Discriminating Individuals with High and Low Immunosenescent Profiles by Using Blood Serum and Their Components Detected by Raman Spectroscopy

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➤ ## Immunosenescence (Immune+Senescence)
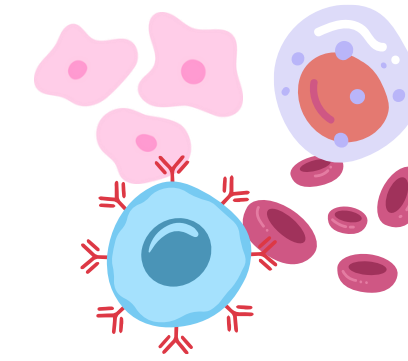
Age-related changes in the immune system

- Abundance and transcriptome data of innate and adaptive immune cells

Senescence occurs on
ALL CELLS!

Immunosenescence only in old age?

- Young age, old immune profile

Immune profile = true biological age of our body?

- Determines body health and the development of diseases.

- Abundances of immune cells and the expression of immune proteins fluctuate with age

RESEARCH ARTICLE

Aging Cell   WILEY

Deciphering the role of immune cell composition in epigenetic age acceleration: Insights from cell-type deconvolution applied to human blood epigenetic clocks

Ze Zhang[1,2,3] | Samuel R. Reynolds[1] | Hannah G. Stolrow[1,2] | Ji-Qing Chen[1,4] | Brock C. Christensen[1,2,3,4] | Lucas A. Salas[1,2,3,4]

"The immune cell composition was found to be a significant factor influencing the variation on epigenetic clock"

Raman/SERS approach:

- Raman spectra follows sample composition
- Minimum sample preparation
- Specificity & Sensitivity: Fingerprint
- Versatile sample type

Raman Spectroscopy (Bruker, Edinburth Instruments)

*SERS: Surface Enhanced Raman Spectroscopy

RESEARCH PAPER

Non-invasive SERS serum detection technology combined with multivariate statistical algorithm for simultaneous screening of cervical cancer and breast cancer

Ningning Gao[1] · Qing Wang[2] · Jun Tang[1] · Shengyuan Yao[1] · Hongmei Li[1] · Xiaxia Yue[3] · Jihong Fu[4] · Furu Zhong[5] · Tao Wang[1] · Jing Wang[6]

BASIC SCIENCE

nanomedicine
Nanotechnology, Biology, and Medicine

Nanomedicine: Nanotechnology, Biology, and Medicine 22 (2019) 102097

Original Article

Raman profiling of circulating extracellular vesicles for the stratification of Parkinson's patients
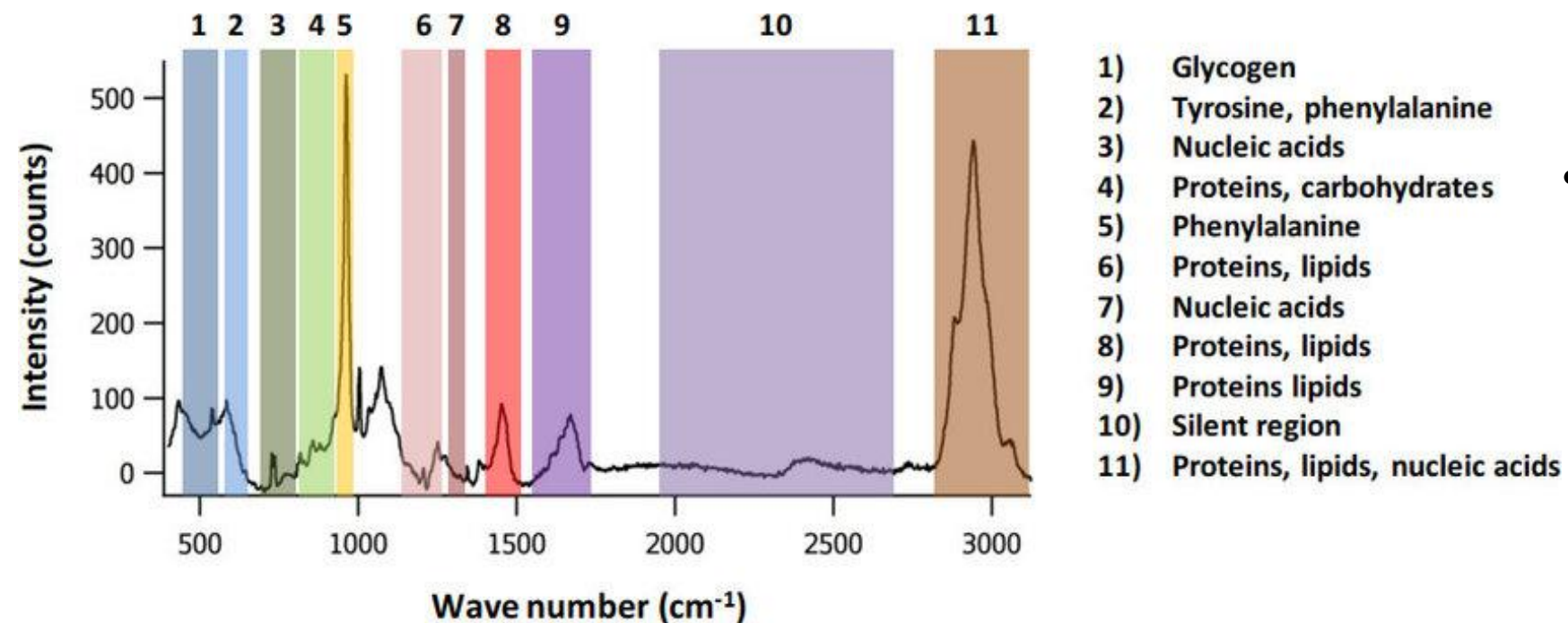
Alice Gualerzi, PhD[a,*], Silvia Picciolini, PhD[a], Cristiano Carlomagno, PhD[a], Federica Terenzi, MD[b], Silvia Ramat, MD, PhD[b], Sandro Sorbi, MD[a,b], Marzia Bedoni, PhD[a]

[a]IRCCS Fondazione Don Carlo Gnocchi, Italy
[b]Università degli Studi di Firenze, Dipartimento di Neuroscienze, Psicologia, Area del Farmaco e Salute del Bambino, Italy
Revised 13 September 2019

2

Raman Spectroscopy Principle

Measure

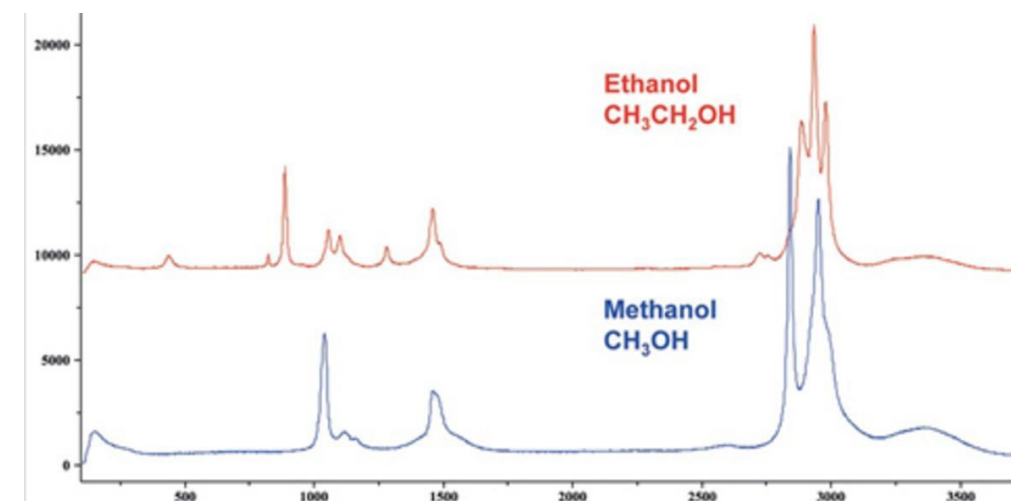Principle or raman scattering (K. Liu et al., 2022)

➢ **What is it?**

- Analytical technique that provide detail information about molecule through fingerprint.

- Non-destructive method with high sensitivity.

1) Glycogen
2) Tyrosine, phenylalanine
3) Nucleic acids
4) Proteins, carbohydrates
5) Phenylalanine
6) Proteins, lipids
7) Nucleic acids
8) Proteins, lipids
9) Proteins lipids
10) Silent region
11) Proteins, lipids, nucleic acids

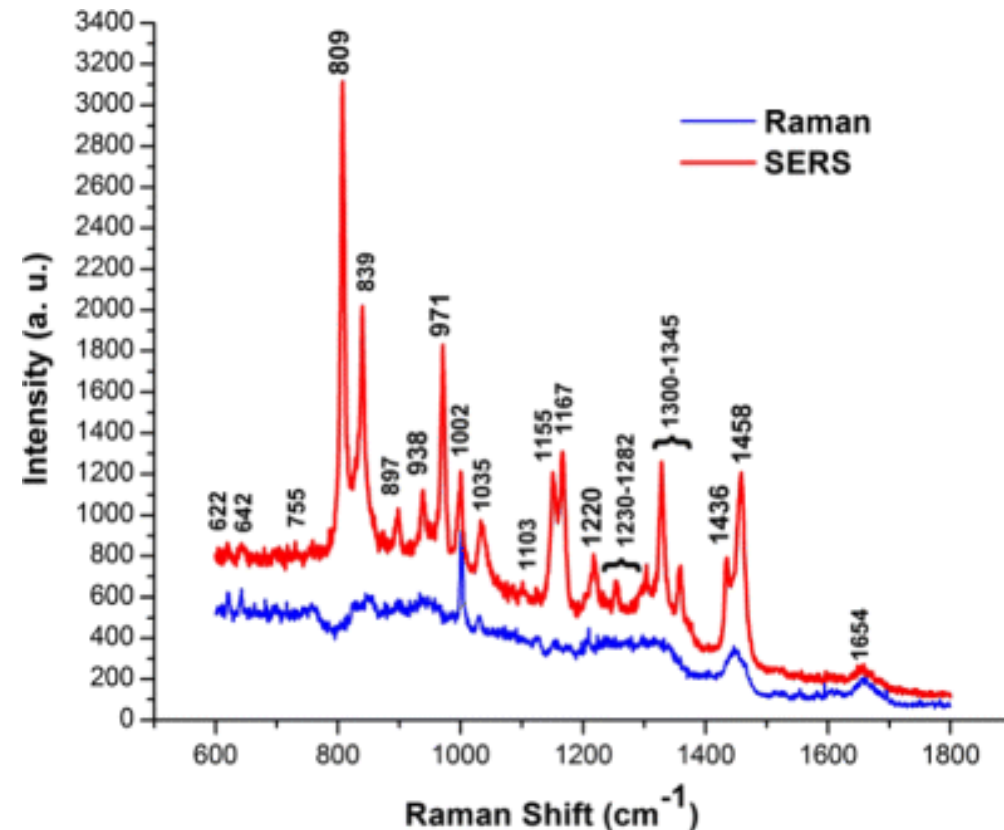Example of a Raman spectrum of a biological sample (Conforti et al., 2024)

➢ **Raman Spectrum**

- Number of peaks, showing the intensity and wavelength position of the Raman scattered light.

Raman spectra of ethanol and methanol (Horiba, 2024)

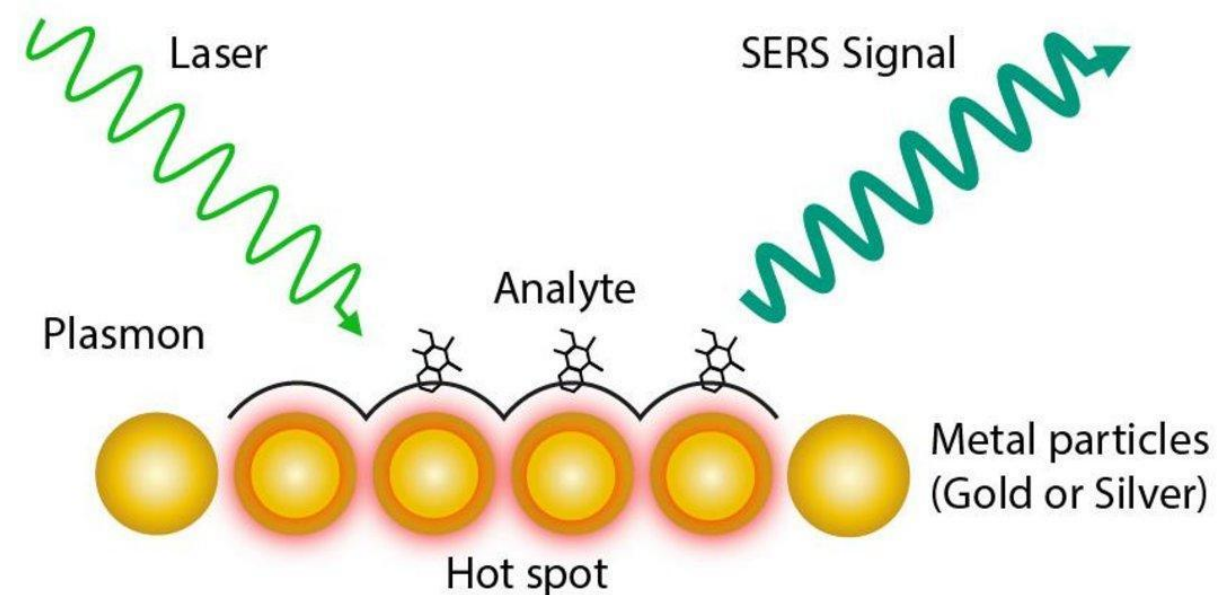# Surface Enhanced Raman Spectroscopy (SERS) Principle

Comparison of the conventional Raman and SERS spectra of a serum sample

Source: https://link.springer.com/article/10.1007/s10103-016-1976-x

➢ ## What is it?

- One of Raman techniques that amplify Raman spectra

- Provides all the advantages of Raman spectroscopy with higher sensitivity

- Enhanced up to 10^{10}

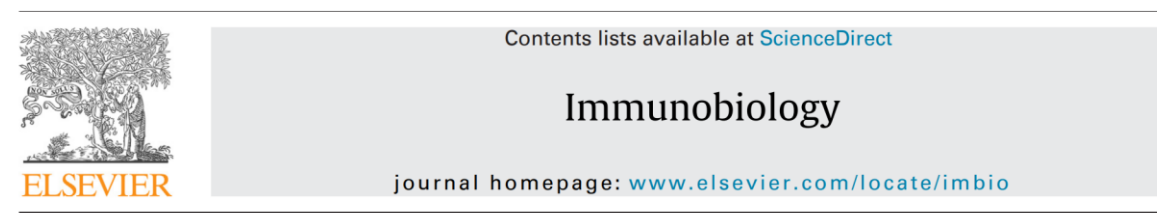Schematization of the Surface-enhanced Raman Scattering (SERS) mechanism (Horiba, 2024)

➢ ## How it works?

- Molecules adhered to metallic surfaces (gold, silver)

- Enhancement via plasmon resonance

- Localized surface plasmons resonates creating "hot spots."

- Hot spots enhance the local electric field near the metal surface

Immunosenescent CD4 + T cell (T helper cell)

Th Cells, CD4+:

- Activated by antigen recognition by MHC Class II molecule

- Coordinating immune response:
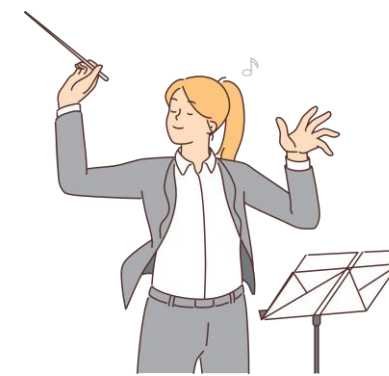   - Cytokine secretion

- Develop long-lived memory cells

Th cells as "conductor"

Changes

T cell dysfunction: Progression of disease

Importance for Early Detection

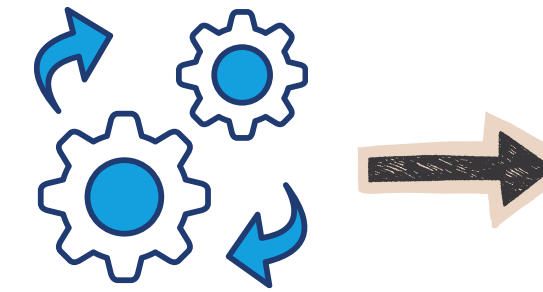Aberrant NKG2D expression with IL-17 production of CD4+ T subsets in patients with type 2 diabetes

Wisitsak Phoksawat[a,b], Amonrat Jumnainsong[b,c], Naruemon Leelayuwat[d], Chanvit Leelayuwat[b,c,*]

Predominant expression of the immunosenescent biomarker CD57 on CD4+ T cells and their subsets in the older people associating with the cardiovascular disease risk factors

Kanda Sornkayasit,[1] Chanvit Leelayuwat,[2] Amonrat Jumnainsong,[2] Patcharaporn Tippayawat,[2] Wipaporn Wongfieng,[3] Rian Ka Praja,[4] Sonwit Phanabamrung,[2] Laong-thip Raknarong,[2] Kanin Salao,[5] Arnone Nithichanon,[6,7] Suwit Chaisri,[8,9] and Wisitsak Phoksawat[6,7,*]

IL-17 and IFN-γ Productions by CD4+ T cells and T cell Subsets Expressing NKG2D Associated with the Number of Risk Factors for Cardiovascular Diseases

Wisitsak Phoksawat[a,b,c], Amonrat Jumnainsong[b,c,d], Kanda Sornkayasit[a,b], Kanoungnit Srisak[a,b], Nantarat Komanasin[e,f], Chanvit Leelayuwat[b,c,d,*]

Open access   **Short report**

KLRG1 marks tumor-infiltrating CD4 T cell subsets associated with tumor progression and immunotherapy response

Journal for ImmunoTherapy of Cancer

Casey R Ager [1,2,3,4] Mingxuan Zhang,[1,5] Matthew Chaimowitz,[3,6] Shruti Bansal,[3,6] Somnath Tagore,[1,7] Aleksandar Obradovic,[1,7] Collin Jugler,[2] Meri Rogava,[1,3] Johannes C Melms,[1,3] Patrick McCann,[3,6] Catherine Spina,[3,6] Charles G Drake,[1,3,4,8,9] Matthew C Dallos,[1,9,10] Benjamin Izar [1,3,7,9,11]

These cells are aberrant, undergo changes and affect the immune system function.

Expression: CD28- / CD57+ / KLRG1+(Immunosenescent); NKG2D+ (Pathogenic)

Cytokine: IL-17; IFN-Gamma

Conceptual Framework

Problem

Immunosenescence health decline

- Reduced Immune Response
- Increased Infection Susceptibility
- Lingering Low-Grade Inflammation

Need of Method for Early Detection of Immunosenescence

- Easy to perform
- Rapid
- Sensitivity, Specificity, Accuracy

Process

Phase I: Immunosenescence Profiling

- Fresh Whole Blood
- Flow Cytometry

Phase II: Raman & SERS Spectra Acquisition

- Serum
- Exosome
- Raman & SERS

Supervised/Unsupervised Analysis

Outputs

- Individual Immunosenescence Profile
- Individual Raman & SERS Spectra
- Machine Learning Performance

Outcomes & Impacts

Immunosenescence detection:
- Rapid
- Minimum invasive
- High performance

Platform for Immunosenescence detection

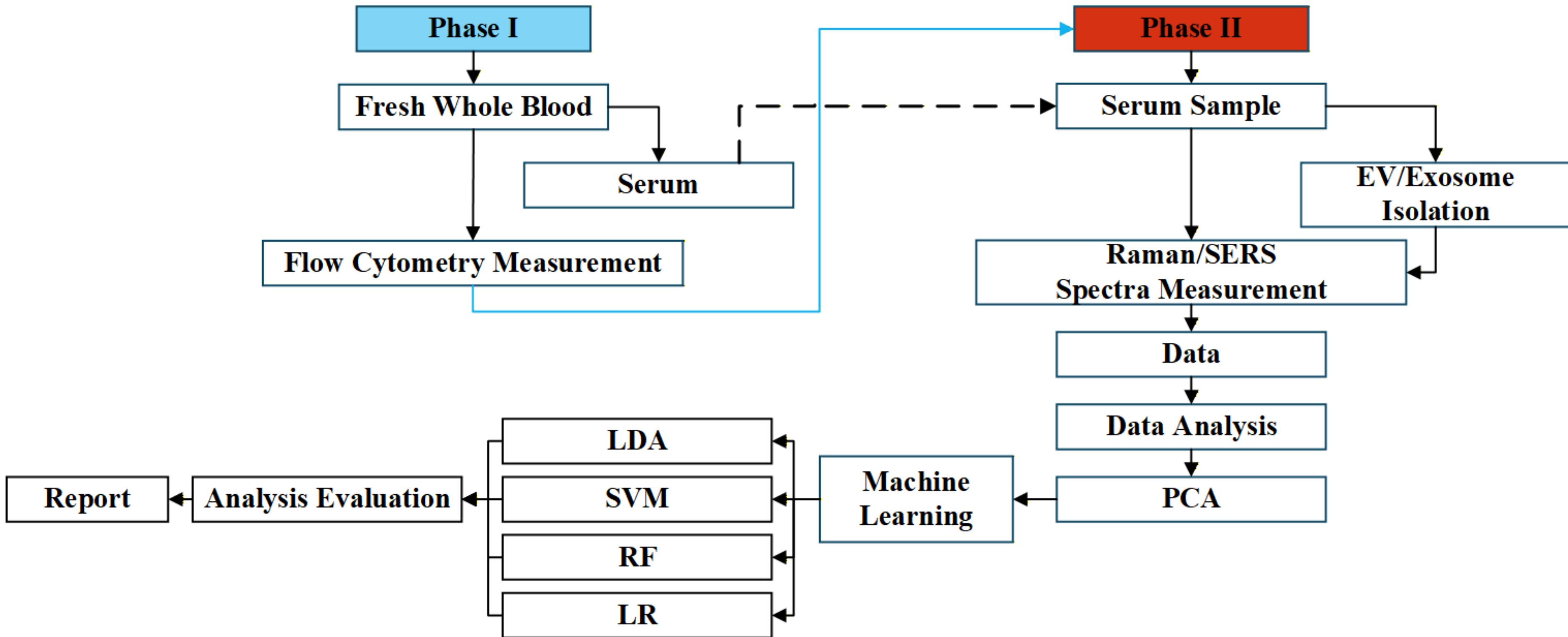Improve health management with precision medicine

Objective

General objective:

- To differentiate the immune profiling from serum and small biomolecule (extracellular vesicle, exosome) of individuals with high and low percentage of pathogenic / immunosenescent CD4+ T cells by using portable Raman spectroscopy/SERS

Hypothesis

Individuals with high and low percentage of pathogenic/immunosenescent CD4+ T cells are possible to be differentiated by analyzing its serum and exosome by using Raman spectroscopy/SERS

Study Design

Anticipated Outcomes

- Raman spectra of serum and extracellular vesicle/exosome from individual with high and low immunosenescent profile

- Raman spectroscopy/SERS as a new tool for detection of high and low profile of immunosenescence in the individual

- New platform to detect immunosenescent condition in clinical samples (serum, extracellular vesicle/exosome)

Marker	Fluorescence
Anti-CD3	FITC
Anti-CD4	APC-Cy7
Anti-CD28	PE-Cy7
Anti-CD314/NKG2D	APC
Anti-CD57	PE
Anti-KLRG1	BV785
Anti-IL17	PerCP-Cy5
Anti-IFNγ	BV421

➢ **Done:**

- Training on principle and running samples
- Order KLRG-1 antibody
- Setting parameter for immunosenescent profiling
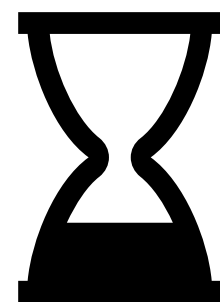
❑ Immunosenescence panel:

- CD57+
- CD28-
- KLRG-1

❑ Cytokine panel:

- IFN-gamma
- IL-17

❑ Pathogenic panel:

- NKG2D+

❖ To do:

- Setting up parameter for KLRG-1
- Data analysis training
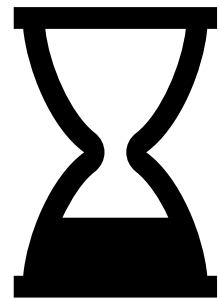- Optimization of the whole panel

10

Raman Spectroscopy (Bruker, Edinburth Instruments)

➢ Done:

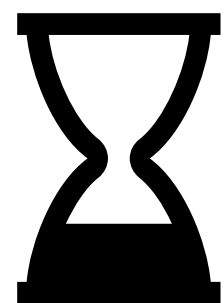- Training on principle and instrument operation

❖ To do:

- Sample trial (dry vs wet comparison), Raman & SERS

- Data analysis trial

Exosome Isolation

➢ Done:

• Searching for exosome isolation kit and comparison

Kit Name	Catalog	Brand	Input	Exosome Storage
Total Exosome Isolation Reagent (from serum)	4478360	Thermo	100uL serum	2°C to 8°C (1 week) ≤20°C for long-term storage
The Original ExoQuick,	EXOQ5A-1	Sysytem Biosciences	250uL serum	Use directly or freezing

❖ To do:

• Order isolation kit

• Trial exosome isolation

Conclusion

○ -

Future Work

➢ Training:

- Flow Cytometry: Setting up parameter for KLRG-1 & Optimization whole panel

- Raman Spectroscopy: Sample preparation, running & optimization

- Exosome Isolation: Order kit & training exosome isolation

➢ Thesis Proposal

➢ EC application

Thesis Plan

#	Activities	2025		2026				2027	
		Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2
1	Coursework	🟩	🟩	🟧					
2	Literature review and planning		🟧	🟧					
3	Proposal Writing		🟧	🟧					
4	Proposal Examination				🟧				
5	EC Application				🟧				
Laboratory									
6	Specimen collection				🟧				
7	Flow Cytometry					🟧			
8	Raman & SERS					🟧			
9	Data Analysis						🟧		
Manuscript preparation									
10	Manuscript preparation & publication							🟧	
11	Thesis Defense								🟧

Credit: 3

🟩	**Done**
🟧	**On process**
🟧	**Future work**

Acknowledgement

Advisor

Asst. Prof. Wisitsak Phoksawat

1st Year Master Student

16