OPTIMISATION OF MULTI-OMIC SPATIAL ANALYSES OF ENDOMETRIOSIS FFPE TISSUES USING MALDI MSI

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INTRODUCTION

- **Endometriosis** is a complex, chronic disease which causes lesion growth of endometrial-like tissues outside of the uterine cavity.
  - Affects ~11.4% of Australian women of reproductive age.[1]
  - Symptoms include chronic pain, painful menstruation and infertility.
  - Diagnosis only currently possible with invasive surgery.
    - = Discovery of **new disease biomarkers urgently required**.

- Both proteins and glycans show much promise as disease biomarkers in both endometriosis and other diseases.[2]
- **Mass Spectrometry (MS)** is an advanced method for the measurement and identification of biomolecules including proteins and glycans.
- **Mass Spectrometry Imaging (MSI)** allows for the analysis of whole FFPE (formalin-fixed paraffin-embedded) tissues allowing for molecular localisation and measurement of spatial distribution.

- MSI permits accurate analysis of endometriotic lesion-specific molecules which can be compared to healthy tissue to **identify disease biomarkers**.

METHOD

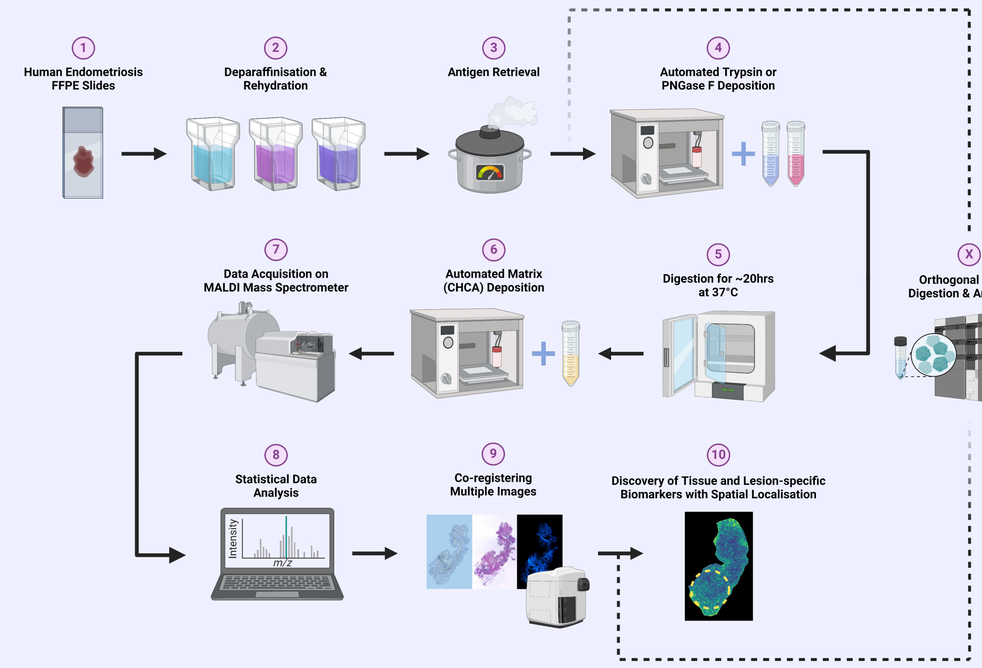

Figure 1 - Diagram illustrating the process of MALDI-MSI sample preparation for FFPE (formalin-fixed paraffin-embedded) tissues and the identification and analysis of protein peptides and N-linked Glycans. Instruments shown include (3) Aptum Retriever 2100, (4/6) HTX TM-Sprayer M3, (7) Bruker SolariX 7T 2XR hybrid ESI/MALDI-FT-ICR MS, (9) Zeiss Axioscan Z.1 Digital Slide Scanner and (X) Shimadzu Prominence Nano HPLC coupled with a Sciex 5600 TripleTOF MS (created with BioRender.com)

- **10 confirmed endometriosis patients** with ≥ 1 deep and ≥ 2 superficial lesions (= 44 sections) AND full-thickness uterine tissues AND a healthy endometrium tissue microarray.
  - Endometriosis and full-thickness uterine tissues sourced from The Royal Women's Hospital Tissue Bank under ethical approval (Project 10/43).

- Method optimisation on full-thickness uterine sections and the healthy endometrium microarray (sourced from CHTN) to define a healthy endometrium, then progress to using diseased endometriosis tissues.[3-5]
- Assess spatial proteomic and glycomic profile differences of deep and superficial endometriotic lesions as well as healthy endometrium samples from MSI data.

- **Statistically assess** using –
  - Principal Component Analysis (PCA) — Define tissue types and regions
  - Linear Discriminant Analysis (LDA) models — Build classification models
  - Two-sided Independent t-test (Student's or Welch's)
  - Receiver Operative Characteristics (ROC) curves
  - Area Under the Curve (AUC) analysis. — Determine discriminant features

- Annotate disease biomarkers via orthogonal protein digestion and LC-MS/MS for proteins and METASPACE annotations for N-glycans.

RESULTS & DISCUSSION

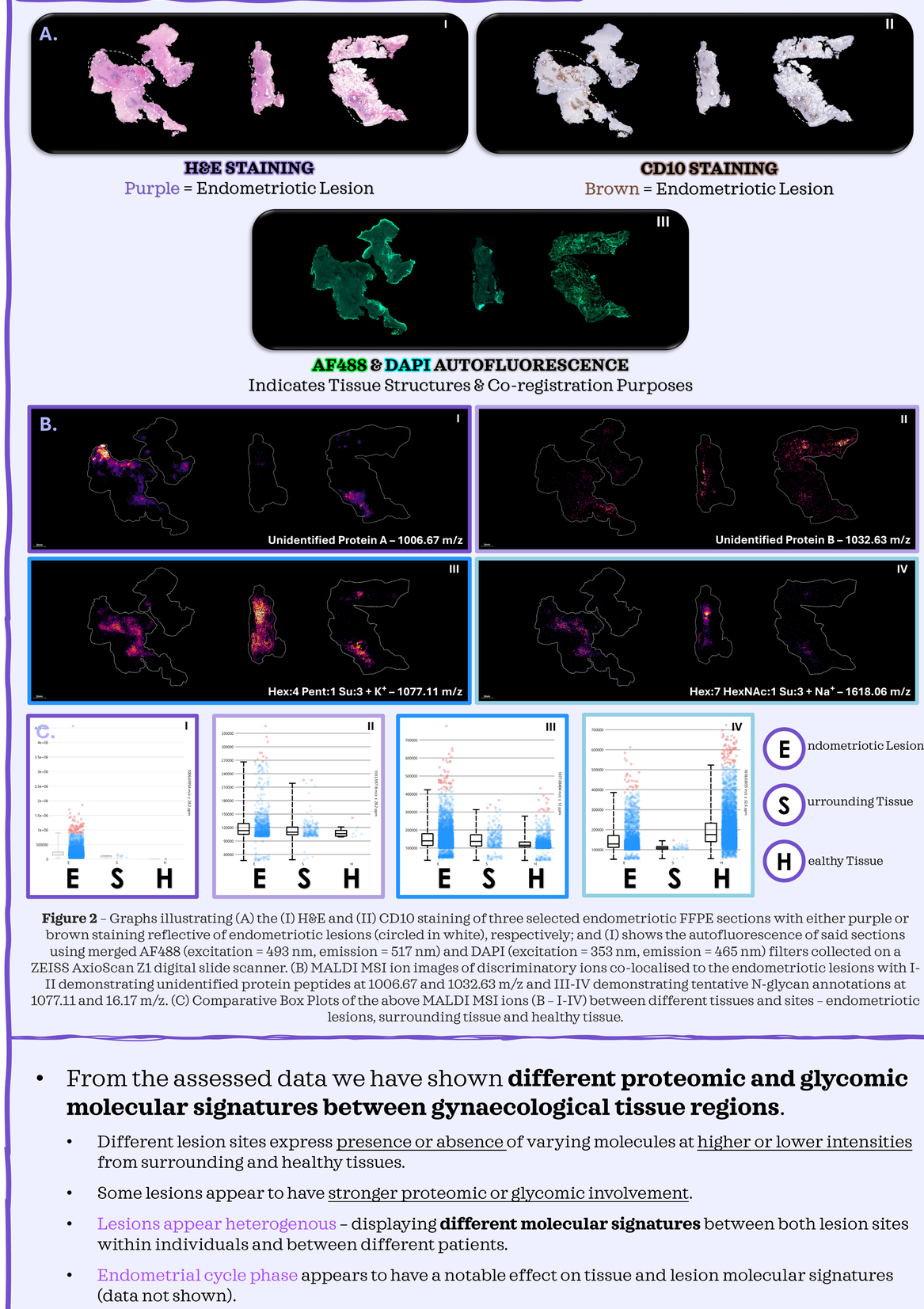

Figure 2 - Graphs illustrating (A) the (I) H&E and (II) CD10 staining of three selected endometriotic FFPE sections with either purple or brown staining reflective of endometriotic lesions (circled in white), respectively; and (I) shows the autofluorescence of said sections using merged AF488 (excitation = 493 nm, emission = 517 nm) and DAPI (excitation = 353 nm, emission = 465 nm) filters collected on a ZEISS AxioScan Z1 digital slide scanner. (B) MALDI MSI ion images of discriminatory ions co-localised to the endometriotic lesions with I-II demonstrating unidentified protein peptides at 1006.67 and 1032.63 m/z and III-IV demonstrating tentative N-glycan annotations at 1077.11 and 16.17 m/z. (C) Comparative Box Plots of the above MALDI MSI ions (B – I-IV) between different tissues and sites – endometriotic lesions, surrounding tissue and healthy tissue.

- From the assessed data we have shown **different proteomic and glycomic molecular signatures between gynaecological tissue regions**.
  - Different lesion sites express presence or absence of varying molecules at higher or lower intensities from surrounding and healthy tissues.
  - Some lesions appear to have stronger proteomic or glycomic involvement.
  - Lesions appear heterogenous – displaying **different molecular signatures** between both lesion sites within individuals and between different patients.
  - Endometrial cycle phase appears to have a notable effect on tissue and lesion molecular signatures (data not shown).
- MSI can therefore allow for the accurate analysis and localisation of differential molecular profiles between endometriotic lesions, surrounding tissues and healthy tissues.
- Next steps include:
  - Further annotation of data to identify affected proteins and glycans.
  - Cross-validation with different sample types.

CONCLUSION

- MSI has the potential to be a vital technology in the discovery of biomarkers and understanding of pathophysiology in diseases such as endometriosis.
  - Tissue biomarkers can then be tested in more non-invasive mediums (blood and urine) to generate diagnostic tests.
  - The heterogeneity of endometriotic lesion sites, however complicates the discovery and assessment of biomarkers.
- Endometriosis needs further research to identify biomarkers and **assist in earlier diagnosis, thus improving quality of life** of those affected.

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