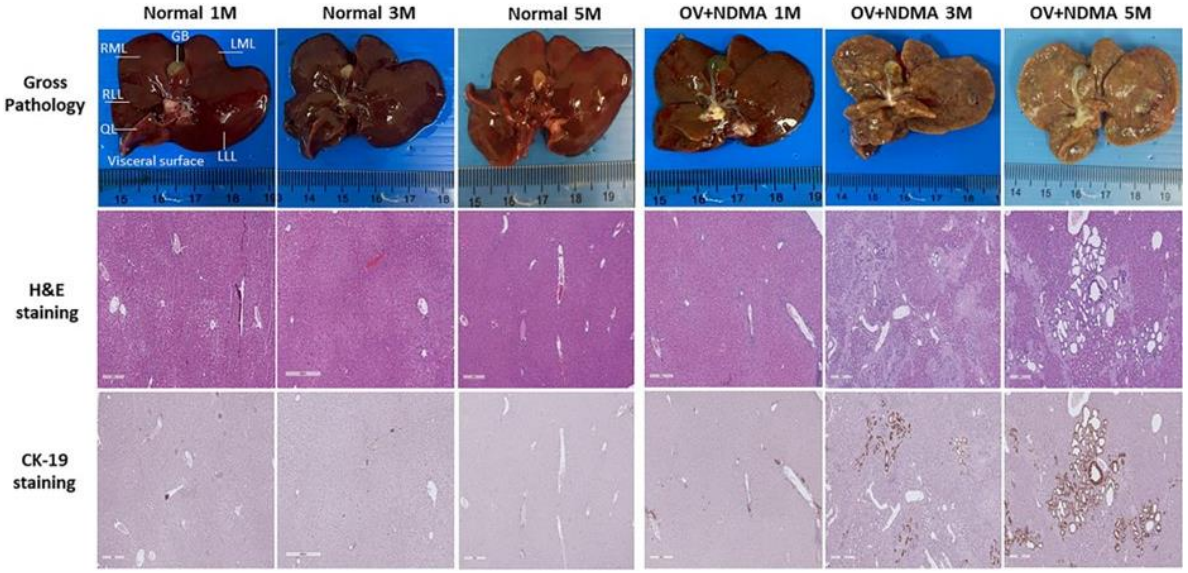



**Fig.1** Illustration of gross pathology, and haematoxylin & eosin and CK-19 staining in representative liver tissues of hamsters. Animals were infected with *O. viverrini* infection combined with NDMA 12.5 ppm in drinking water for 6 weeks. Immunohistochemical staining for cytokeratin 19 (CK-19) showed cytoplasmic staining of tumor cells in OVNDMA treated at 3 and 5 month groups while no staining was observed in normal group (Magnification × 4).

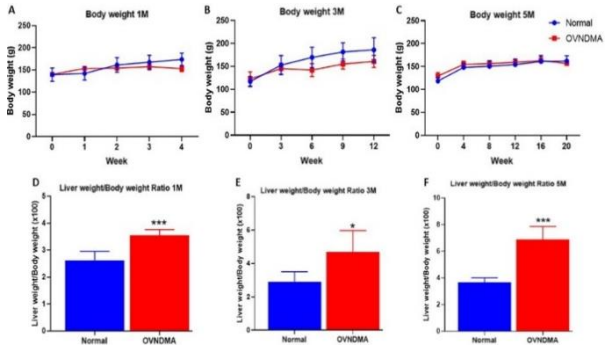



**Fig.2** Induction of CCA in hamsters was induced by a combination of *O. viverrini* infection and NDMA 12.5 ppm in drinking water for 6 weeks. Body weigh gain at 1 month **(A)** 3 month **(B)** 5 month **(C)**. Liver weight to body weight ratio in normal and OV+NDMA group at 1 month (**D)** 3 month **(E)** 5 month **(F)**. Data are mean ± SD and analyzed by student t-test. **P* value < 0.05 compared to normal group. **P* value < 0.05 compared to normal group. ****P* value < 0.001 compared to normal group.

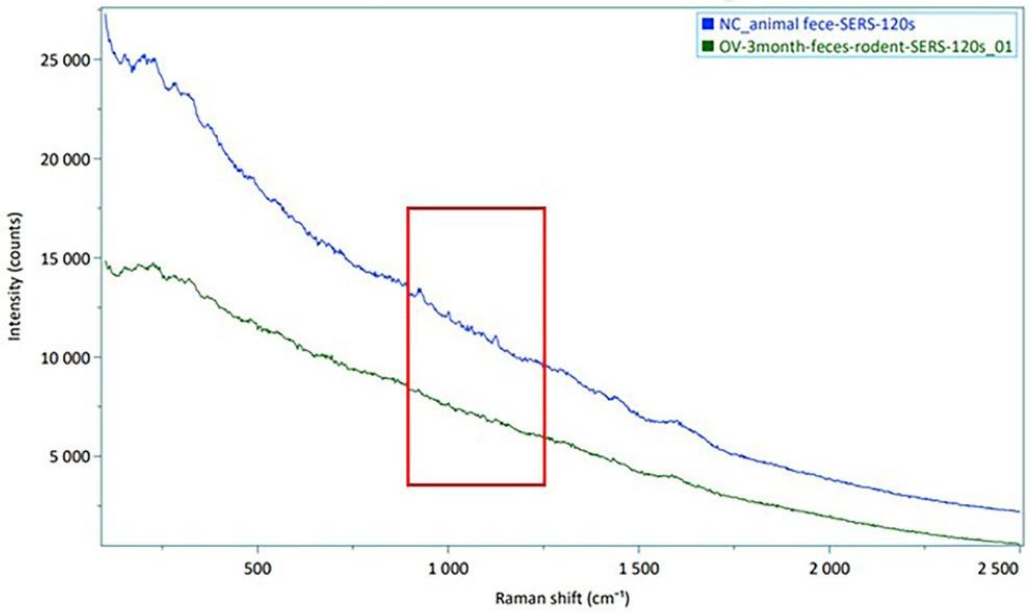



**Fig.3** Raman peak separate early CCA at 3 months between CCA and normal by Raman spectroscopy. Upper line is CCA, and under line is normal.

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

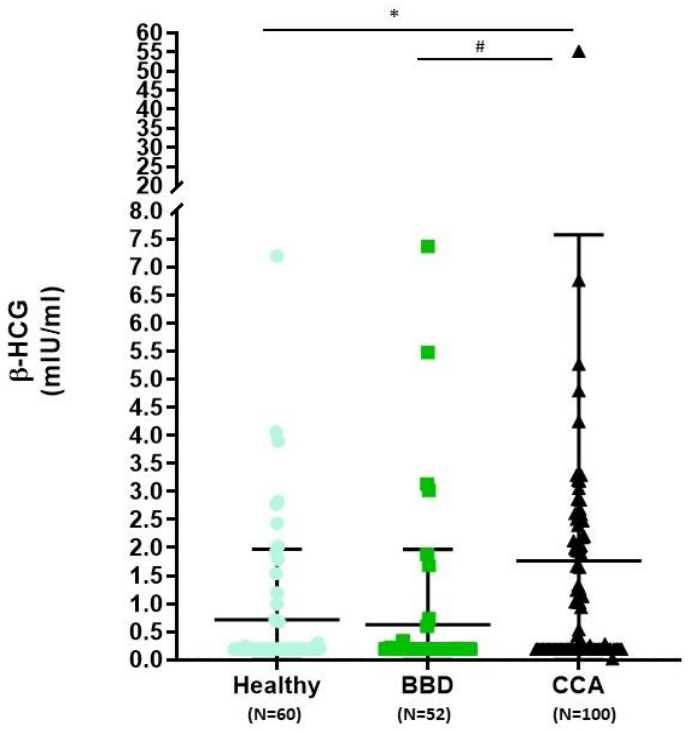



**Fig. 4** Determination of β-HCG levels in human serum. Serum β-HCG levels in healthy (N=60), Bening biliary disease (N=52), and CCA patient (N=100) were determined using electrochemiluminescence immunoassay. * P<0.05 compared with healthy group. #P<0.05 compared with BBD group.

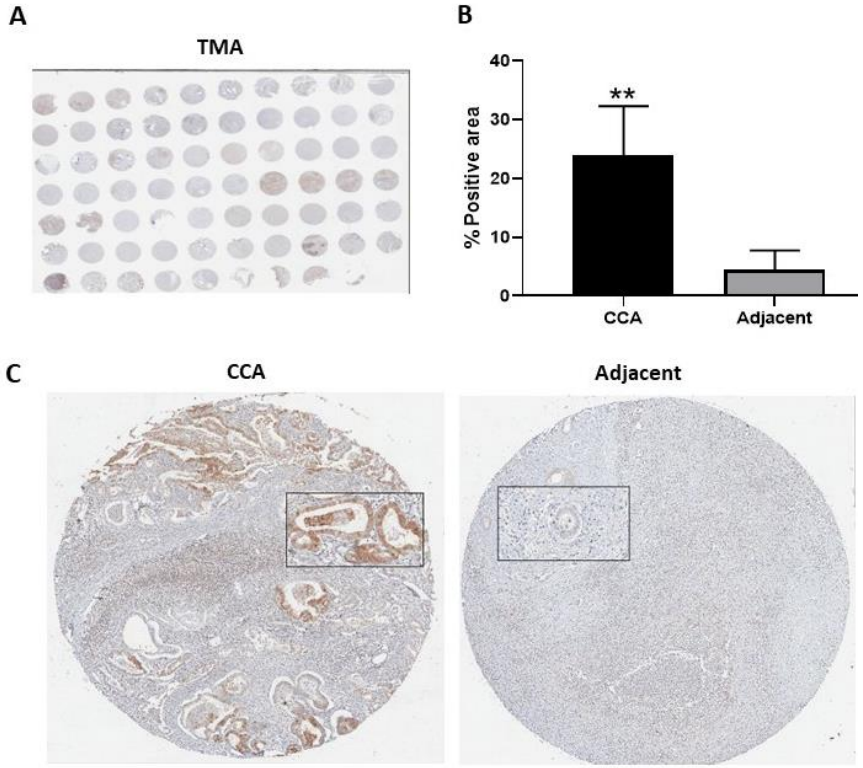



**Fig. 5** Immunohistochemical staining for β-hCG in cholangiocarcinoma and adjacent tissues. Staining pattern of the TMA section (A). Immunoreactive positive area (%) in cancerous tissues was higher than in adjacent tissues (B). Images showing β-hCG staining in CCA (C), and adjacent tissues (D). ** P<0.001 compared with adjacent tissue.

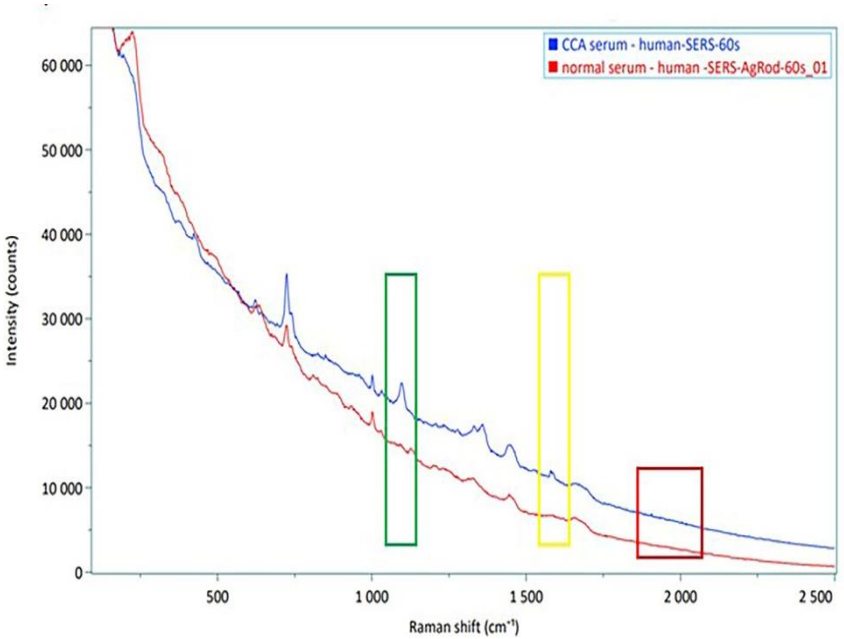



**Fig.6** Raman peak separates serum CCA from the patients from normal serum by Raman spectroscopy. Upper line is CCA, and under line is normal.

# Project Activities: Budget plan for ENKKU-MDKKU in year 1

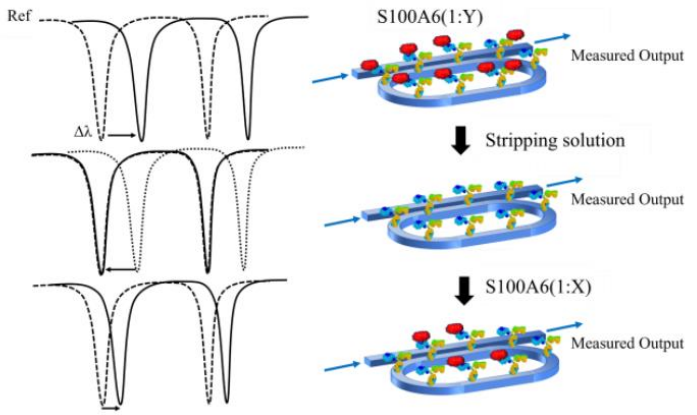
| Items | Activities | Budget |
|---|---|---|
| Hamsters' CCA setting, samples collection and histopathological confirmation | 1.1 Animal model setting for time profile of CCA development (inflammation, initiation tumor and metastasis stages); animal, fish, reagent, chemicals, supplier, materials, rental fee for animal rearing, fish digestion, metacercaria identification and collection<br>1.2 Samples collection; materials, supplier and rental fee for animal scarification & sample collection<br>1.3 Histopathological study; antibodies, sectioning tissue, supplies & materials, reagents for H&E staining and IHC staining | 3,500 USD |
| Discovery of Raman peak for CCA diagnosis | 2.1 Raman spectroscopy measurement; device, chip, materials, standard protein, rental fee for machine and measurement<br>2.2 Proteomics analysis; chemical, reagents, buffer, etc.<br>2.3 Data analysis | 3,000 USD |
| Patients sample collection | 3. Collection of various samples from the patients: healthy, CCA, HCC, and other cancers; materials, volunteer compensation, physician, surgeon, etc., | 2,500 USD |
| Identification & evaluation of biomarker in CCA patients: training set | 4.1 Raman spectroscopy measurement; device, chip, materials, standard protein, rental fee for machine and measurement<br>4.2 Electrochemical sensor for CCA diagnosis; chemical, reagents, buffer, etc.<br>4.3 Data analysis | 2,500 USD |
| Verification of electrochemical sensor for CCA diagnosis: verification set | 5.1 Evaluation of electrochemical & photonic sensor in the patients' samples, device, chip, reagent, etc.<br>5.2 Standard method measurement by ELISA; i.e., materials, chemical, buffer, reagents etc. | 2,900 USD |
| SERS development: Synthesis | 6.1 Compulsory chemicals: HAuCl4 (Tetrachloroauric(III) acid trihydrate ), buffer solutions, binding chemicals, other functionalized materials, deionized water and related instruments<br>6.2 Equipment to support portable sensors, e.g., optical filter and/or small spectrometer | 3,500 USD |
| Verification of electrochemical sensor for CCA diagnosis: verification set | 7.1 Raman spectroscopy measurement & characterization<br>7.2 Other compulsory characterization: Dynamic Light Scattering (DLS) and/or UV-Vis Absorption Spectrometer and related equipment | 1,000 USD |
| KKU facilities services | 8. Facilities and services for Research Instrument Center (RIC) KKU | 2,100 USD |

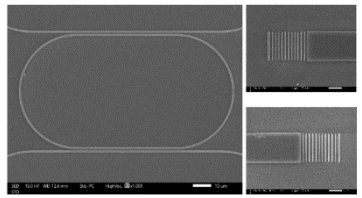
# Project Activities: **CMU; Biosensing platform based on silicon photonic development**

## Related technologies by CMU prior to ASEAN IVO 2023

1. **Silicon Nitride sensor device**
2. **Antibody coating technique on silicon nitride** *(S. Udomsom, et.al., Coatings 2021, 11, 595)*
3. **Silica nanoparticles coating**
4. **Itaconic protein crosslink technology** *(Application Number 2301000943)*
5. **Microfluidics channel by 3D printed mold, and by silicon chip mold** (with TMEC, Thailand)
6. **Demonstration of S100A6 sensing (one of the CCA candidate biomarkers)** (with KKU, Thailand) *(S. Udomsom et al., in Progress in Biomedical Optics and Imaging - Proceedings of SPIE, 2023,vol. 12395)*

**Principle of Resonator based sensor**
**Detection of optical wavelength shift due to bio-analytes**



**Basic silicon nitride sensor device (Previously fabricated)**

## 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**
3. **Protein coating techniques for CCA biomarkers.**
4. **Sensor prototype development**

## Plan and progress over 2 years

### (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 |

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

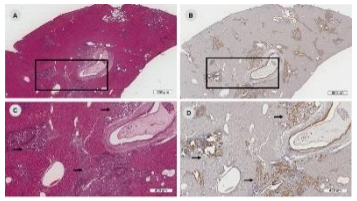
Budget for CMU activities in Year 1 approved by NICT secretariate

Activities for year 2 will be presented at a later report

Expected outputs
1 patent/petty patent
1 publication

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

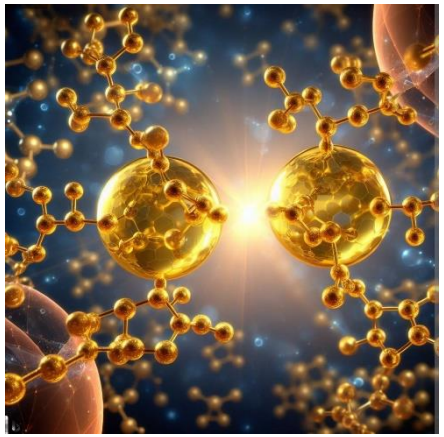
Collect and verify CCA samples: urine, serum

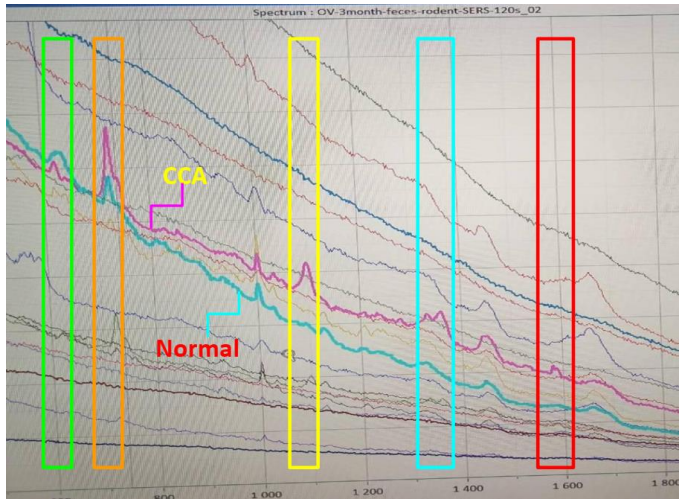


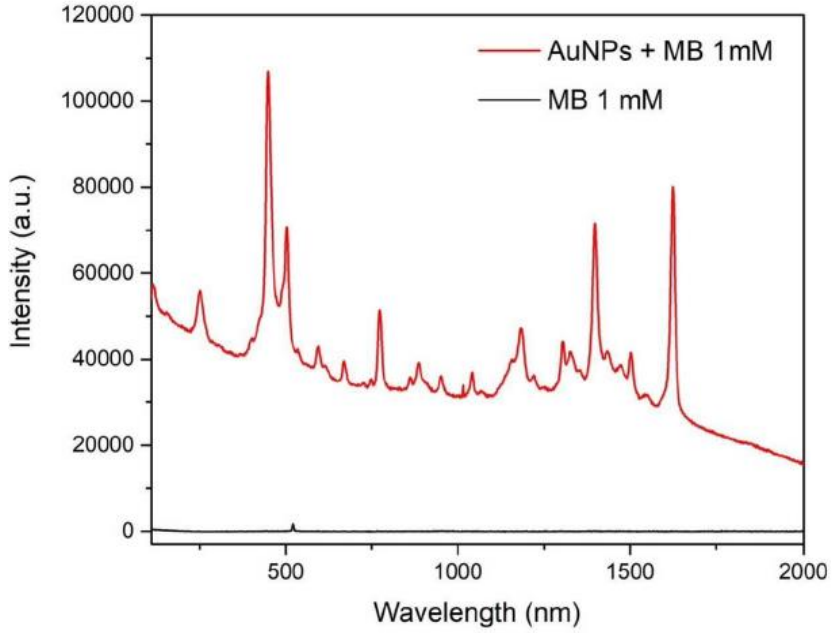
| Budget usage | Budget |
|---|---|
| Collection of various samples from the patients: healthy, CCA, HCC, and other cancers; materials, volunteer compensation, physician, surgeon, etc., | $25000 |
| Identification & evaluation of biomarker in CCA patients: training set | $3500 |
| Total | $5000 |

Budget plan for year 1

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



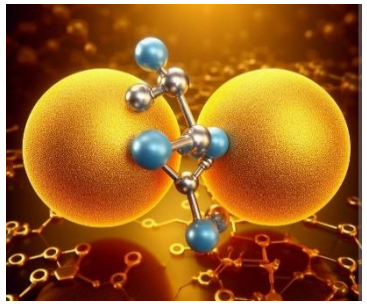




**Future work**

1) add technique to bind two nanoparticles to gain greater enhancement

2) Verify enhancement performance



# R&D Results: MDKKU; application and Research Development

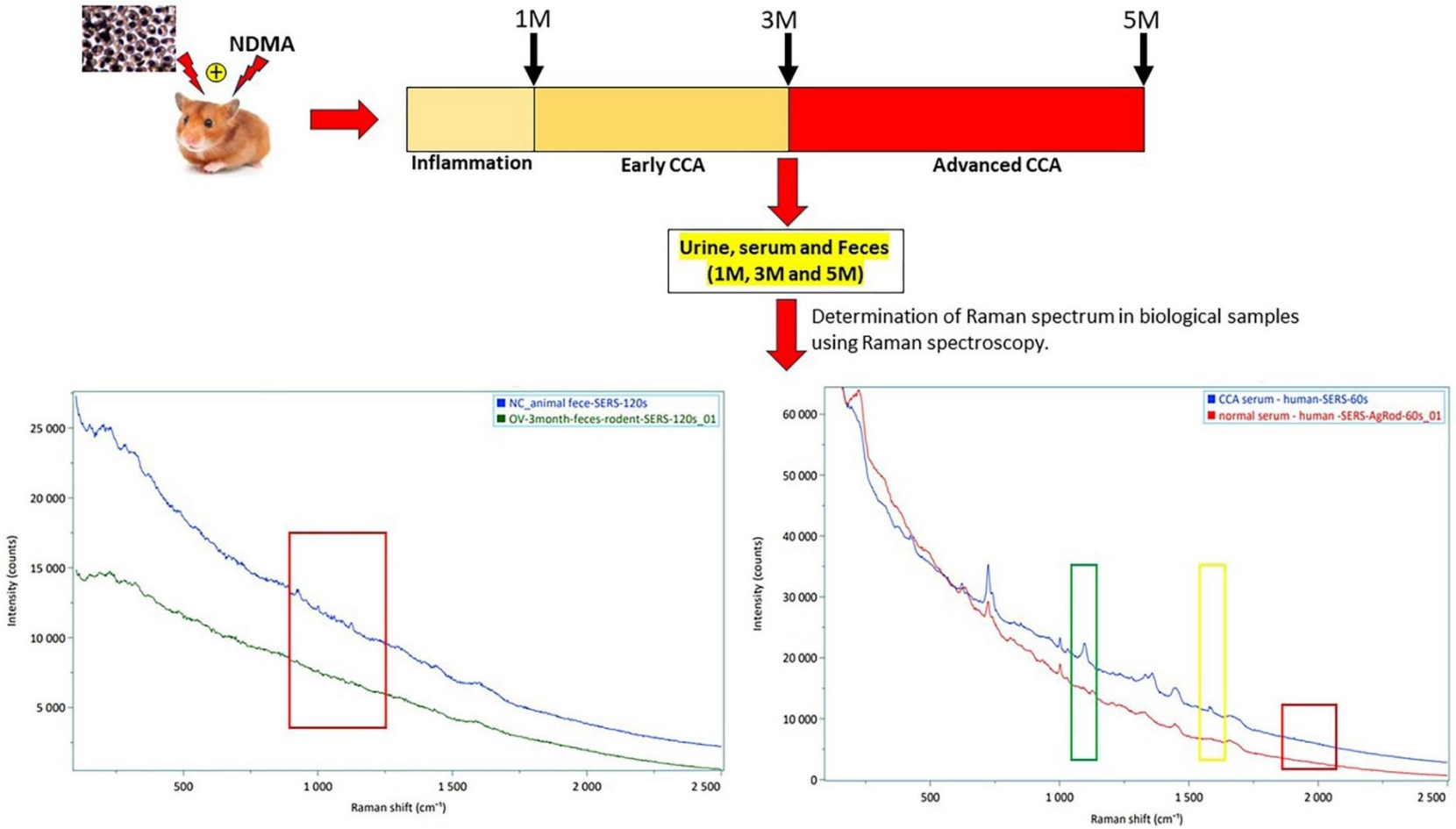



**Fig.5** Application and research development. After induction of CCA in hamsters by a combination of *O. viverrini* infection and NDMA 12.5 ppm in drinking water for 6 weeks, urine, serum and feces were collected at 1 month, 3 month and 5 month, respectively. The difference of raman spectrum in biological samples at each stage of CCA development were determined using raman spectroscopy and commercial surface enhance raman spectroscopy chip.

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

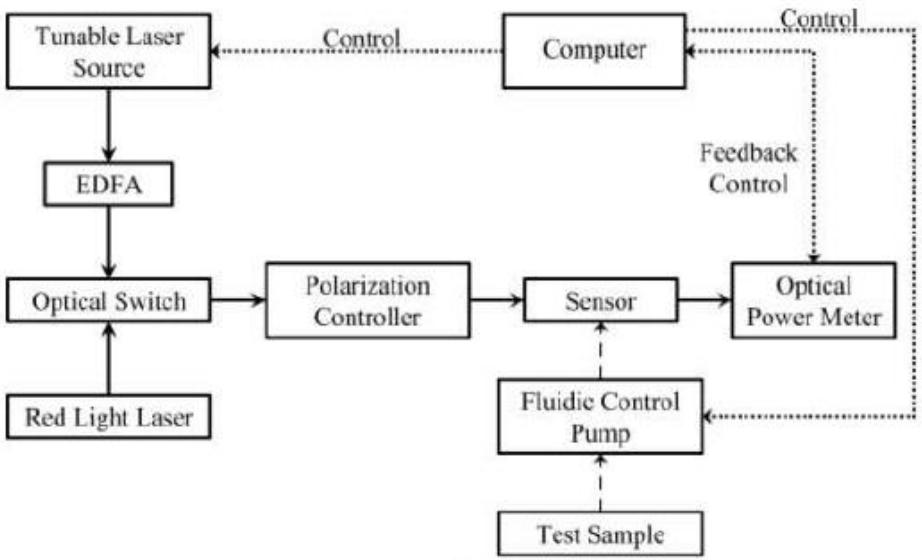



**Figure 5** (a) Experiment setup (b) Sensor chip inside the fluidic package

# Scientific Contribution & Social impact:

Since the project has only been running for 6 months, we do not yet have a scientific contribution (international conferences and journal articles) or a societal impact. However, we plan to have the following international conferences, journal articles and social impact in year 1:

- Presentation of our scientific contribution at an oncology conference or meeting.
- Publication of an international journal on CCA diagnosis by Raman spectroscopy.
- Publication on an international journal on SERS chip development to improve Raman spectroscopy for CCA diagnosis.
- Report and publish the electrochemical & photonic sensors for CCA diagnosis through CARI Institute website and KKU reporter to achieve social impact.

# Conclusion:

| Team | Recent output |
|---|---|
| MDKKU | - Establish early stage and tumor progression of hamster's CCA<br>- Sample collection of CCA patients<br>- Discovery Raman spectrum for CCA diagnosis by Raman spectroscopy<br>- Evaluate targeted diagnostic marker of CCA in CCA patients |
| ENKKU | - Develop reagent to enhance SERS<br>- Develop electrochemical sensor for CCA diagnosis<br>- Verify electrochemical sensor for CCA diagnosis |
| CMU | - Design and develop photonic sensor for CCA diagnosis |
| LAO | - Sample collection of CCA patients |

# Future works:

| Team | Future works |
|---|---|
| MDKKU | - Verify early stage and tumor progression of hamster's CCA using Raman spectrophotometer<br>- Sample collection of CCA patients<br>- Evaluate Raman spectrum and electrochemical & photonics sensors for CCA diagnosis |
| ENKKU | - Develop the precision nanoparticle distance control technique (novel)<br>- Improve reproducibility<br>- Increase sensitivity (enhancement)<br>- Specificity already implied from Raman fingerprint |
| CMU | - Design and develop photonic sensor for CCA diagnosis<br>- Improve reproducibility of photonic sensor |
| LAO | - Sample collection of CCA patients<br>- Evaluate sensor to detect CCA diagnosis |