

## INTRODUCTION

### ME/CFS – challenging diagnosis

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is characterized by debilitating fatigue that profoundly impacts patients' lives. Diagnosis of ME/CFS remains challenging, with most patients relying on self-report, questionnaires, and subjective measures to receive a diagnosis, and many never receiving a clear diagnosis at all.

### Single-cell Raman spectroscopy – a label-free phenotypic technique

Raman spectroscopy is a non-invasive and label-free approach to probe molecular vibrations in a sample. A single-cell Raman spectrum (SCRS) is a phenotypic fingerprint of all biomolecules in that cell and could potentially differentiate between various cell types and give insights into underlying biology<sup>1</sup>.

### Blood cells as a target

Peripheral blood mononuclear cells (PBMCs) obtained from ME/CFS patients exhibited altered mitochondrial function, indicating differences in the energetic function<sup>2</sup>. As ME/CFS may have a systemic energy issue, we hypothesized that single-cell analysis of PBMCs might reveal differences in ME/CFS compared to healthy and other disease groups<sup>3</sup>.

### Our study design

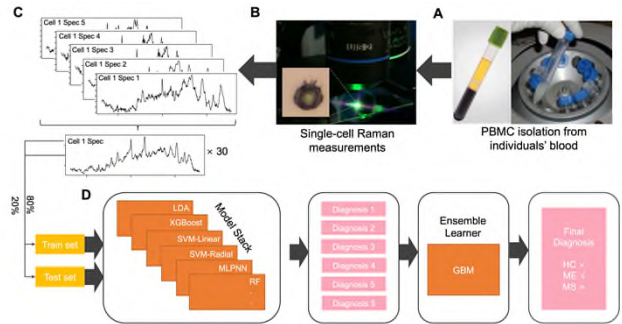
Samples: 98 individuals' blood from UK ME/CFS Biobank

HC (healthy controls): n = 16

MS (multiple sclerosis; positive control): n = 21

ME (mild): n = 25; ME (moderate): n = 15; ME (Severe): n = 21

## METHODS

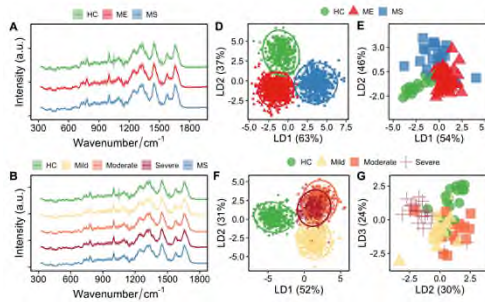


(A) PBMCs isolated from blood samples. (B) Raman spectra of single PBMCs from 98 individuals measured. (C) Between 5–7 spectra measured in each cell which then averaged to one spectrum for one cell (D) SCRS from 98 individuals were then split into a train set (80%) and a test set (20%). The train set was used to train an ensemble learner and the independent test set was input into the trained learner for diagnosing the cell as HC, ME or MS.

## RESULTS

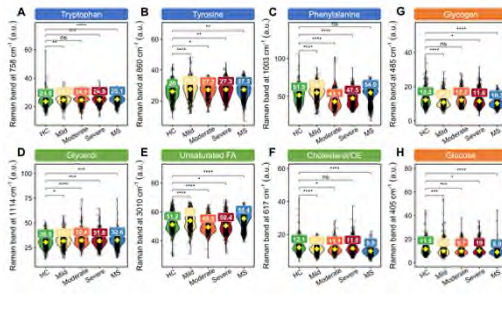
### 1) SCRS differentiate different cohorts

SCRS from 2155 single cells obtained from 98 individual subjects differ among cohorts at both single-cell level and individual level.



### 3) Quantification of biomolecules in different cohorts

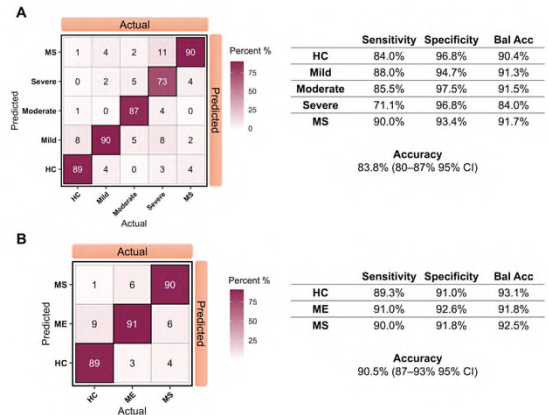
Relative quantification of biomolecules in PBMCs related to aromatic amino acids, lipid metabolism and energy metabolism was found distinct in controls and ME patients.



### 2) A cell-based diagnostic test using SCRS of PBMCs and an ensemble learner

Ensemble learner performance on an independent test set breakdown by (A) five classes with **84% accuracy** and (B) three classes with overall **91% accuracy**. Matrix entries are shown as percentage values.

The three-class classification model shows a performance of **diagnosing ME/CFS with 91% sensitivity and 93% specificity**, an overall accuracy at 91% with 87–93% at 95% confidence interval.



## CONCLUSION

Our results demonstrate that Raman profiles of blood cells can distinguish between healthy individuals, disease controls, and ME/CFS patients with high accuracy (91%), and can further differentiate between mild, moderate, and severe ME/CFS patients (84%). Additionally, we identified specific Raman peaks that correlate with ME/CFS phenotypes and have the potential to provide insights into biological changes and support the development of new therapeutics. This study presents a promising approach for aiding in the diagnosis and management of ME/CFS. With ongoing plans of expanding our cohort size and bringing in other cohorts of patients with similar co-morbidities, Raman spectroscopy coupled with other techniques, such as metabolomics and proteomics, can further expand our knowledge on the diseases and potentially identify common disease pathways or drivers.

## REFERENCES

- Xu, J. et al. Unveiling Cancer Metabolism through Spontaneous and Coherent Raman Spectroscopy and Stable Isotope Probing. *Cancers* (Basel) 13, (2021).
- Tomas, C. et al. Cellular bioenergetics is impaired in patients with chronic fatigue syndrome. *PLoS One* 12, e0186802 (2017).
- Xu, J. et al. A new approach to find biomarkers in chronic fatigue syndrome/myalgic encephalomyelitis (CF/ME) by single-cell Raman micro-spectroscopy. *Analyst* (2018) doi:10.1039/C8AN01437J.