

## Thesis Progression Title

# Optimization of serum and plasma concentration in sample preparation step using Raman spectroscopy and multivariate analysis

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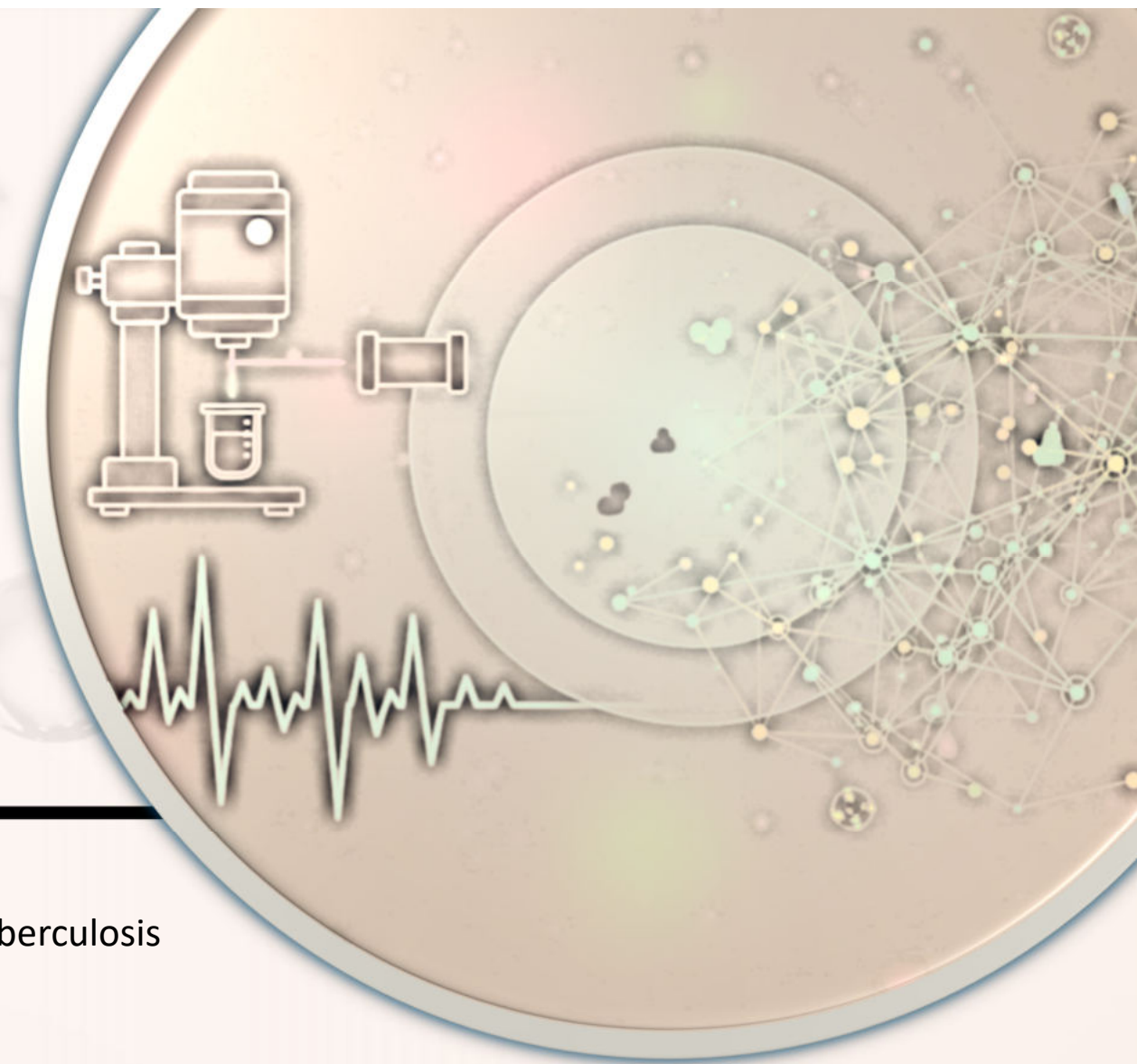
### Thesis Title

Comparative feasibility of plasma, serum, and other clinical specimens for Tuberculosis diagnosis using Raman spectroscopy and machine learning

**Mr. Patipan Wongboothong**

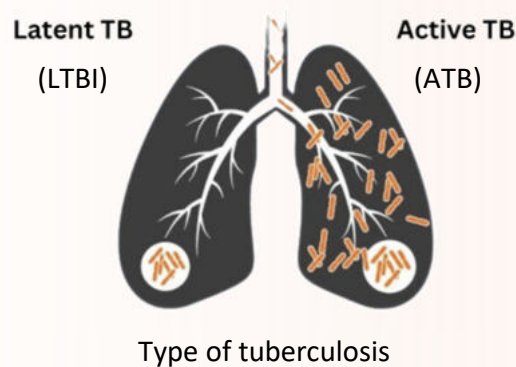
2<sup>nd</sup> Year Ph.D Student of Precision Medical Microbiology, Faculty of Medicine, KKU

**Advisor: Prof. Kiatchai Faksri, Ph.D**

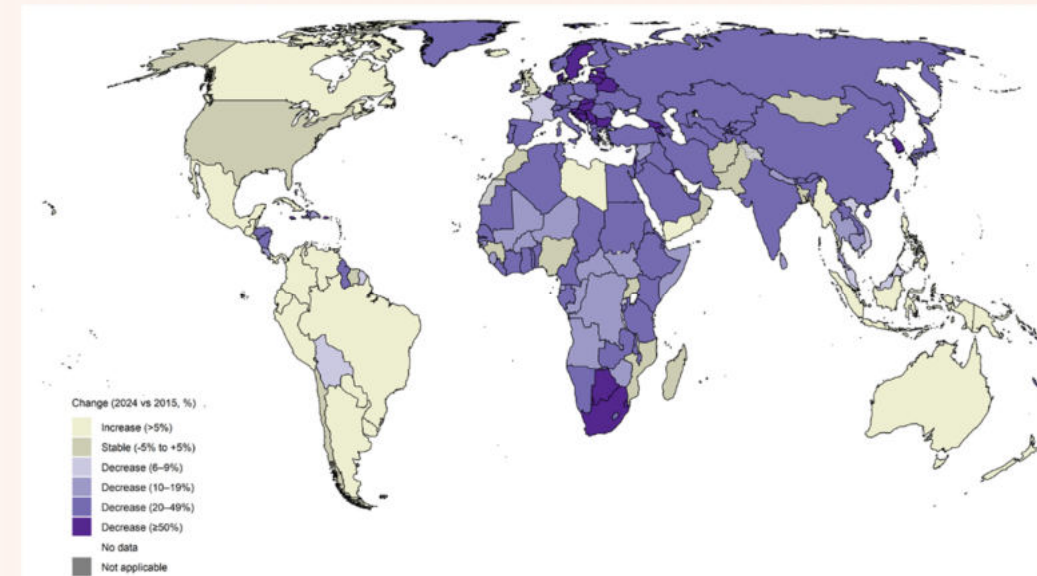


## Tuberculosis

- TB is an illness caused by organisms of the *Mycobacterium tuberculosis* (MTB) complex.
- A total of **10.8 million people fell ill** with TB, and **1.25 million people died** from TB in 2023 worldwide.
- **22 billion US\$** is needed annually for TB prevention, diagnosis, treatment, and care.
- Approximately **one-quarter** of the world's population has latent tuberculosis infection (LTBI).
- WHO ranks TB as one of 15 causes of death worldwide.  
(World Health Organization, 2025)



Estimated TB incidence rates, 2024

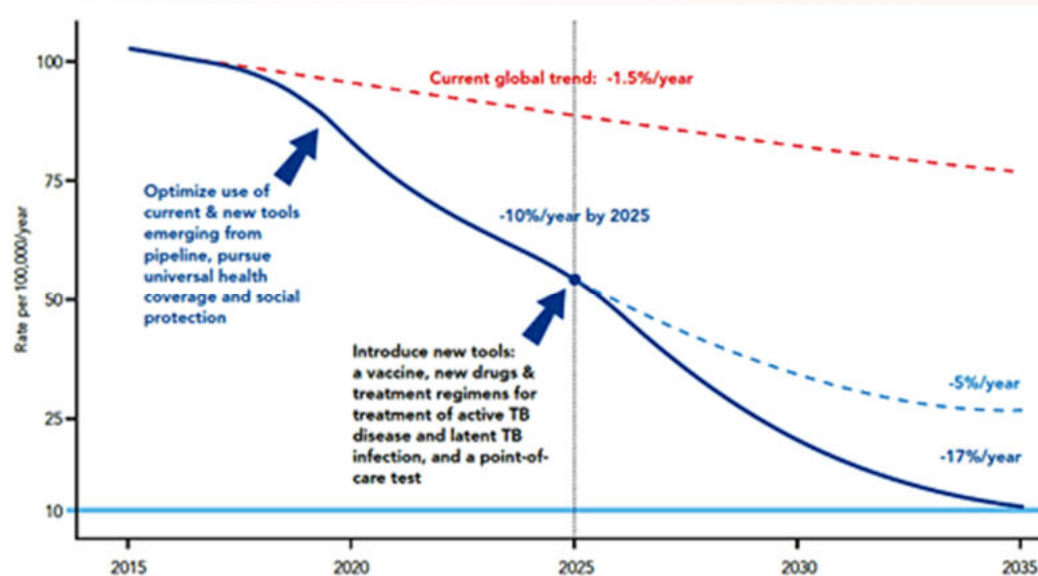


Change (%) in the estimated TB incidence rate, 2024 compared with 2015

## Tuberculosis



Estimated TB incidence rates, 2024



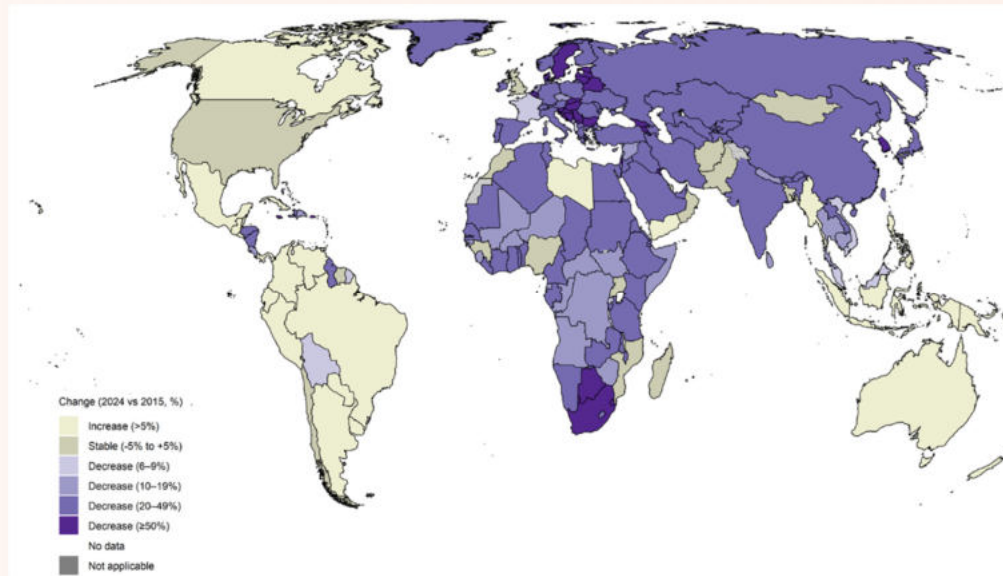
2015 (0%)

2020 (-20%)

2025 (-50%)

2035: End TB

Goal of End TB strategy



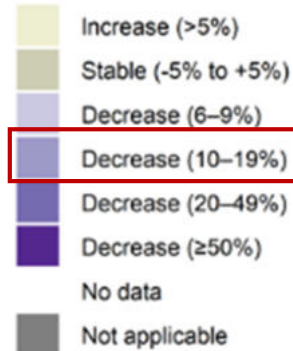
Change (%) in the estimated TB incidence rate, 2024 compared with 2015

Only few countries is close to or reached the 2025 milestone of the End TB Strategy.



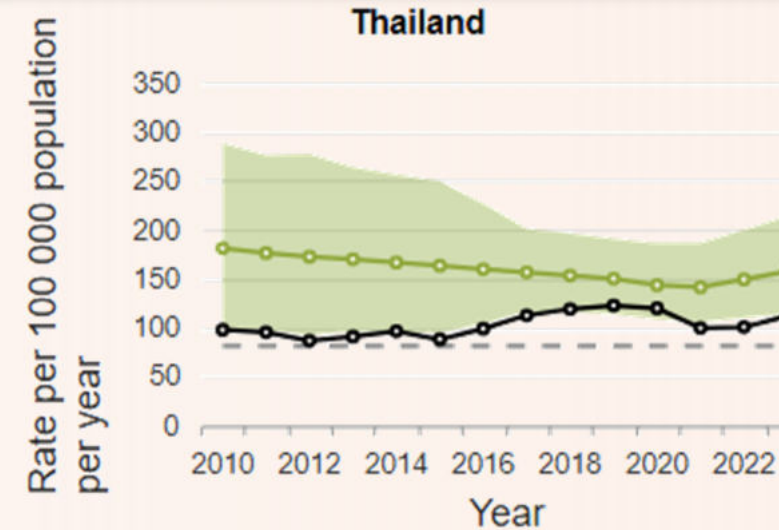
## Tuberculosis

Change (2024 vs 2015, %)



Change (%) in the estimated TB incidence rate, 2024 compared with 2015

- TB mortality rate in Thailand is high, averaging **over 30 deaths per day** or around 11,330 deaths yearly (11% estimate mortality).
- Only about 72,000, or 65%, are diagnosed. The remaining 35%, represent unreported TB patients, posing a significant risk of spreading the infection.



### Situation in Thailand

Thailand is a **High TB burden country**, with approximately 103,000 cases (143 in 100,000 population) (2021)

### Problem

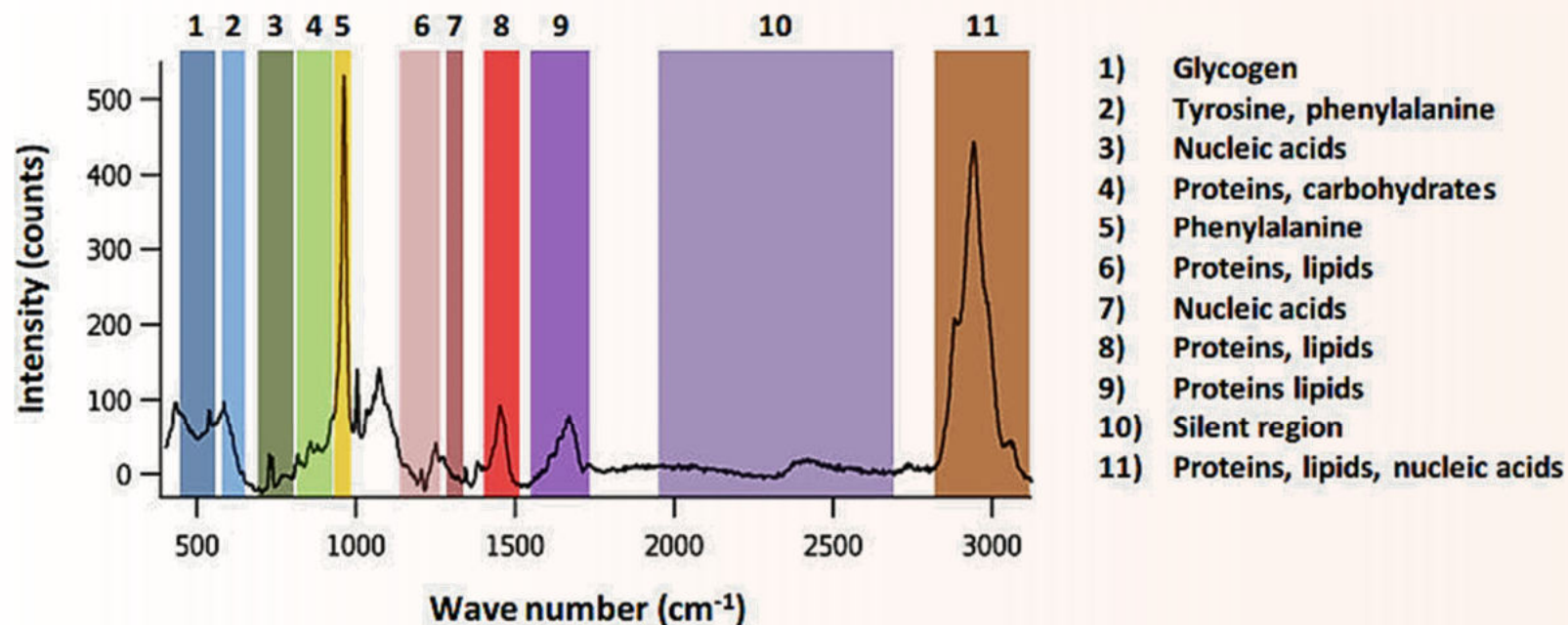
- 1/4 is a reservoir
- There is a gap that can't reach those 35% in the population.

## Raman Spectroscopy

- Raman is an analytical technique that uses the vibration patterns of light to provide unique "fingerprints" for molecular identification.
- This method is **non-destructive** and exhibits **high sensitivity**.



The figure represents the different types of Raman spectroscopy



Example of a Raman spectrum of a biological sample, highlighting characteristic Raman bands and the related molecular assignment (Conforti et al., 2024)

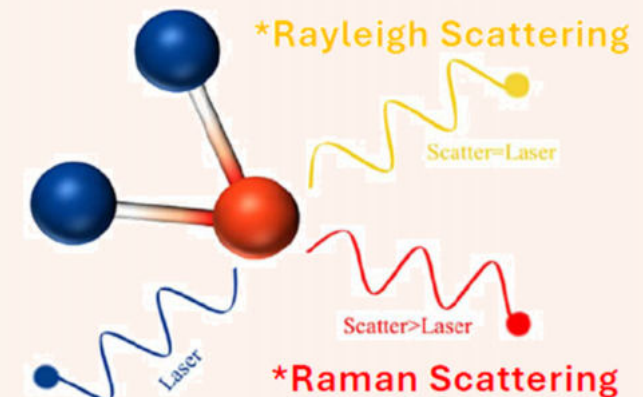


Figure represents a schematic diagram of the simple principles of the Raman scattering effect.

## Surface-Enhanced Raman Spectroscopy (SERS)

- SERS is a *technique* that can **amplify** Raman spectra, allowing for greater sensitivity in detecting low-concentration analytes.
- Can be enhanced up to  $10^{10}$  -  $10^{14}$  and can detect even a single molecule theoretically.

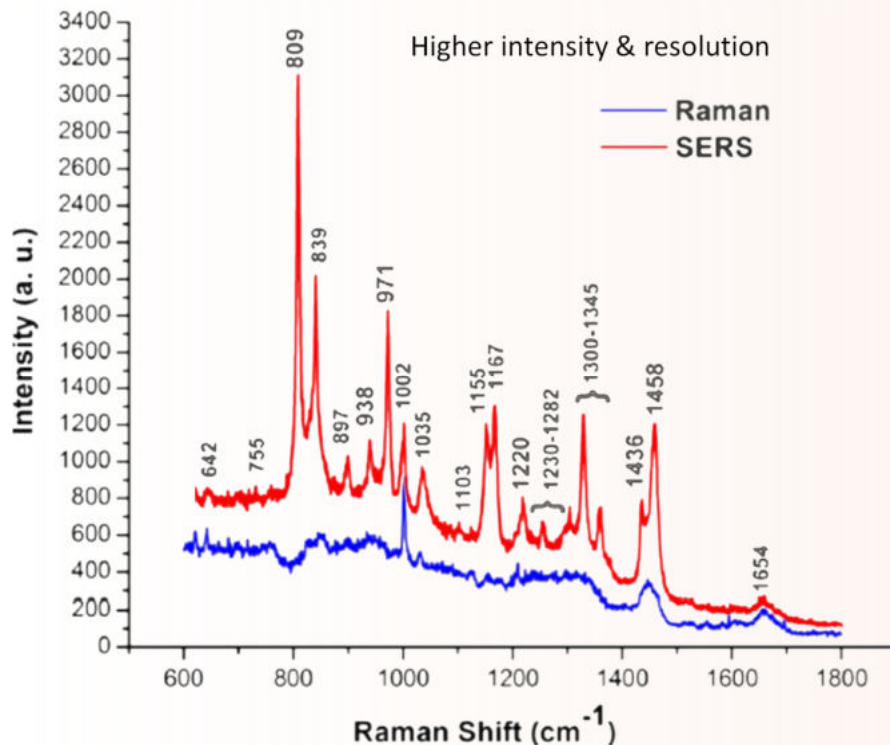


Figure represents different between SERS and Raman spectra

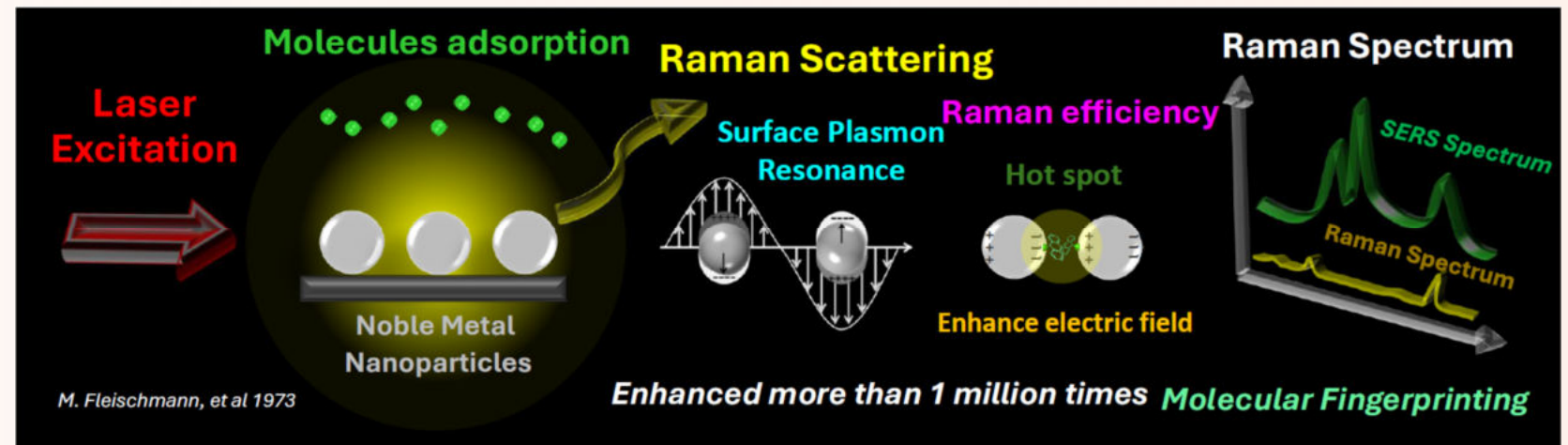
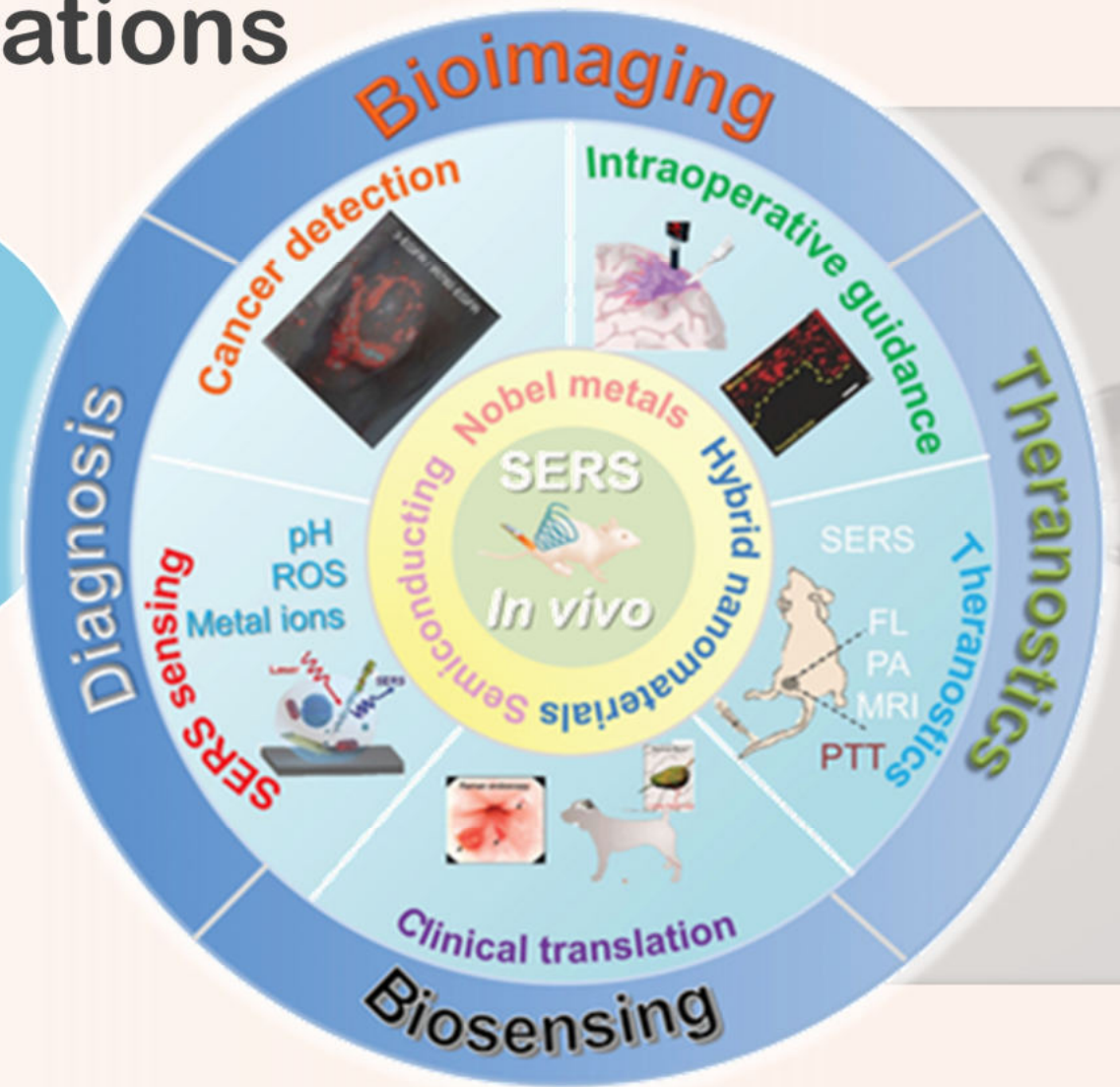
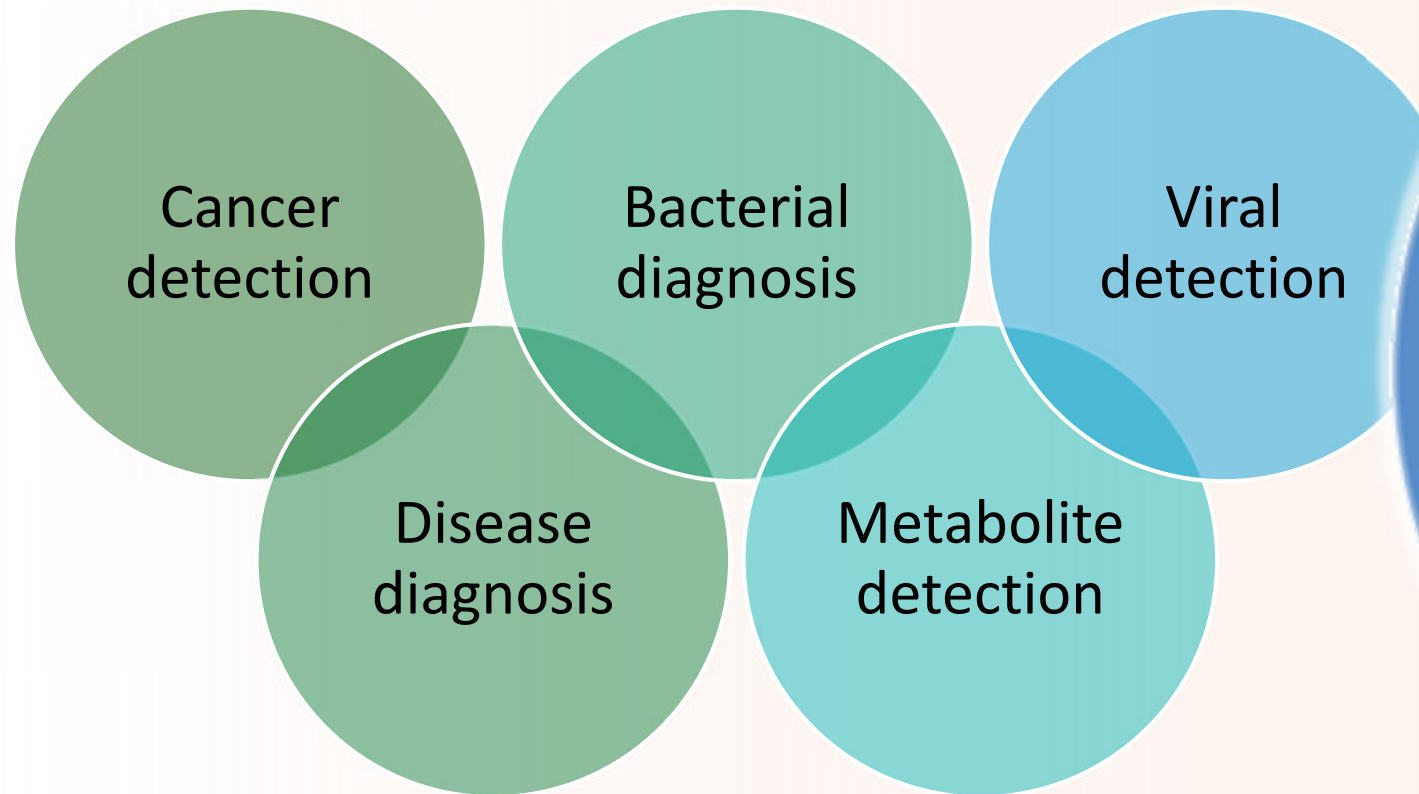


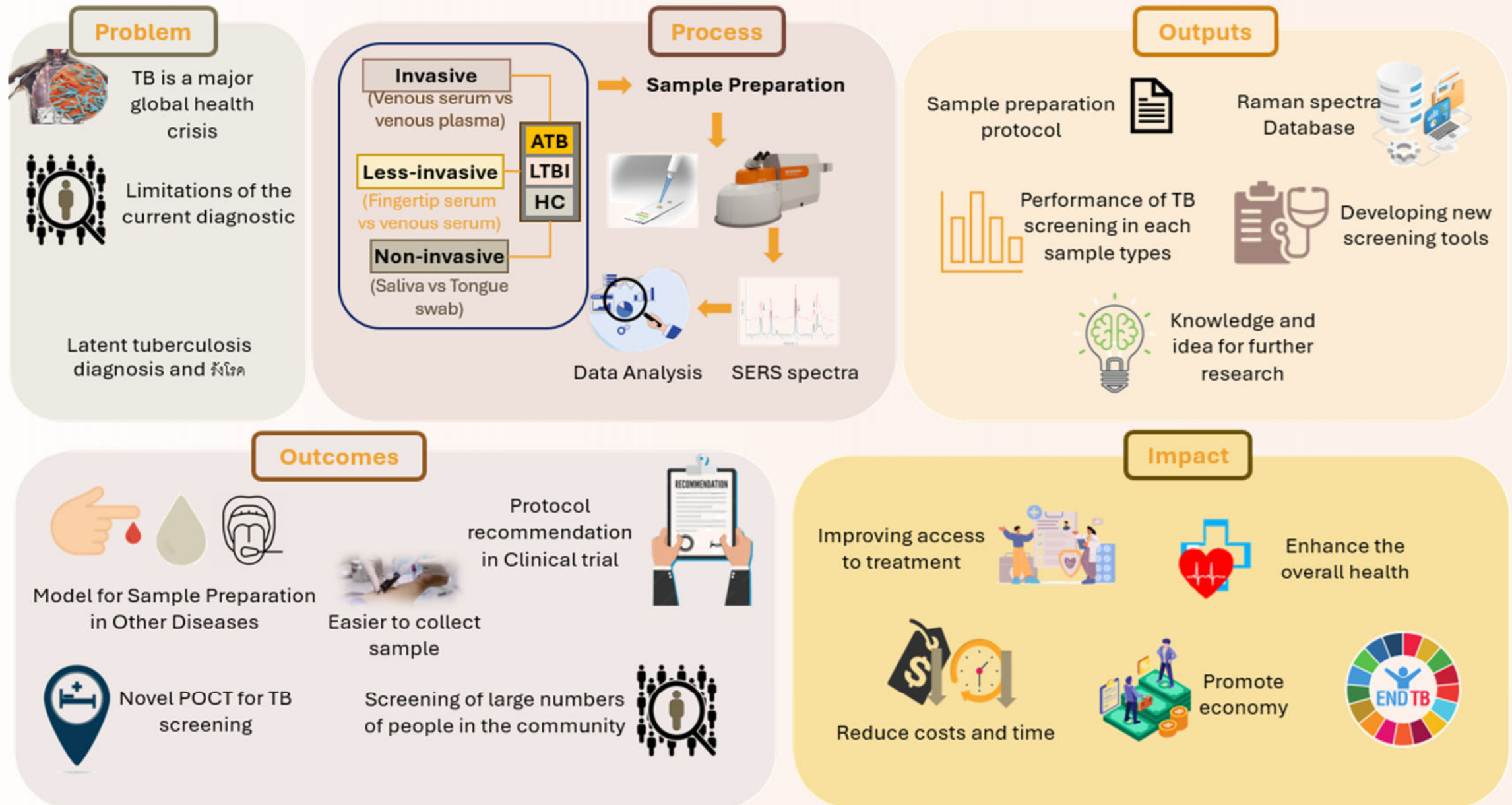
Figure represent mechanism for enhanced Raman signals from SERS (Nuntawong N., 2024)



# SERS in Biomedical Applications



# Conceptual Framework





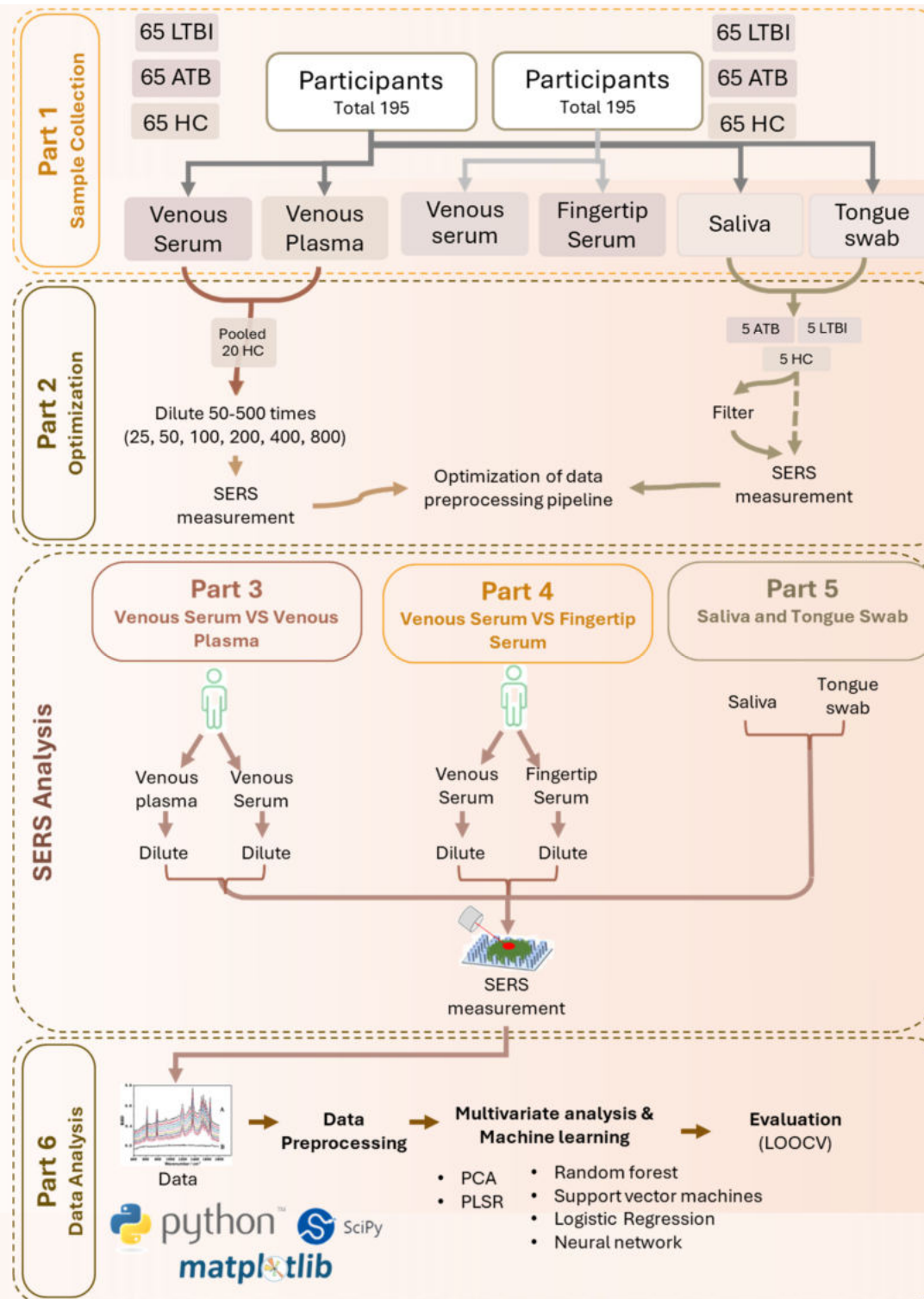
# Objectives

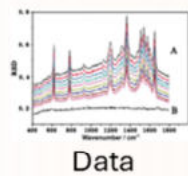
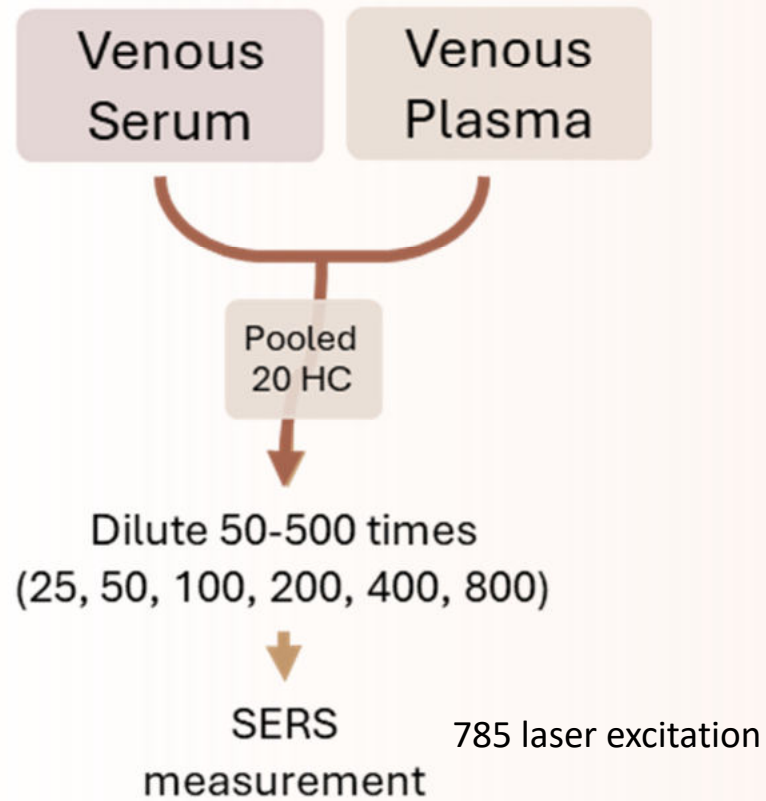
To establish the optimal venous blood matrix by comparing the diagnostic performance of **serum and plasma** with SERS and machine learning to distinguish between TB groups.

To evaluate the feasibility of point-of-care translation by comparing **fingertip serum to venous serum** with SERS and machine learning to distinguish between TB groups.

To investigate the viability of screening by evaluating **saliva and tongue swab** samples with SERS and machine learning to distinguish between TB groups

# Study Design





Data  
Preprocessing

Multivariate analysis

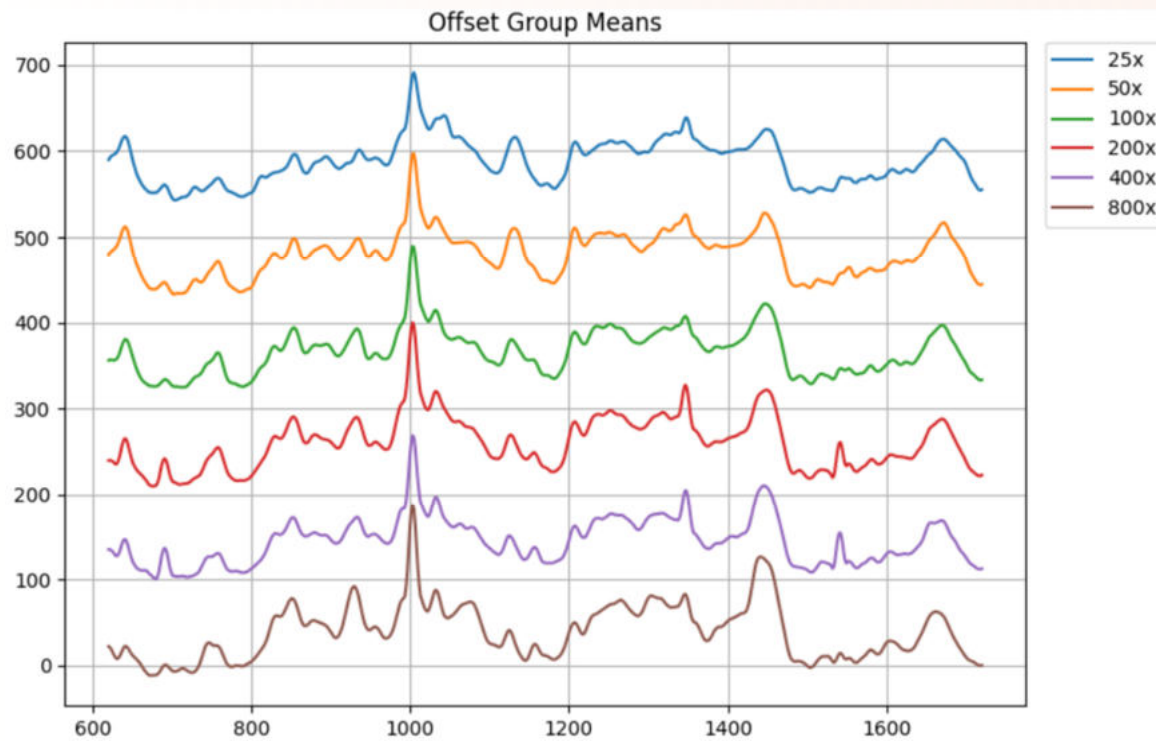
- PCA
- PLSR

Preprocessing Pipeline:

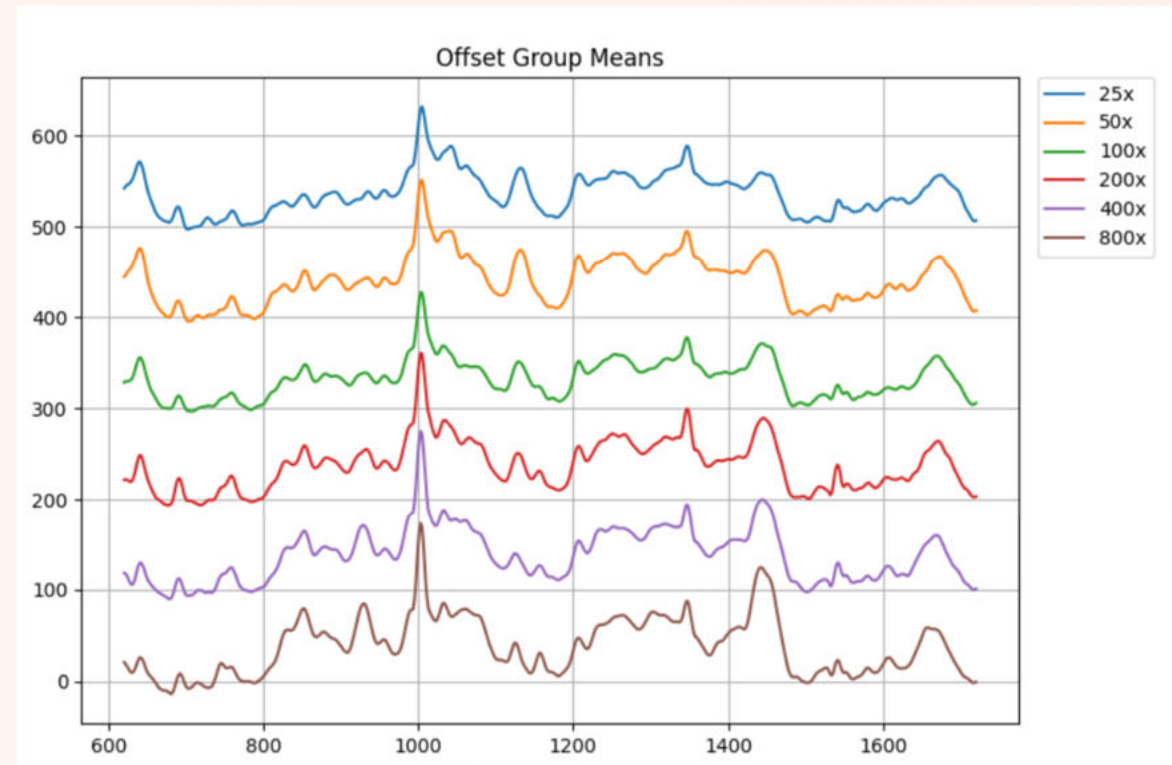
- Step 1: Spike Removal: Kernel: 5
- Step 2: Outlier Removal: SD Multiplier: 3.0
- Step 3: Smoothing: Window: 11, Polyorder: 2
- Step 4: Baseline Correction: Lambda: 100000, p: 0.01
- Step 5: Crop Spectrum: Start: 620, End: 1720



## SERS spectra

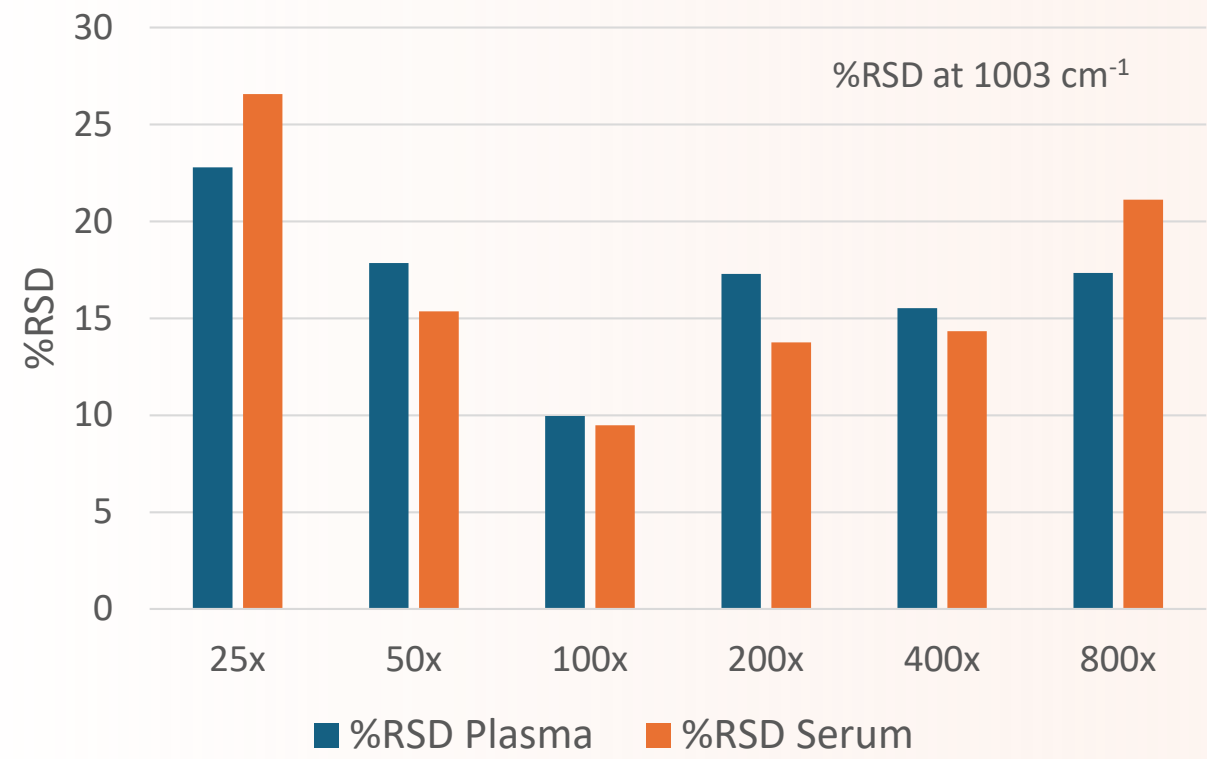


Serum SERS spectra (preprocessed)



Plasma SERS spectra (preprocessed)

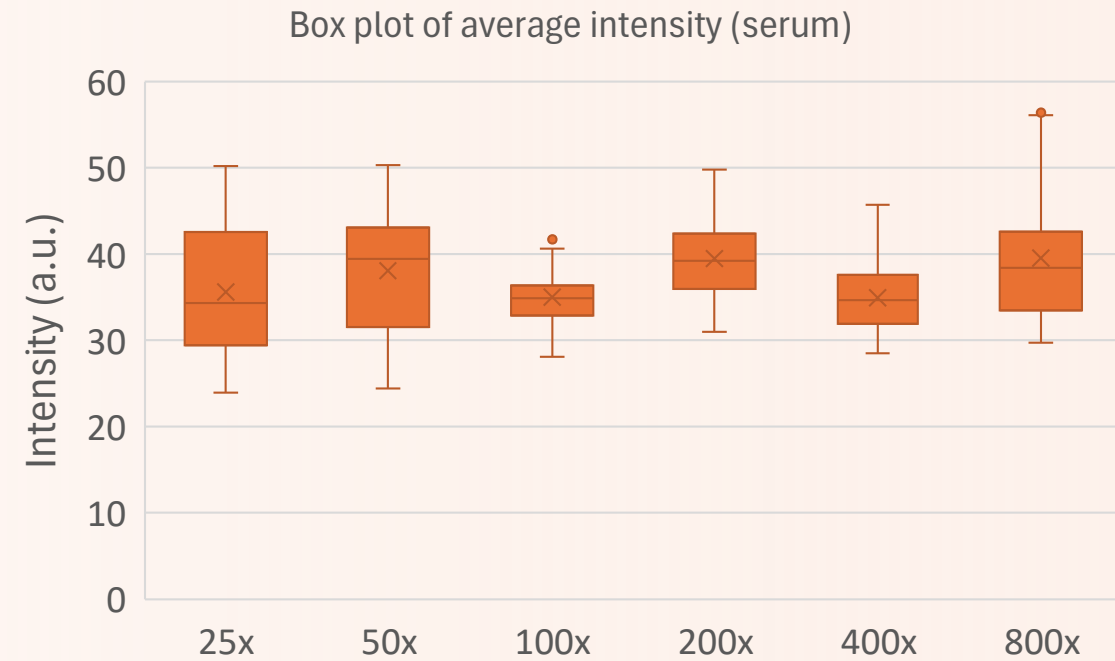
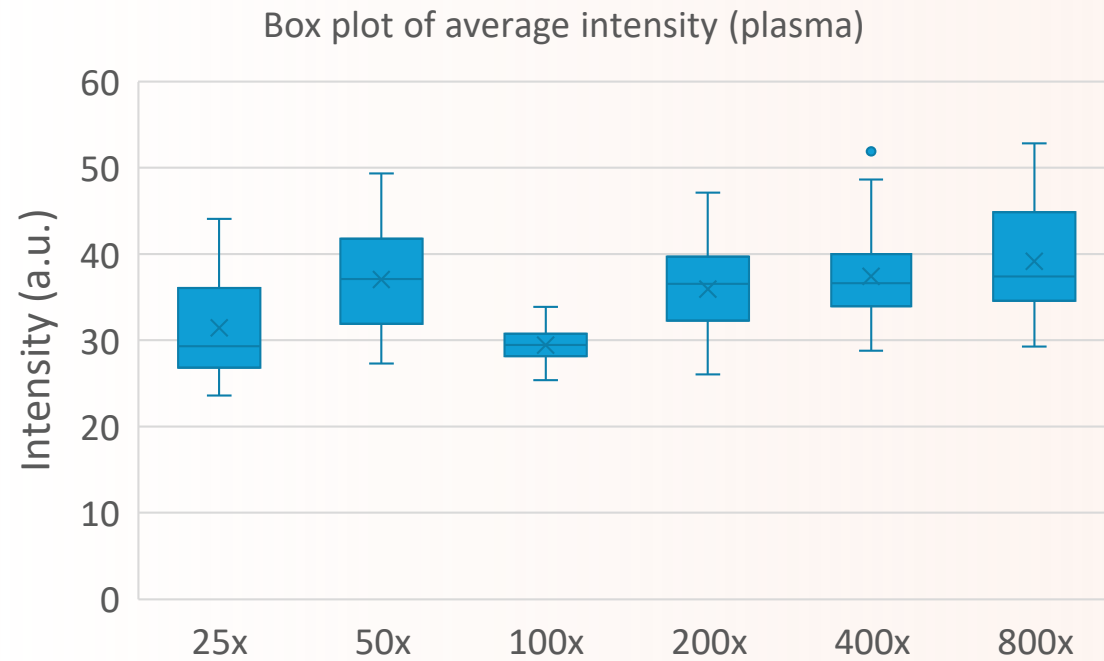
- SERS spectra of serum and plasma is similar in appearance
- At  $1003\text{ cm}^{-1}$  is where peak is spiking the highest



%RSD is calculated based on average intensity at 1003 cm<sup>-1</sup>

The graph show that at concentration of 100, both plasma and serum achieve the lowest %RSD compared to other dilution.

A

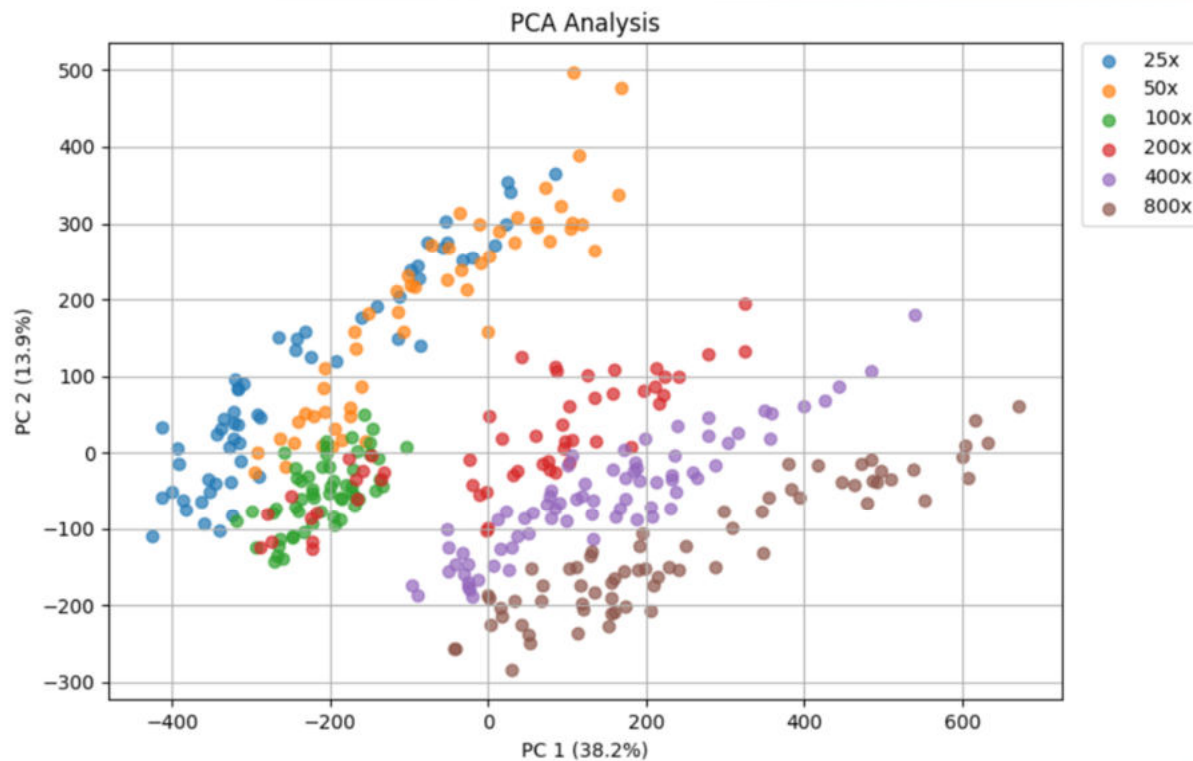


The intensity of all wavelength are average and plot into box plot.

Both graph show at the concentration of 100 show the lowest Standard deviation, indicating the reproducibility of SERS spectra.

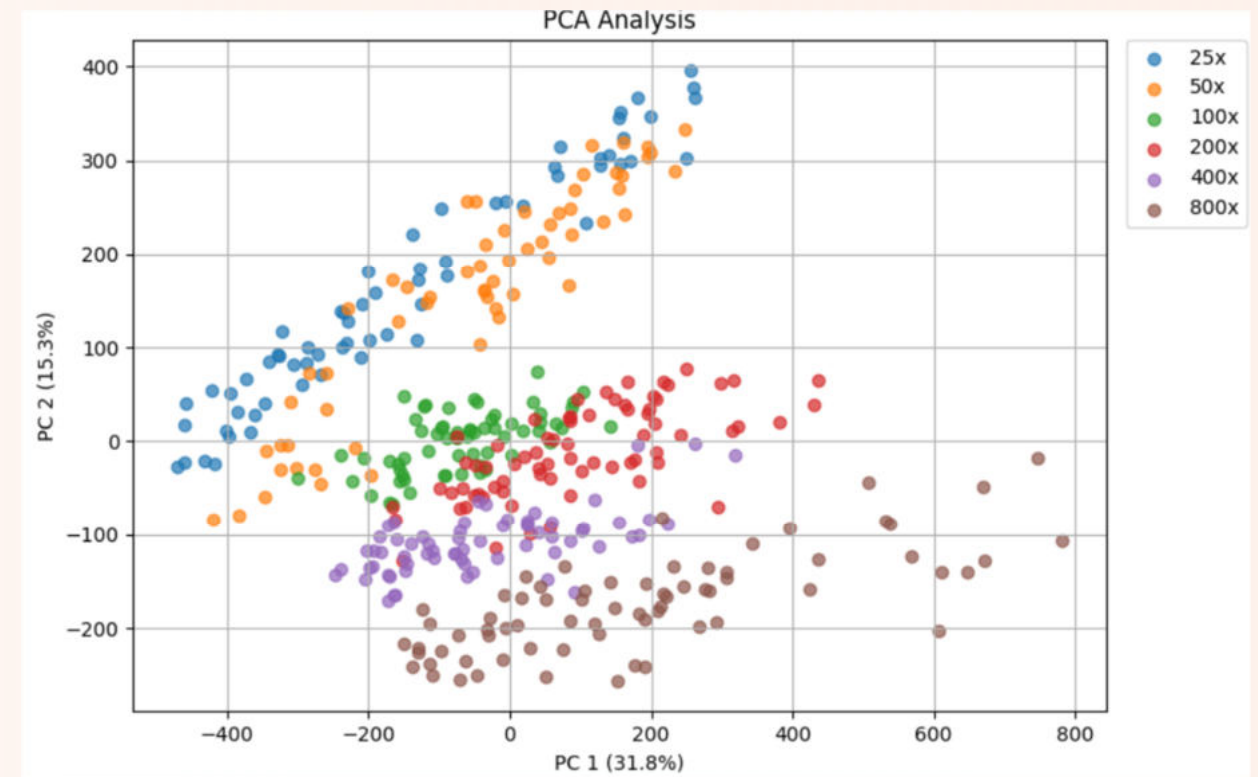


## PCA Analysis



Plasma PCA (preprocessed)

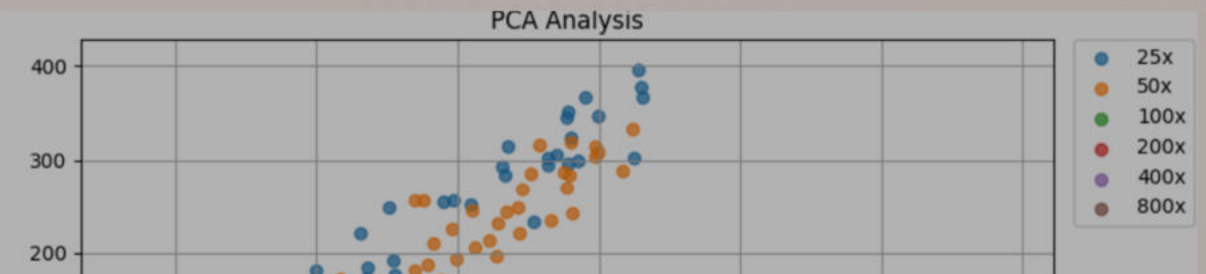
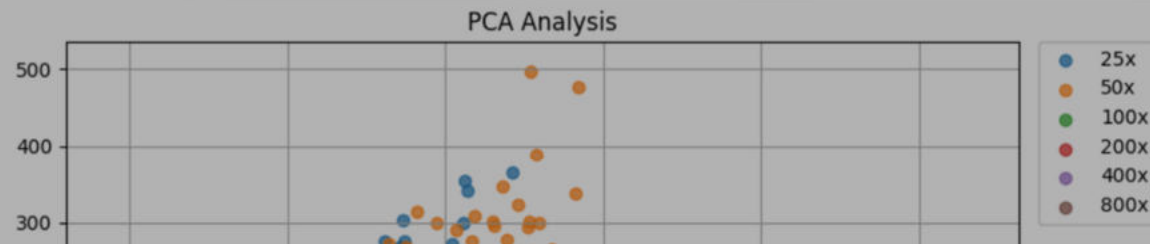
The result from PCA plots also support that at dilution factor 100 are the most tightly packed together.



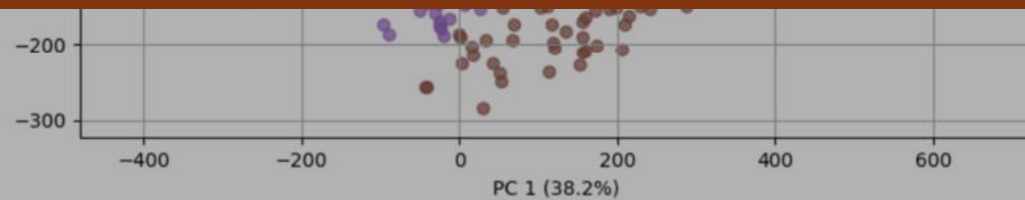
Serum PCA (preprocessed)

Clustering = Highly reproducible and consistency of spectrum

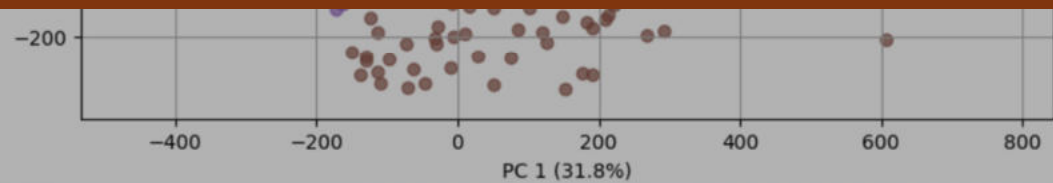
## PCA Analysis



The suitable concentration for **venous serum and plasma** for SERS analysis is dilution factor of 100



Plasma PCA (preprocessed)



Serum PCA (preprocessed)

The result from PCA plots also support that at dilution factor 100 are the most tightly packed together.

Clustering = Highly reproducible and consistency of spectrum

# Thesis Plan

- Done
- Pending
- Further

Activities	Time																
	2567		2568				2569				2570				2571		
	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	
Literature Review																	
Proposal Writing																	
Sample Collection																	
Qualified Examination																	
Comparison between Serum and Plasma																	
Optimization of blood sample																	
Optimization of preprocessing steps																	
Dissertation Proposal Examination																	
Sample preparation and SERS analysis																	
Data analysis																	
Manuscript 1 writing																	
Comparison between Fingertip serum and Vein serum																	
Sample preparation and SERS analysis																	
Data analysis																	
Manuscript 2 writing																	
Comparison between saliva and tongue swabs																	
Optimization of saliva and tongue swab preparation																	
Optimization of preprocessing steps for Saliva and Tongue swabs																	
Sample preparation and SERS analysis																	
Data analysis																	
Manuscript 3 writing																	
Dissertation Defense Examination																	



# Acknowledgement



**Advisor**

**Prof. Dr. Kiatichai Faksri**





**Thank you**  
for your attention