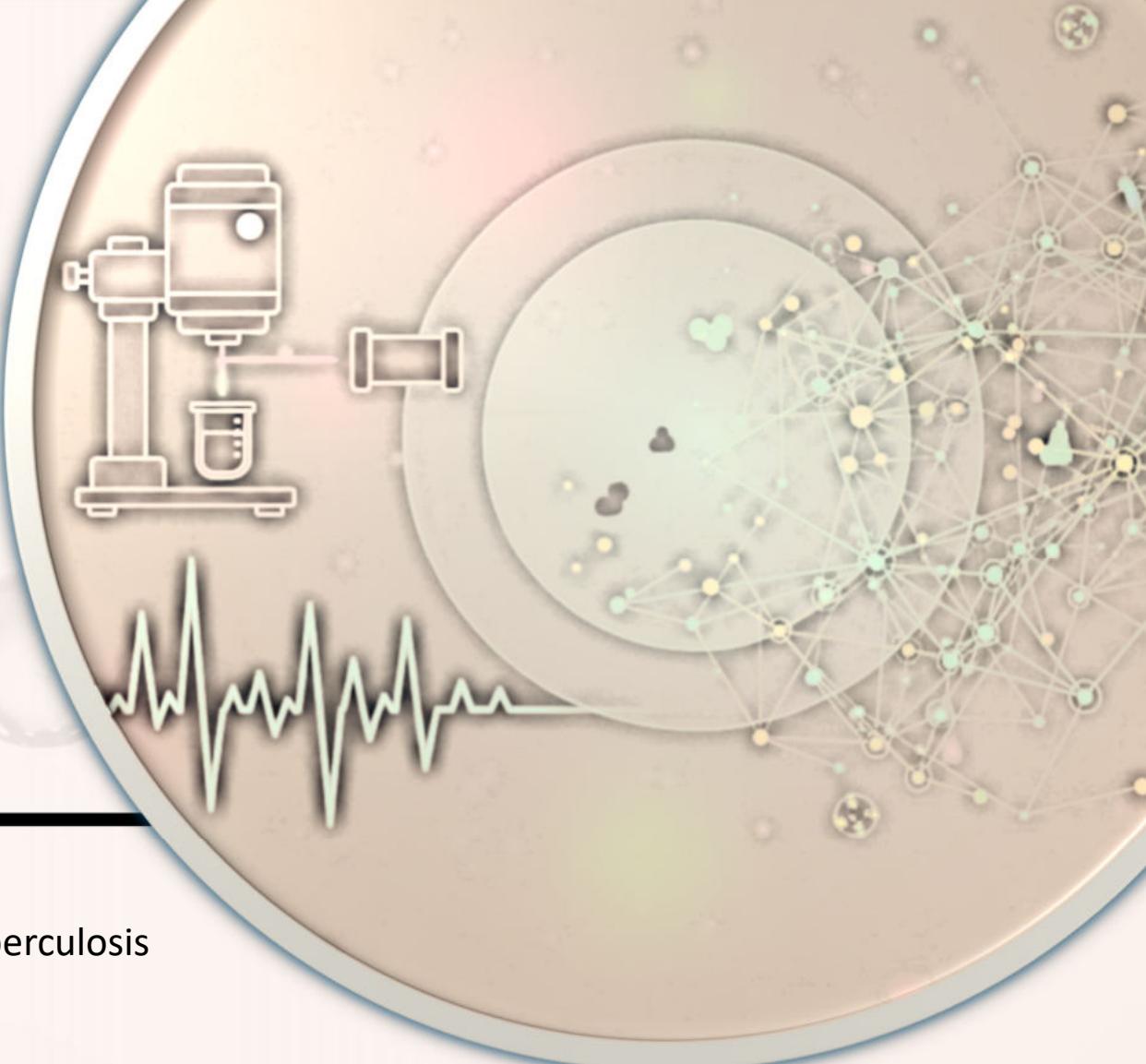


Thesis Progression Title

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

Thesis Title

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



Mr. Patipan Wongboothong

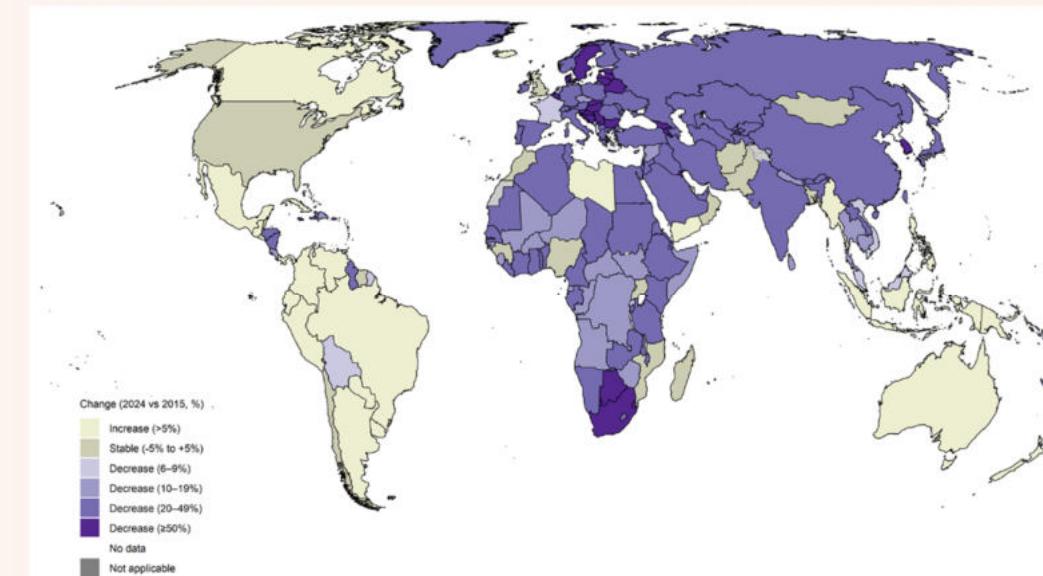
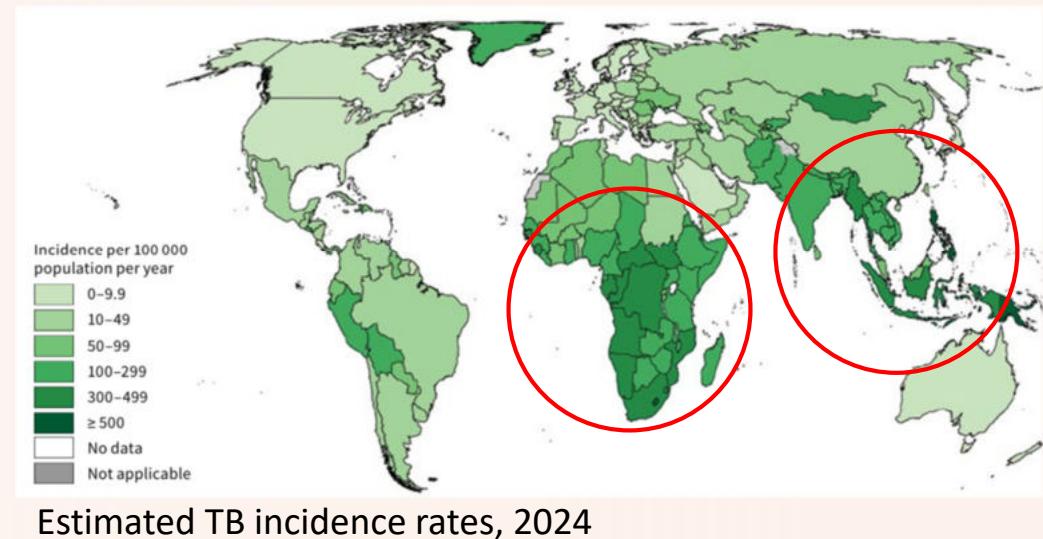
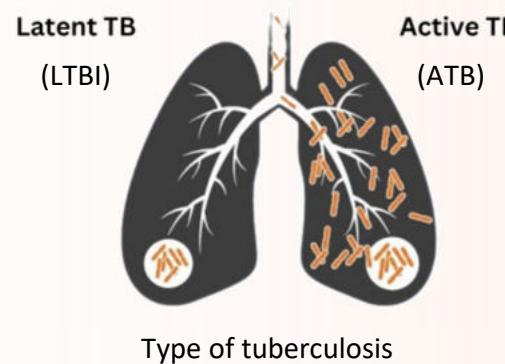
2nd Year Ph.D Student of Precision Medical Microbiology, Faculty of Medicine, KKU

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)



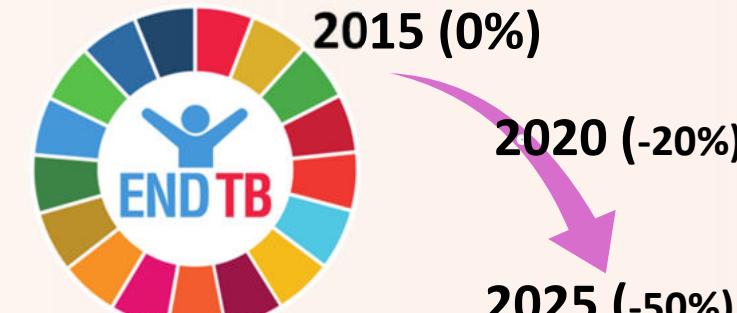
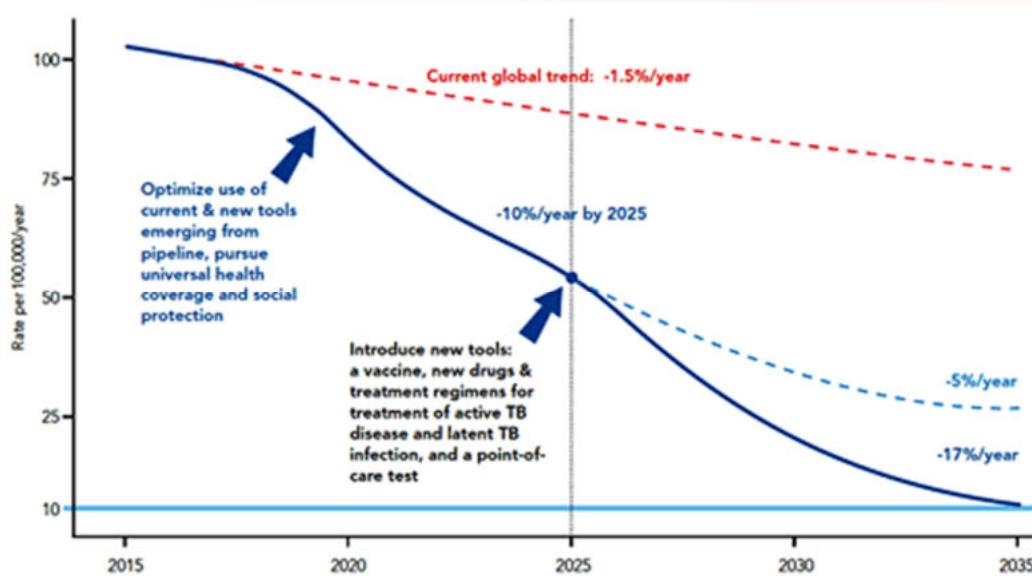
Introduction

Page 3

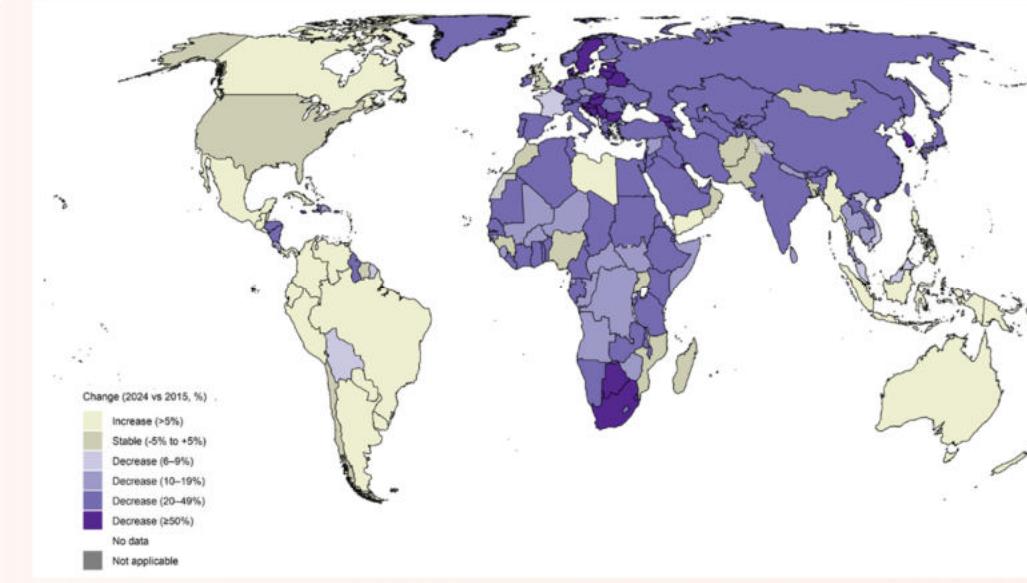
Tuberculosis



Estimated TB incidence rates, 2024



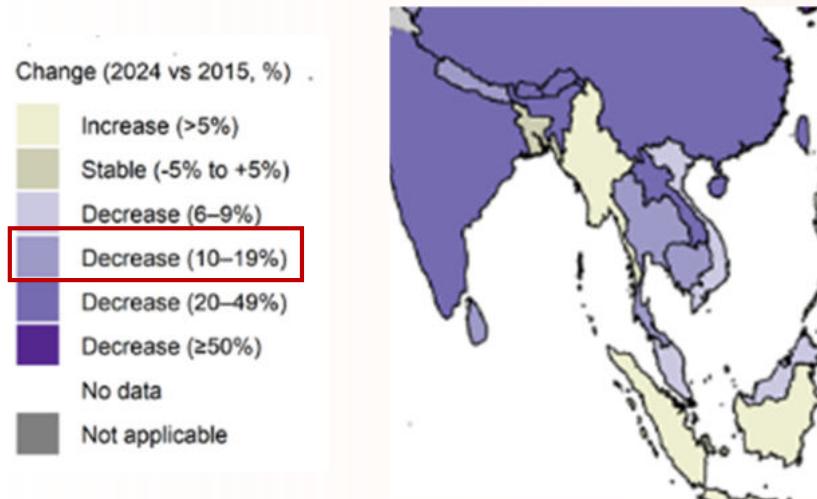
Goal of End TB strategy



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



Situation in Thailand

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

- 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.

Problem

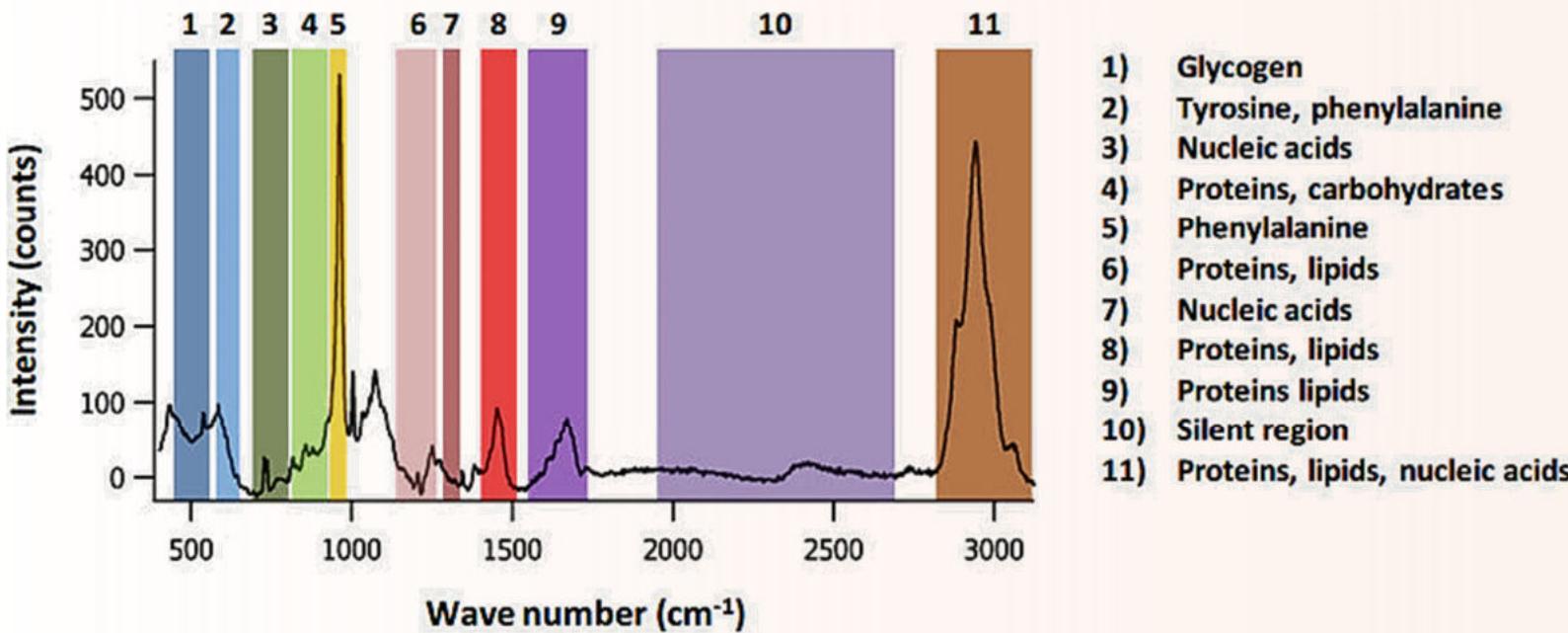
- 1/4 is a reservoir
- There is a gap that can't reach those 35% in the population.

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)

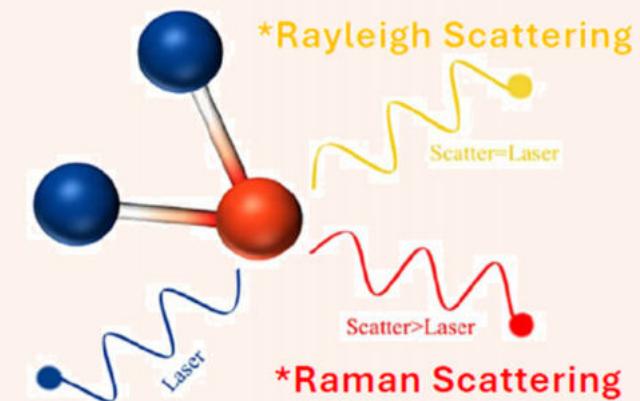
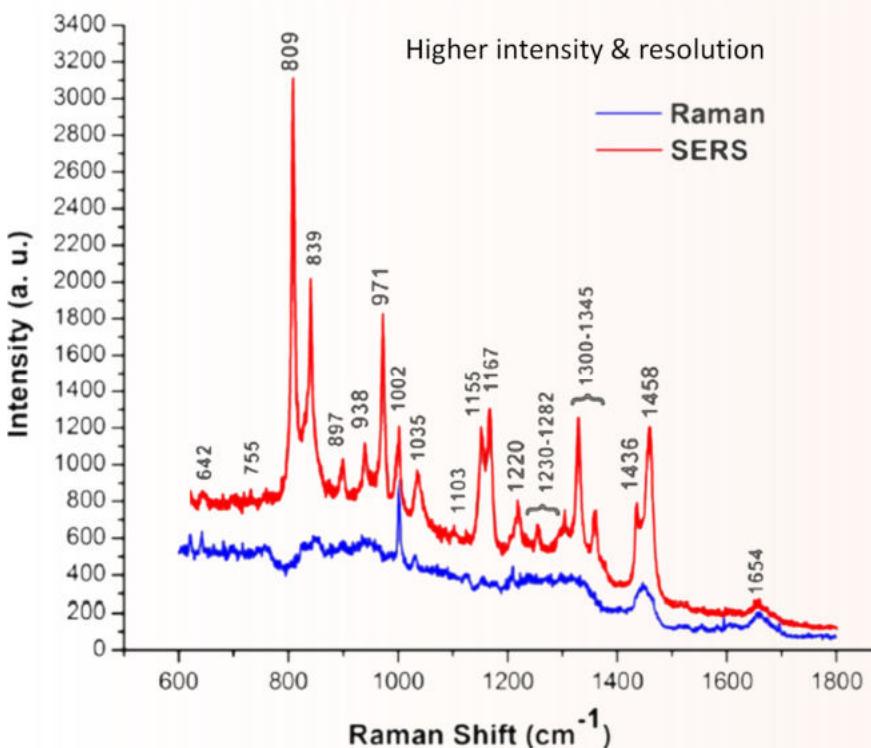
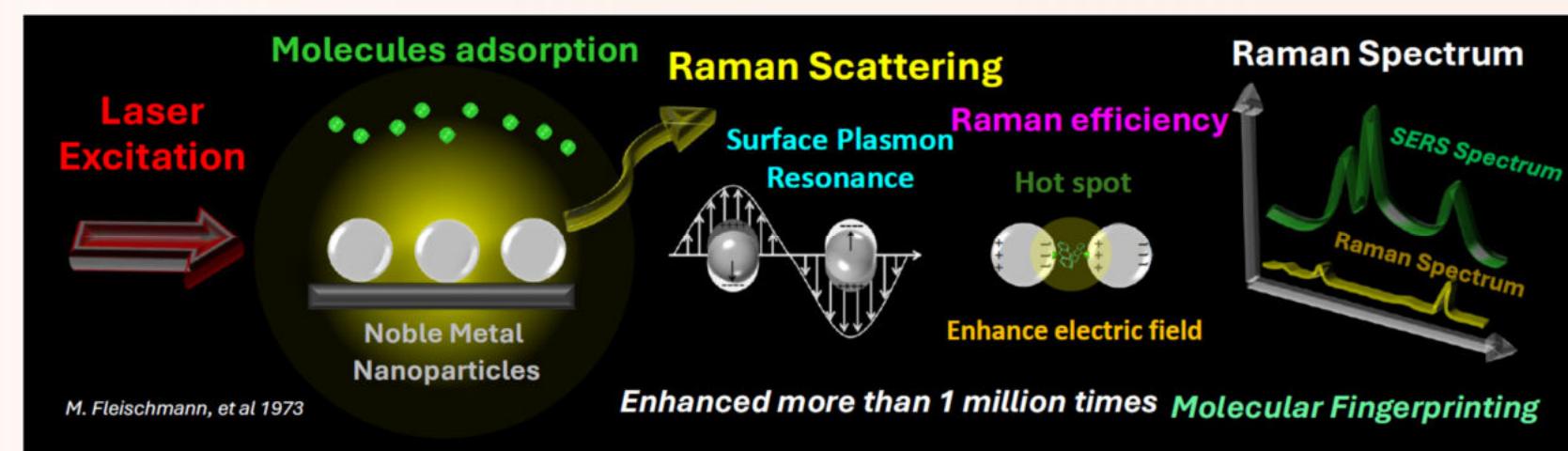


Figure represents a schematic diagram of the simple principles of the Raman scattering effect.

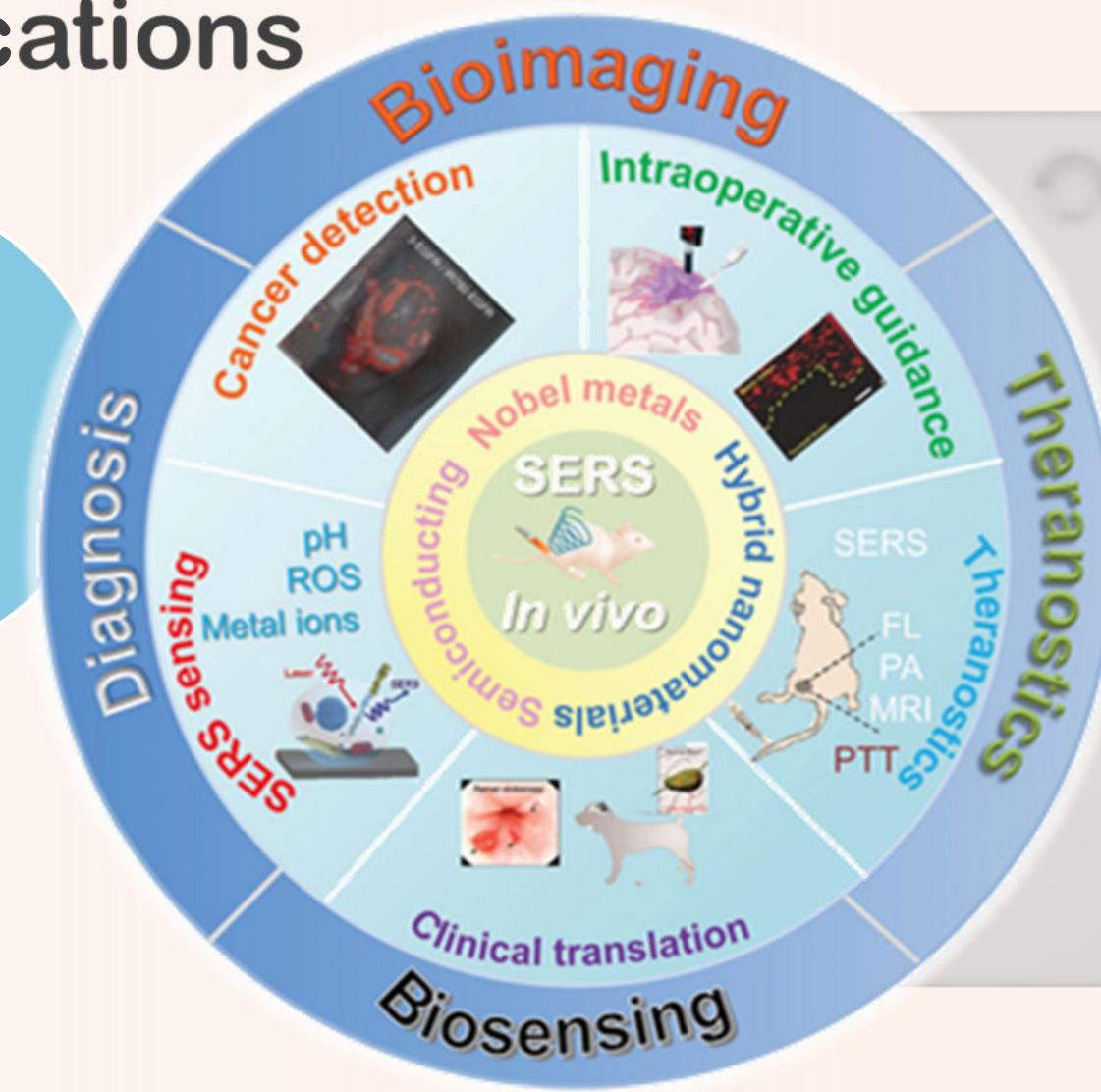
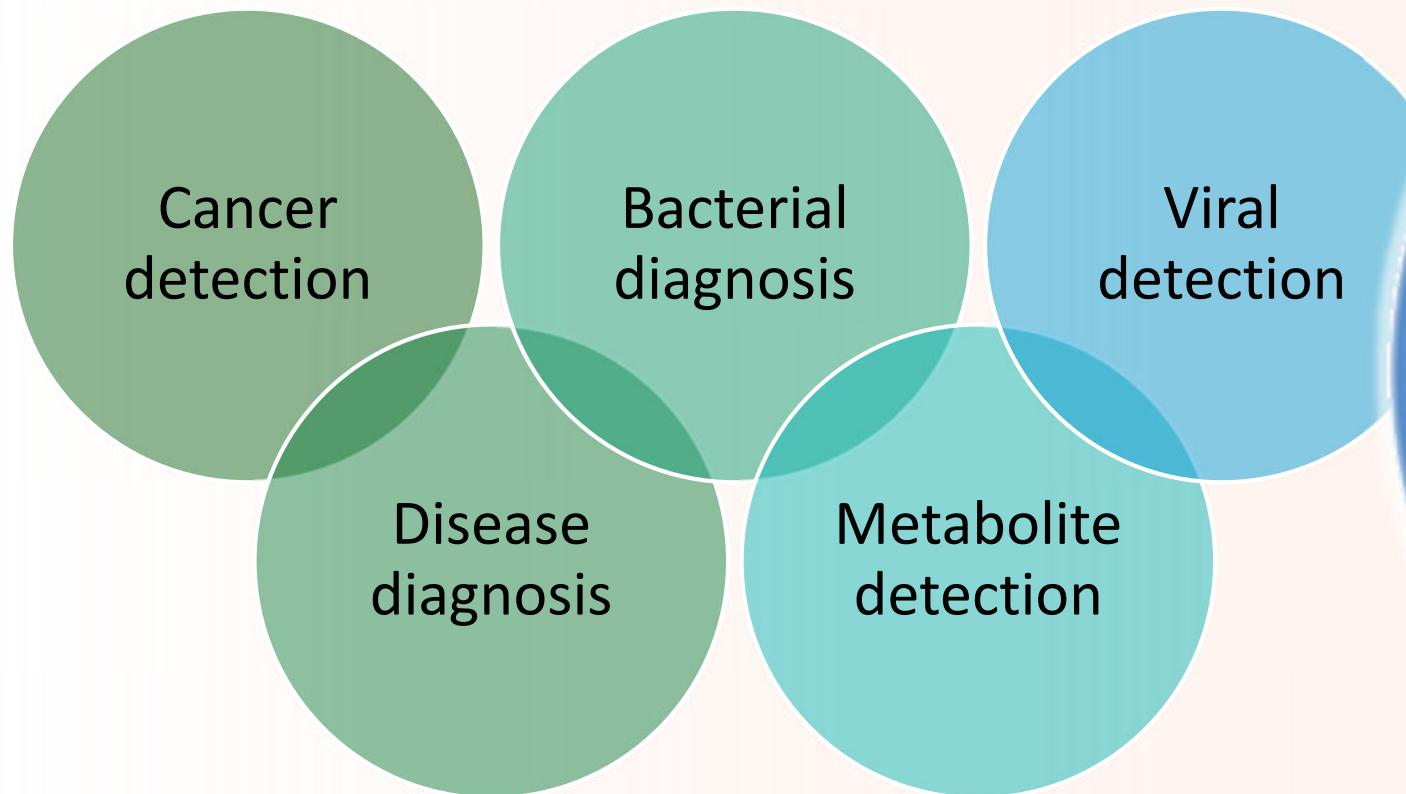
Surface-Enhanced Raman Spectroscopy (SERS)



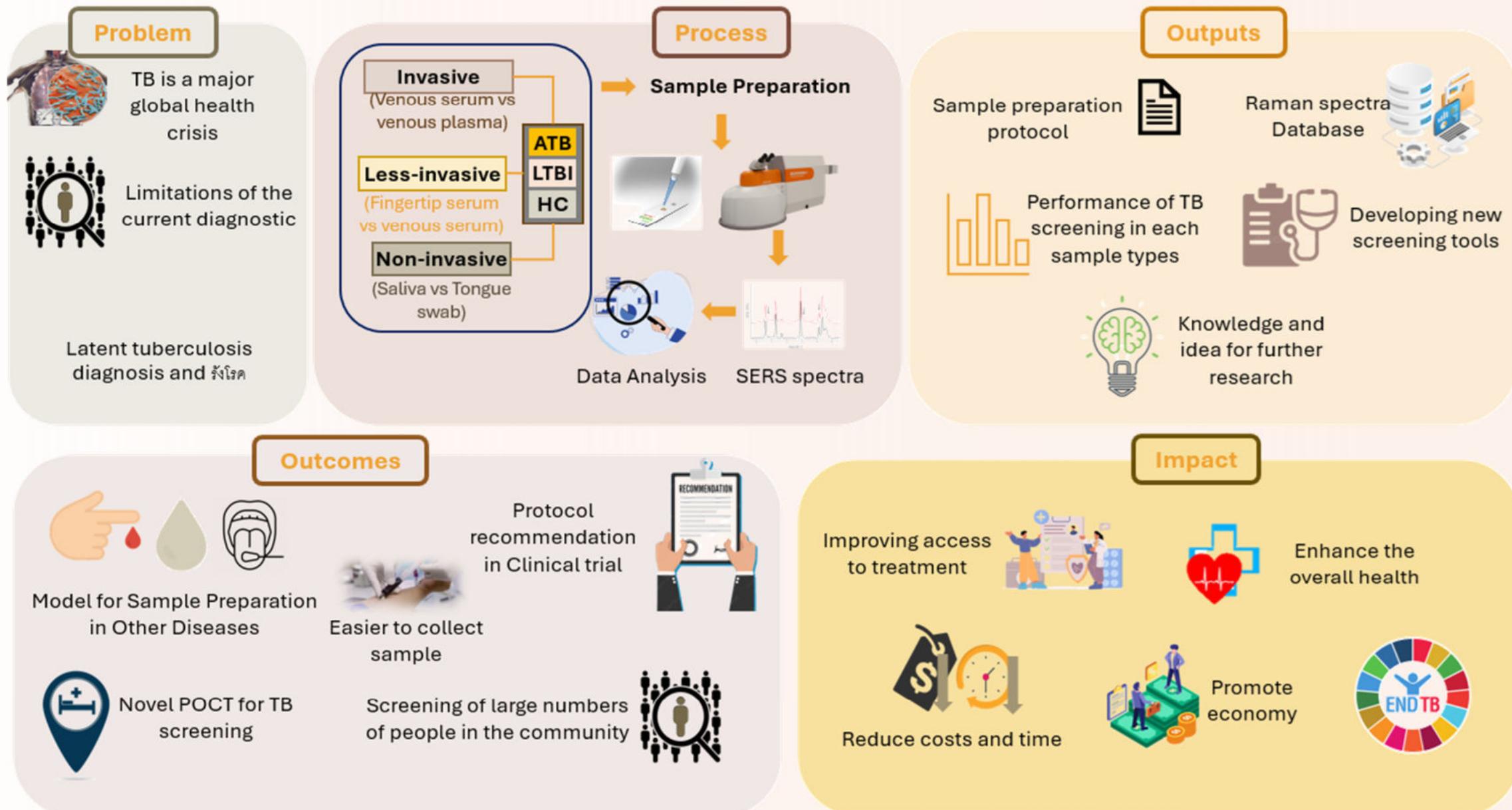
- SERS is a *technique* that can **amplify** Raman spectra, allowing for greater sensitivity in detecting low-concentration analytes.
- Can be enhanced up to $10^{10} - 10^{14}$ and can detect even a single molecule theoretically.



SERS in Biomedical Applications



Conceptual Framework



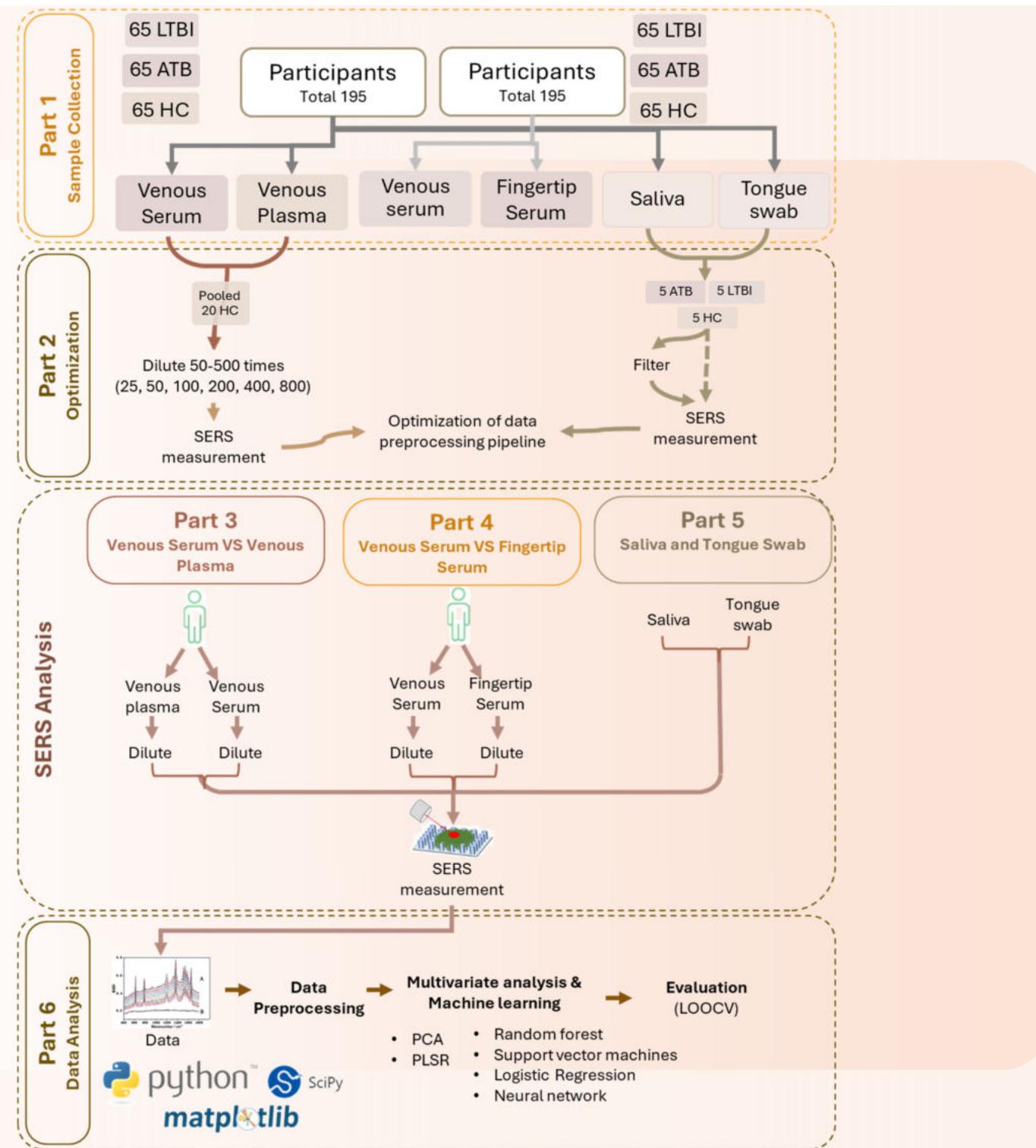
Objectives

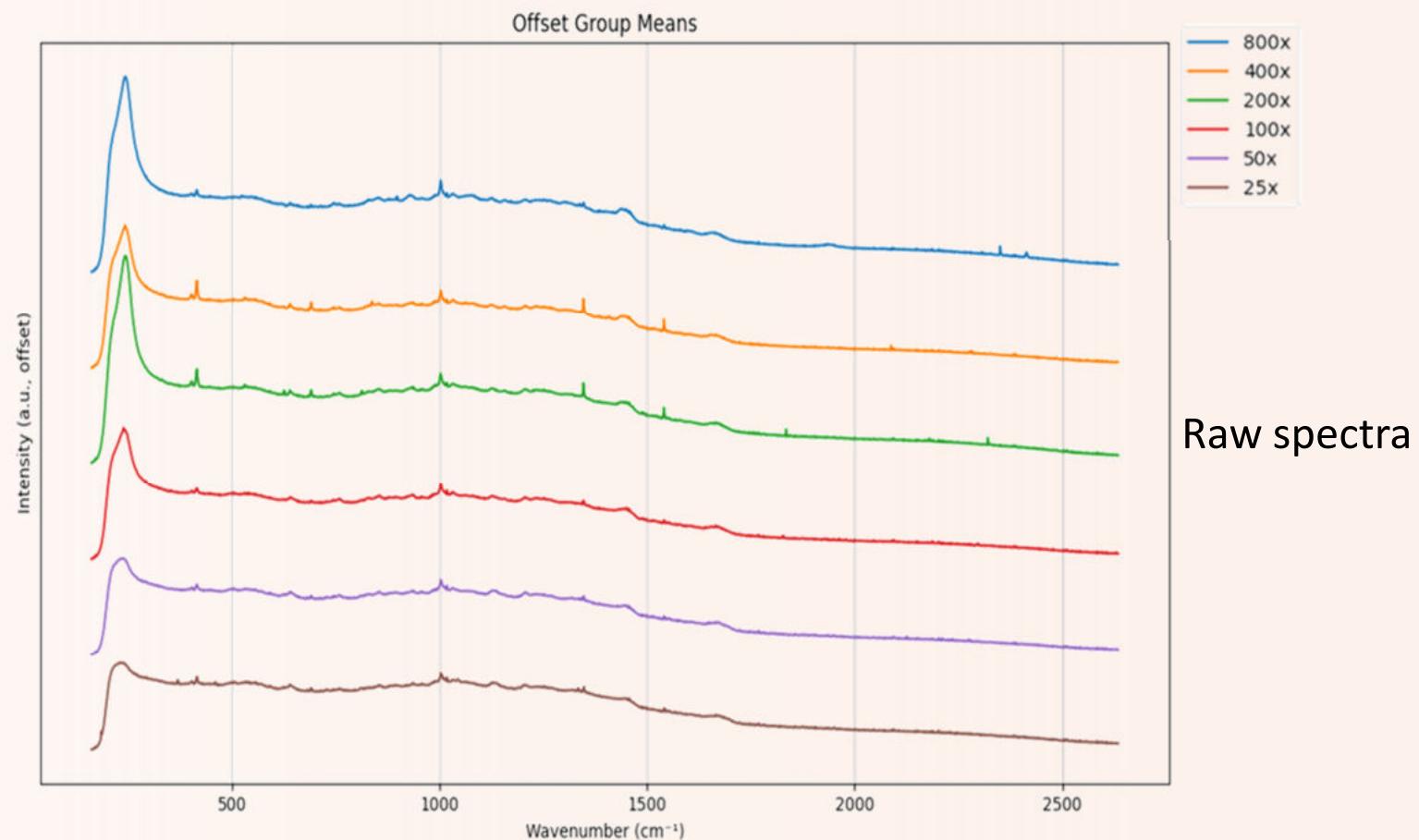
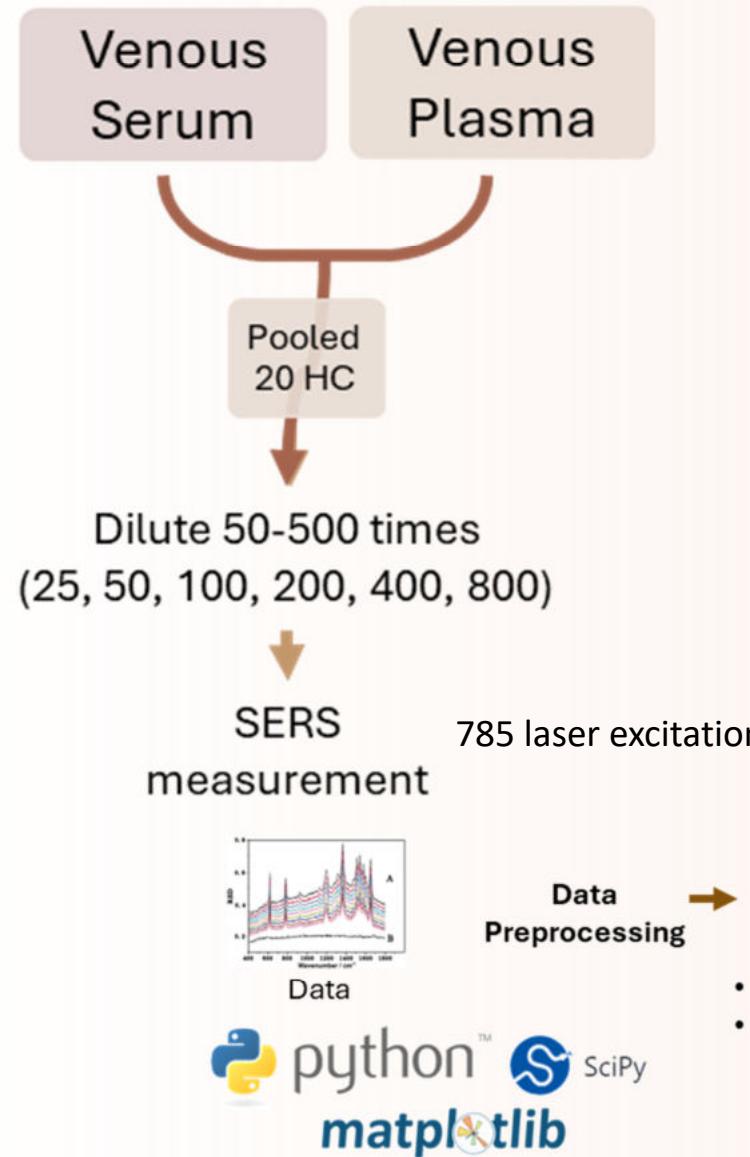
To establish the optimal venous blood matrix by comparing the diagnostic performance of **serum** and **plasma** with SERS and machine learning to distinguish between TB groups.

To investigate the viability of screening by evaluating **saliva** and **tongue swab** samples 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.

Study Design





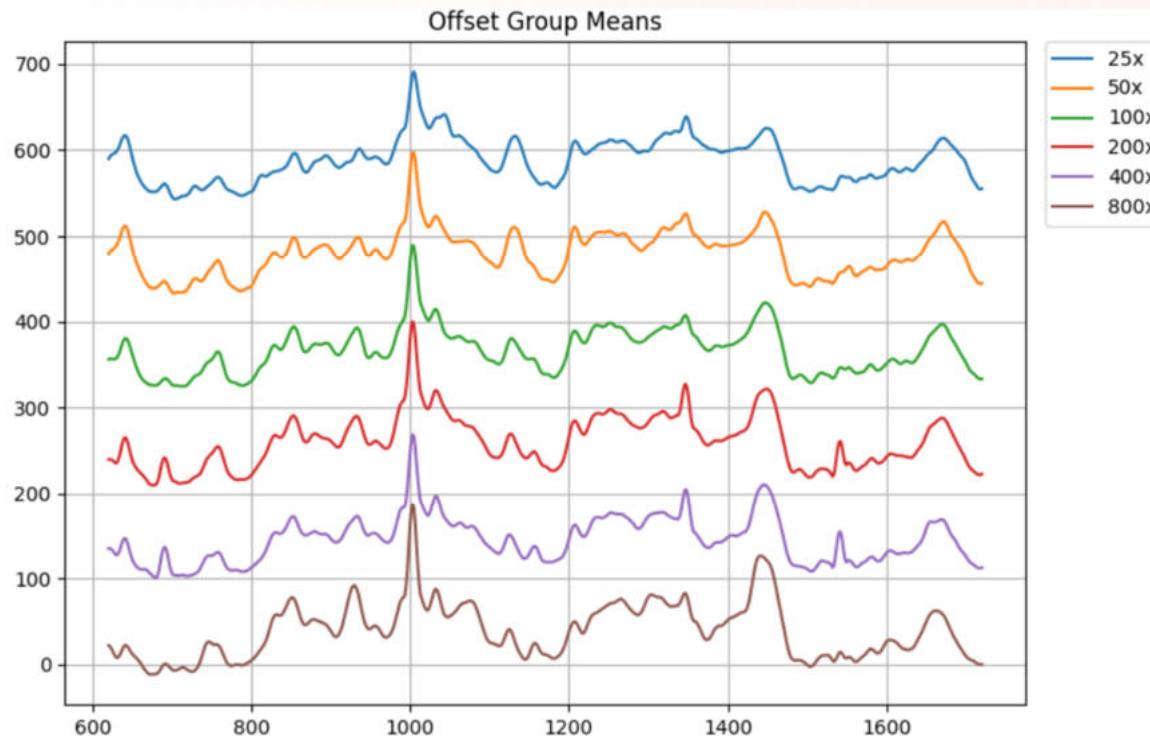
Data Preprocessing → **Multivariate analysis**

- PCA
- PLSR

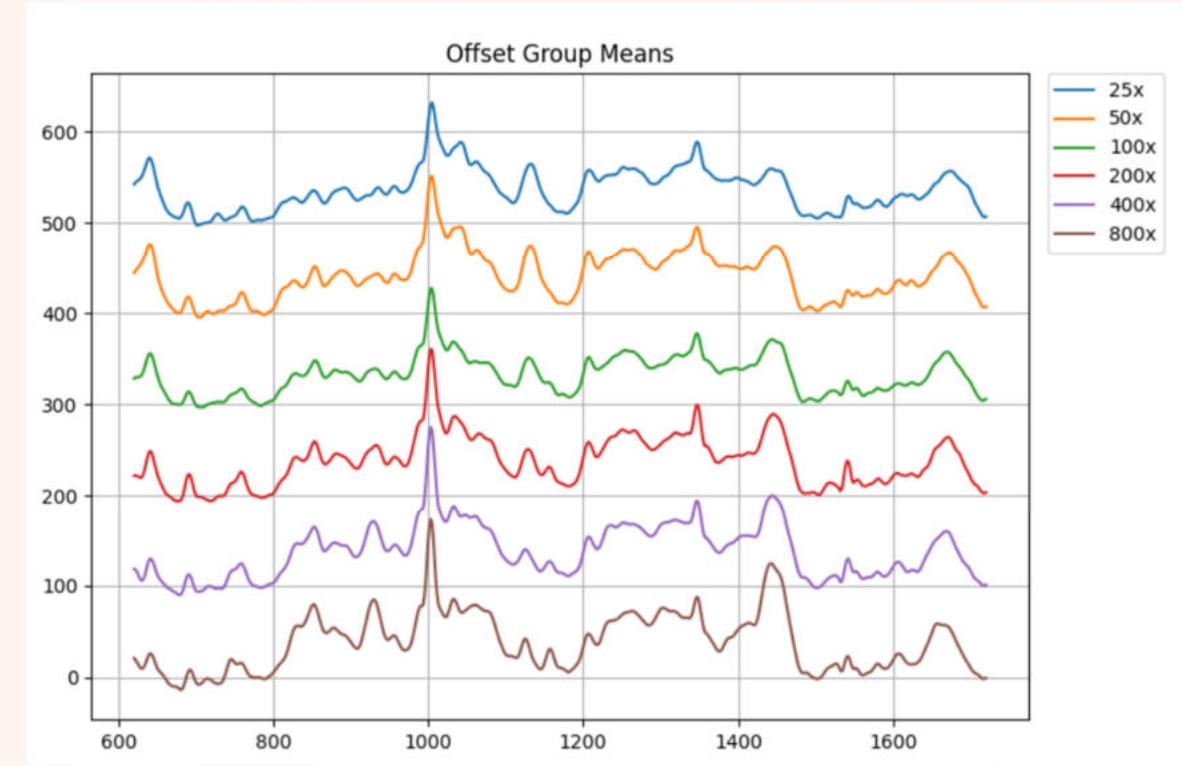
Preprocessing Pipeline:

- 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

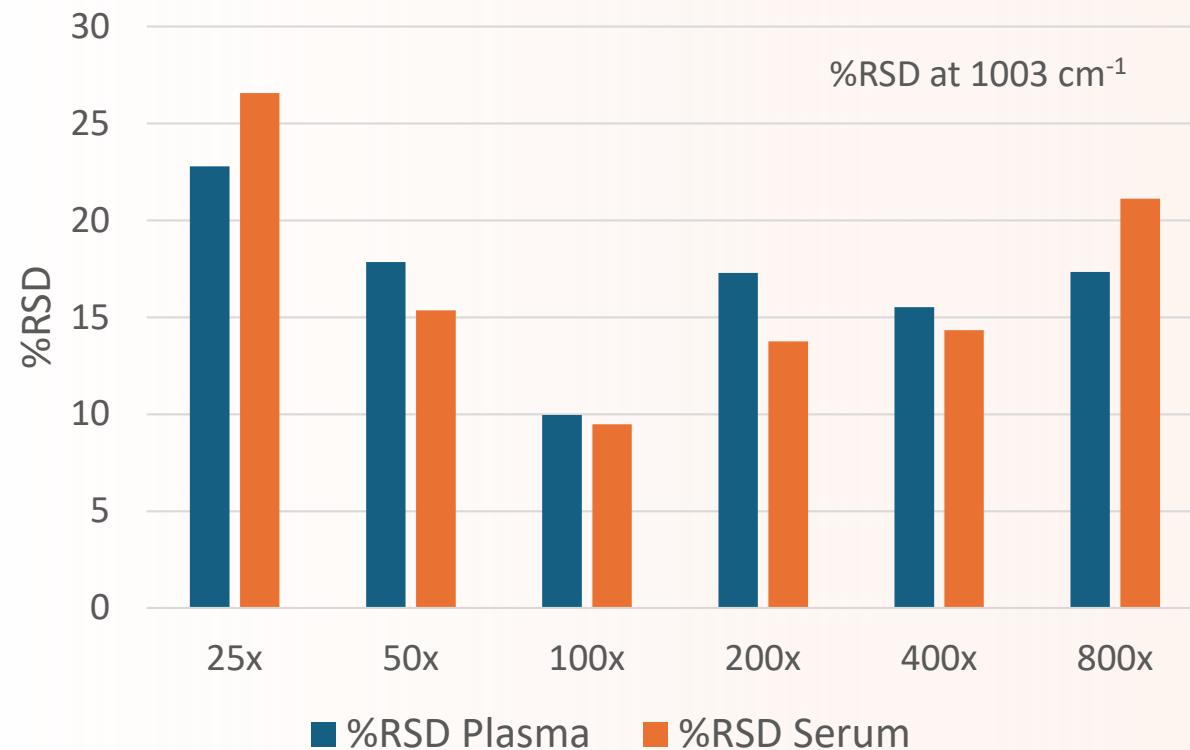


Serum SERS spectra (preprocessed)



Plasma SERS spectra (preprocessed)

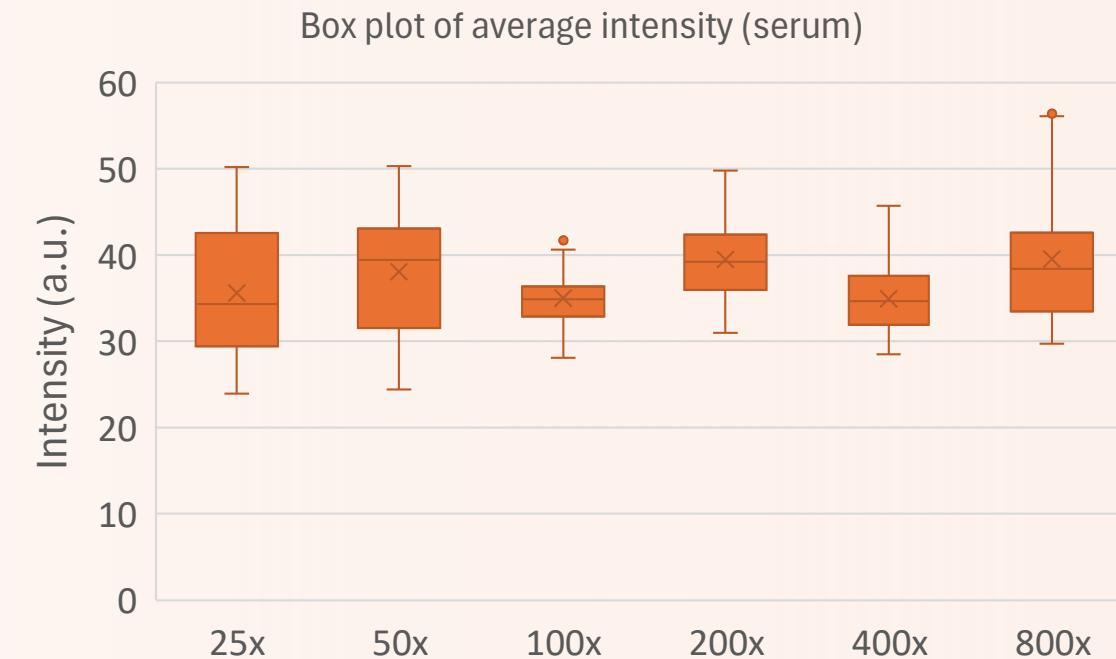
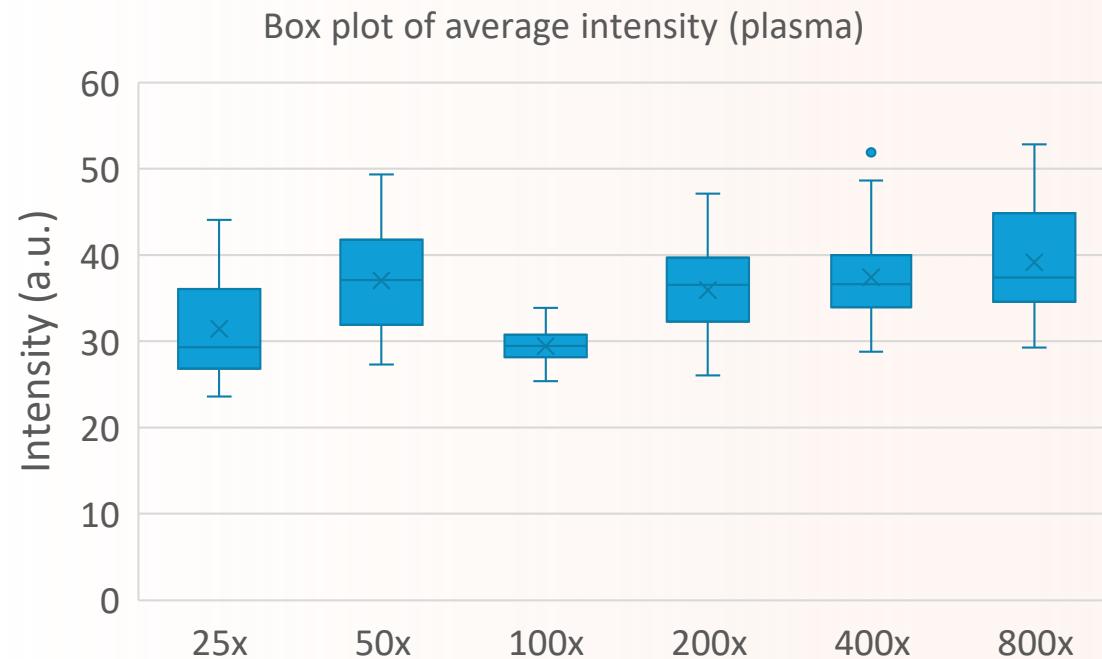
- SERS spectra of serum and plasma is similar in appearance
- At 1003 cm^{-1} is where peak is spiking the highest



%RSD is calculated based on average intensity at 1003 cm^{-1}

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

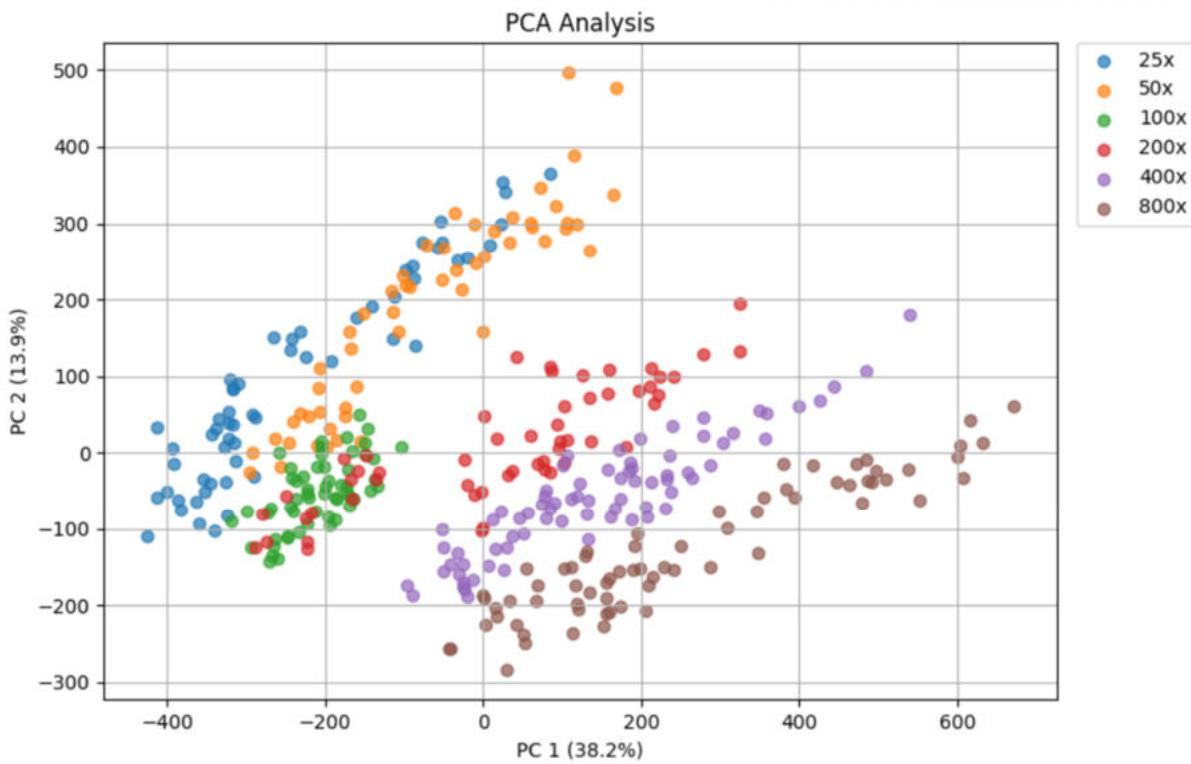
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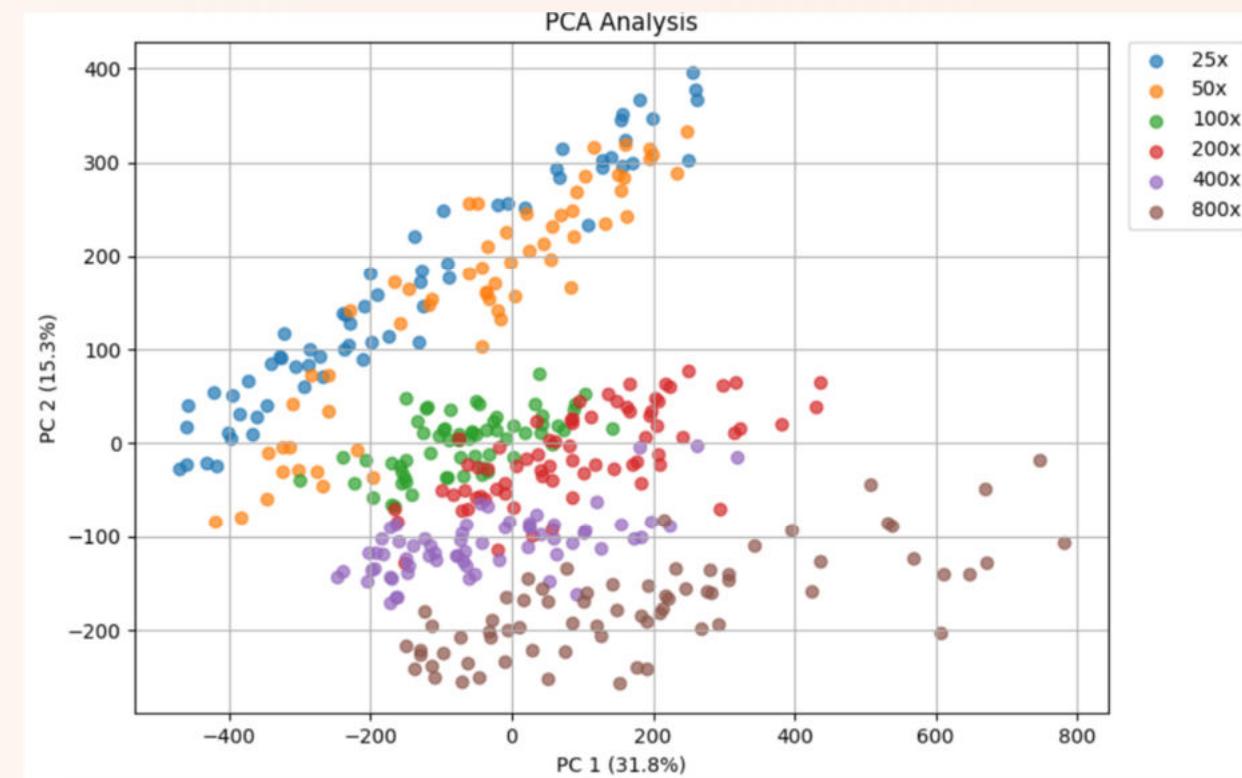
The intensity of all wavelength are average and plot into box plot.

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

PCA Analysis



Plasma PCA (preprocessed)

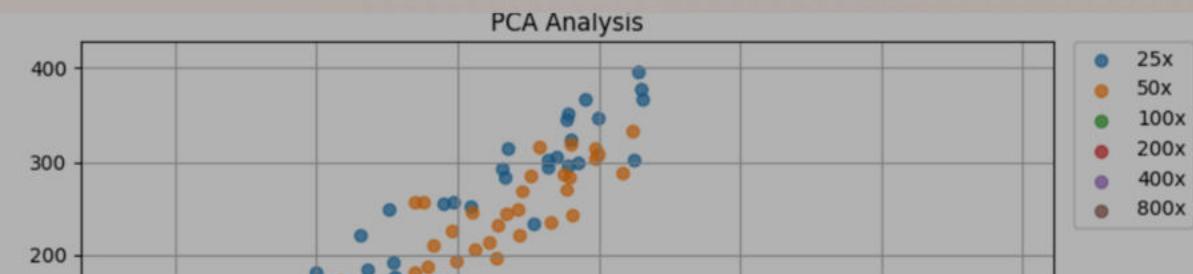
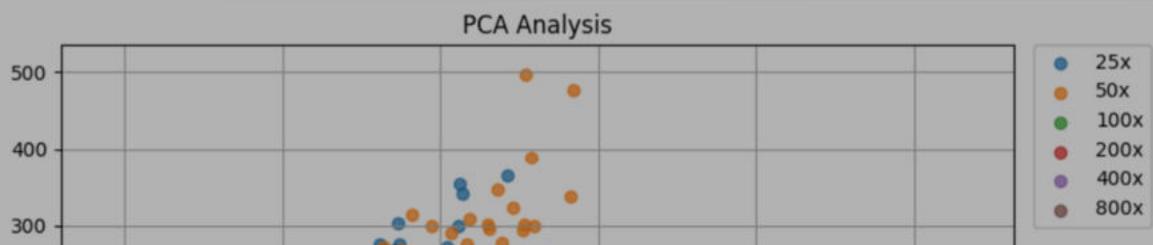


Serum PCA (preprocessed)

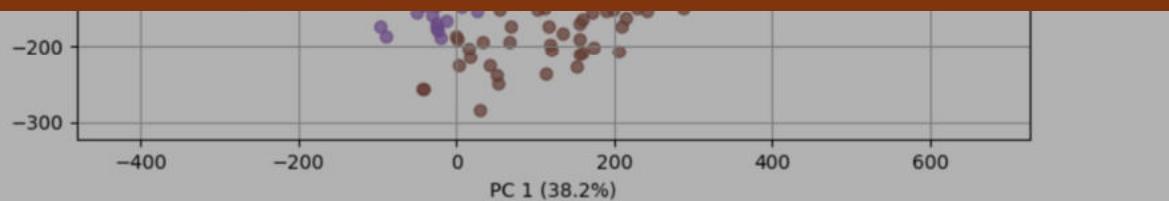
The result from PCA plots also support that at dilution factor 100 are the most tightly packed together.

Clustering = Highly reproducible and consistency of spectrum

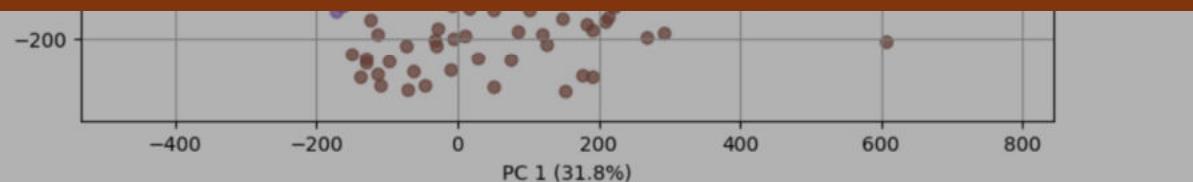
PCA Analysis



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



Plasma PCA (preprocessed)

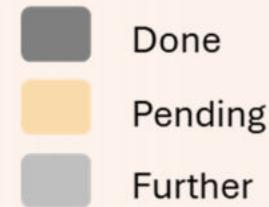


Serum PCA (preprocessed)

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



Activities	Time												
	2567		2568				2569		2570			2571	
	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**