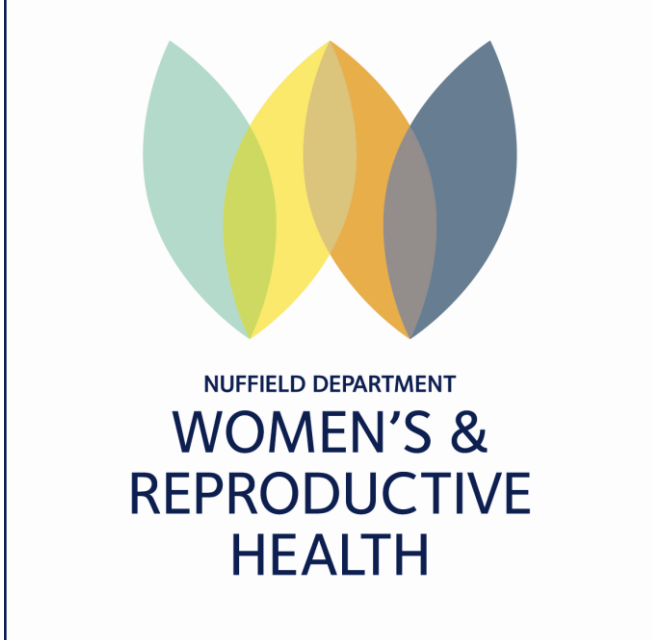


Investigating abnormal immune responses in post-infection conditions using Raman microscopy



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Background

Raman microscopy provides a non-invasive, label-free method of investigating the immune system using smaller sample volumes. The Morten lab previously used this methodology with Peripheral Blood Mononuclear Cells (PBMCs), and it has now been refined to investigate specific immune cells that may be more directly linked to Paediatric Acute-onset Neuropsychiatric disorder (PANS). The literature suggests that ongoing T cell and/or B cell activity against self-antigens could play a role in PANS patients who experience a more chronic or deteriorating pattern of symptoms (Frankovich *et al.*, 2017). The role of neutrophils is also an area of interest within our lab groups work on post infection conditions. Raman microscopy detects changes in immune cells, such as metabolic changes, and should allow thorough investigation of potentially abnormal immune responses in post-infection conditions such as PANS.

Introduction to Raman Microscopy

Raman microscopy utilises lasers to investigate the unique molecular fingerprints of a sample.

- A single-frequency laser, in this case a green 532nm laser, is fired at the sample
- The 'Raman effect' was discovered in 1928 by Sir C. V. Raman and refers to when a molecule absorbs some of the light to excite a molecular vibration. This results in different inelastically scattered light being emitted from the sample, which has a different wavelength than the laser. This is known as 'Raman scattering'. An increase in wavelength is referred to as Stokes scattering, and a decrease is referred to as anti-Stokes scattering
- Scattered light from the laser hitting the sample can be measured. The difference between the consistent laser frequency and this altered detected signal is known as the 'Raman shift' and is uniquely associated with the molecule

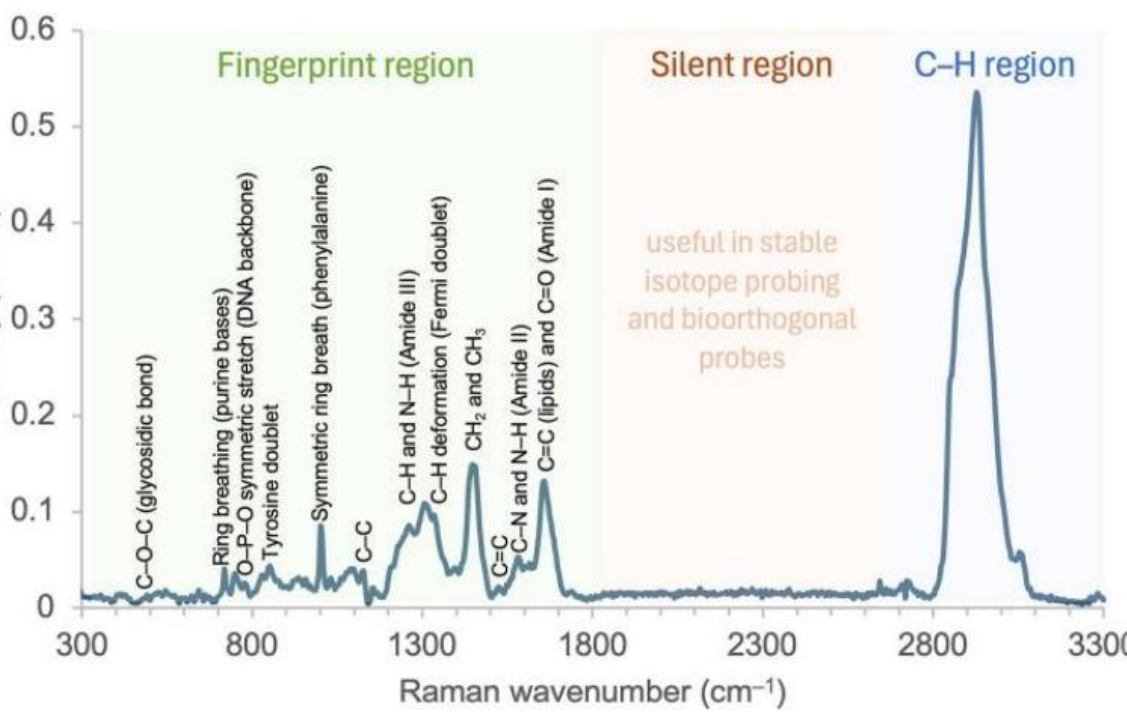


Figure 1. Example full spectra 300-3800 cm⁻¹. Example Raman spectra with approximate areas of interest labelled. The fingerprint region is the portion of the spectrum where molecular vibrations are well defined. Figure from Xu and Morten, 2024.

- Raman shifts can then be used to build a complete Raman spectrum. The detected scattered light is translated into peaks on a complete spectrum that reflects all the detected signals resulting from the laser hitting the sample
- These frequencies are associated with molecules and their specific bonds. Therefore, a Raman spectrum can have its peaks act as a fingerprint for specific molecules, and it can be used to identify and quantify samples

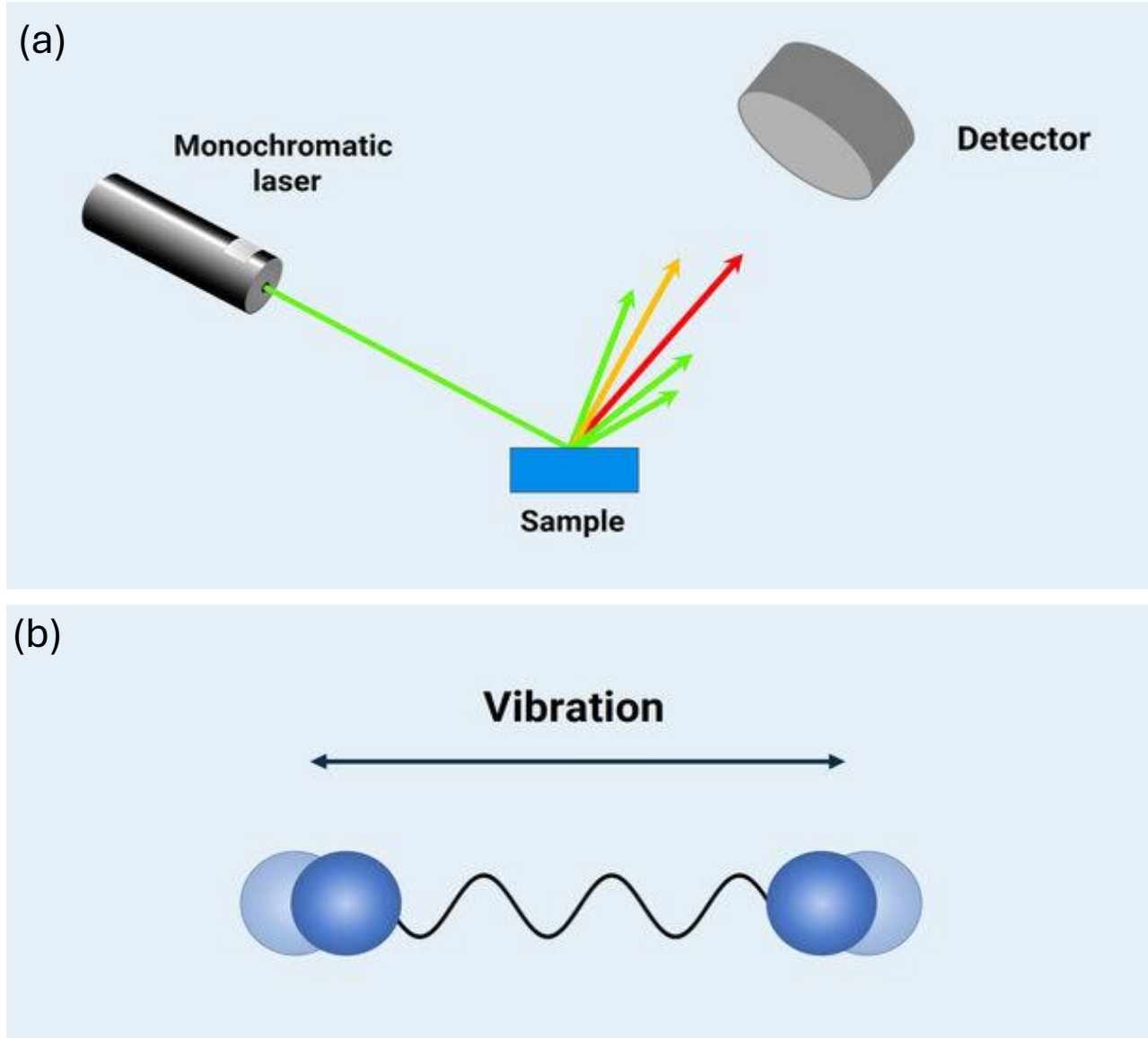
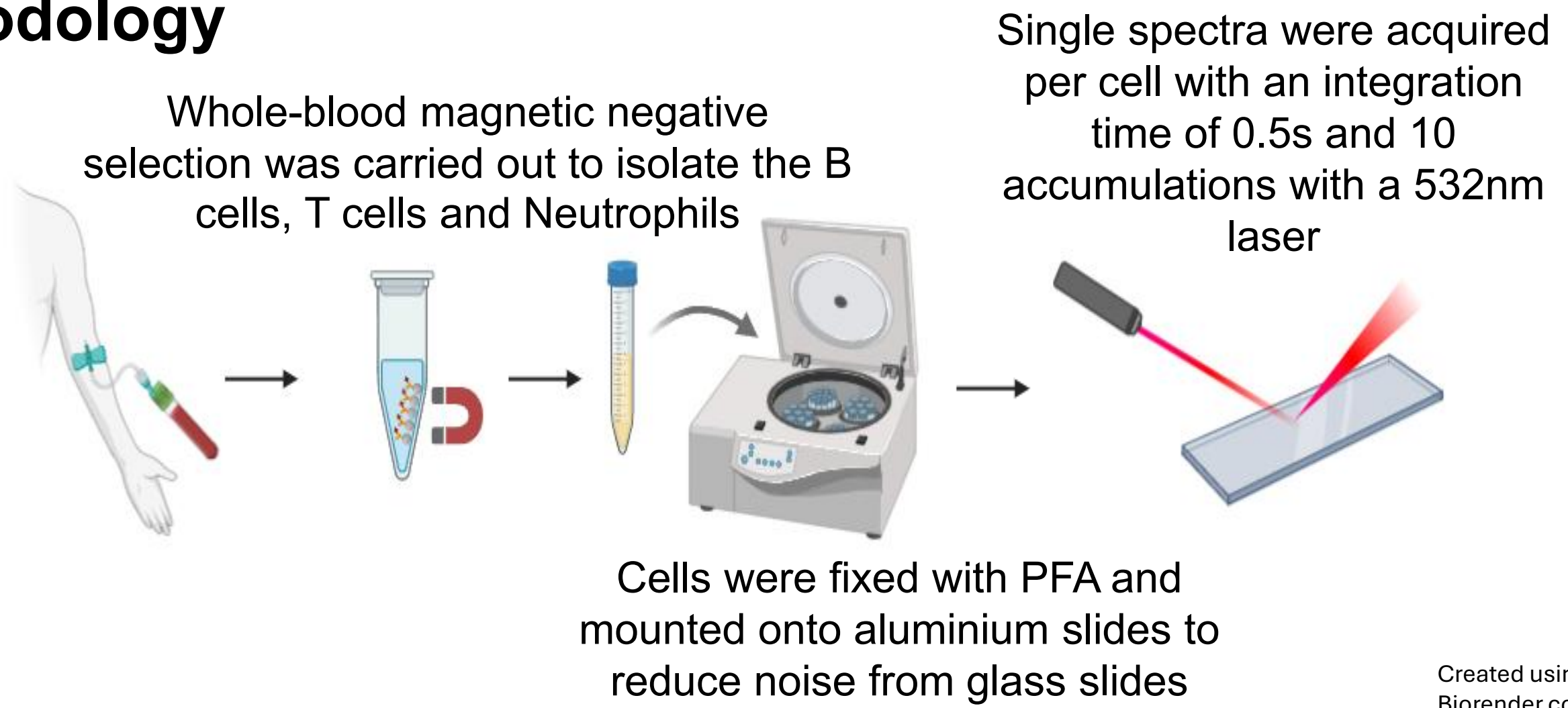


Figure 2. (a) Diagram of the Raman detector setup. The monochromatic laser is a single-frequency light source. Data in the Morten lab is usually collected using the 532nm green laser (b) **Bond vibration.** Excitation of a molecular bond when hit by the laser (Modified from (Bruker, No date) Accessed 2026).

Objectives

- Develop a Raman microscopy protocol for the analysis of immune cell subtypes and establish the effectiveness of using negative selection to isolate specific immune cells from whole blood
- Determine whether Raman microscopy can differentiate B cells, T cells and neutrophils and investigate regions of interest of their spectra
- Establish healthy immune-cell Raman spectra as a baseline for comparison with future patient samples from post-infectious conditions, including PANS
- Investigate the use of Raman microscopy to detect immune-cell stimulation following lipopolysaccharide (LPS) treatment

Methodology



Results

Preliminary analysis of Raman data. Cosmic ray removal, shape fitting to 100 and normalisation to area 1 were all carried out.

Healthy blood cells

- A Partial Least Squares Discriminant Analysis (PLS-DA) was carried out to focus on peaks separating the cell types and reduce interference from noise
- PLS-DA was able to differentiate cell types with an $R^2 = 0.87$ and $Q^2 = 0.62$, confirming strong predictive power but with more data needed to create a more consistent model (Figure 3b)
- PLS-DA Variable Importance in Projection (VIP) analysis identified multiple discriminatory Raman regions, particularly in the regions between 830–850, 1000–1020cm⁻¹ and 1200–1230 cm⁻¹ (Figure 3a)
- The C–H stretching region (~2800–3000 cm⁻¹) can aid spectral discrimination by reflecting broad lipid- and protein-associated differences, but its lower chemical specificity provides less detailed molecular insight, so we focus on the fingerprint region
- 830–850 cm⁻¹ repeatedly appears as a high-VIP region in the B cell spectra despite not being ranked with the first 15 scored (Figure 3d)

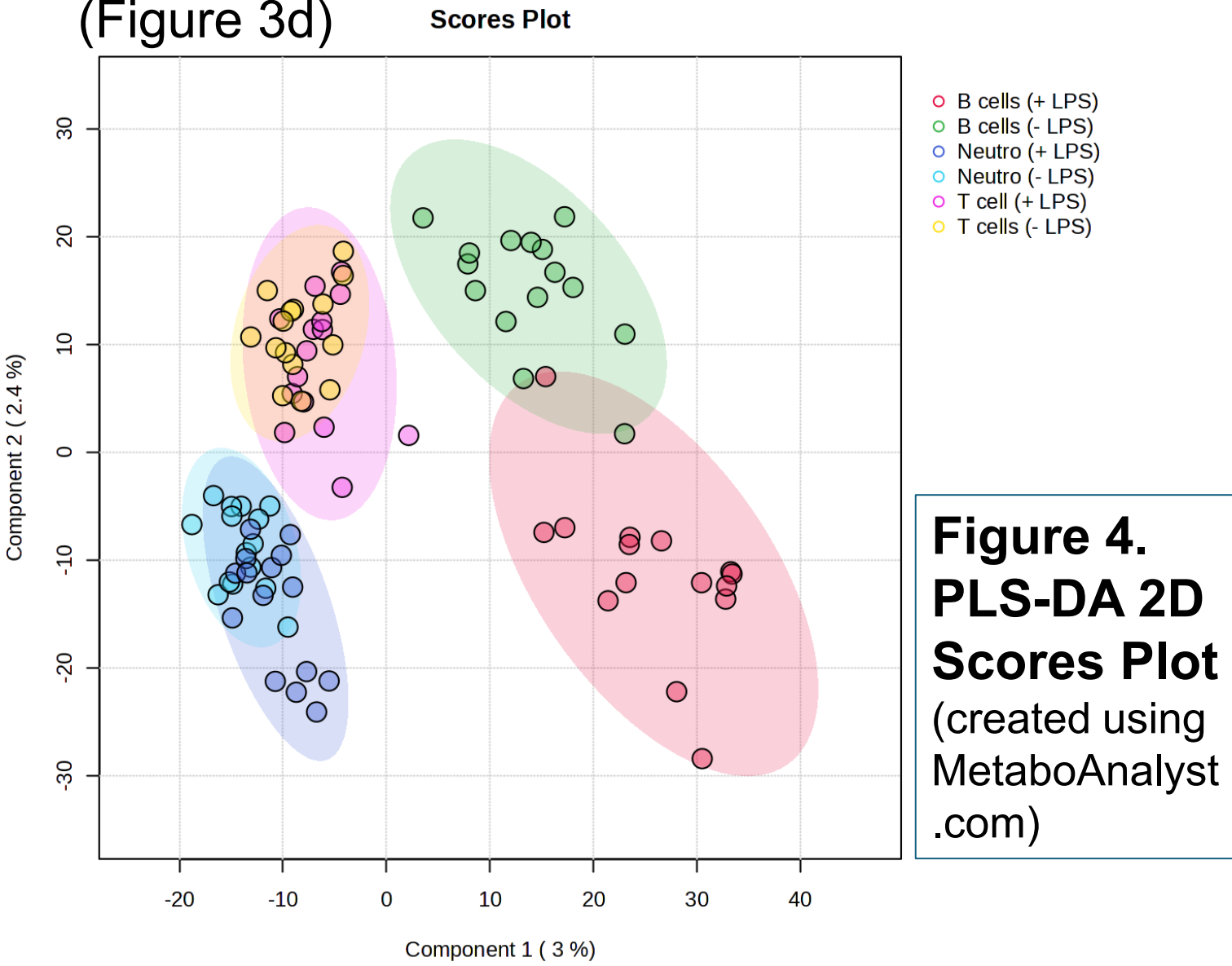
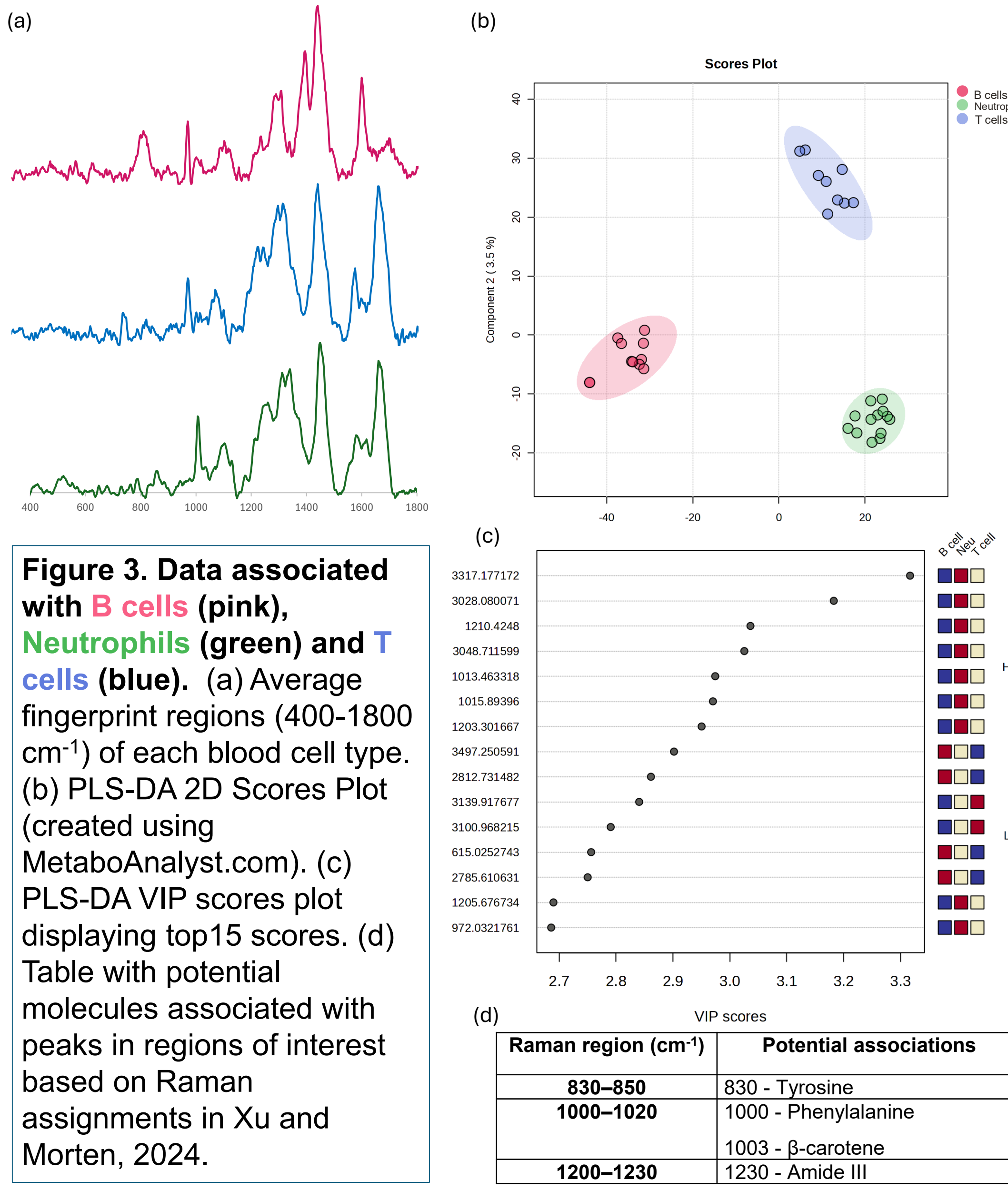


Figure 4. PLS-DA 2D Scores Plot (created using MetaboAnalyst .com)

Lipopolysaccharide (LPS) stimulated healthy blood cells

To test whether stimulation of immune cells can be detected using our methodology

- Cells were kept in RPMI medium for 3 hours, with the treated cells in media at a concentration of 2 μ L/mL of LPS
- Raman microscopy detected LPS-associated spectral changes, particularly in B cells, indicating sensitivity to immune-cell stimulation
- However, moderate model fit and predictive ability ($R^2 = 0.58$, $Q^2 = 0.35$) indicate that additional data and biological replicates are required to validate the response and the sensitivity of the Raman microscope (Figure 4)



Discussion

- Raman microscopy was able to differentiate between immune-cell types, supporting its potential for establishing patient-specific spectral baselines and, with further development has the potential to identify cell populations within PBMCs
- This could be particularly useful in longitudinal studies, where immune-cell spectra are tracked over multiple time points and could be compared during periods of symptom stability and symptom flare
- PANS may be a valuable future application, where Raman microscopy could help investigate changes in immune-cell profiles alongside symptom severity and other biological measurements
- A key limitation is that biological Raman spectra contain overlapping wavelengths, which reduces certainty when assigning individual peaks to specific biomolecules. The technique may therefore be most useful for tracking broader spectral changes rather than relying on single-peak assignments
- Immune-cell stimulation work was previously limited by HTA ethics restrictions. With further ethics now in place, future work can investigate stimulation over longer time frames and with larger datasets. With further funding, this approach could be expanded into a longitudinal PANS study

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