Combining SCENITH and spectral flow cytometry to establish a novel assay to enable high resolution metabolic profiling

Kristina Tomkova[1], Jonathan W. Lewis[1] ,Thomas A. Nicholson[1], Helen M. McGettrick[1], Thomas A. Jackson[1], Simon W. Jones[1], Jon Hazeldine[1]

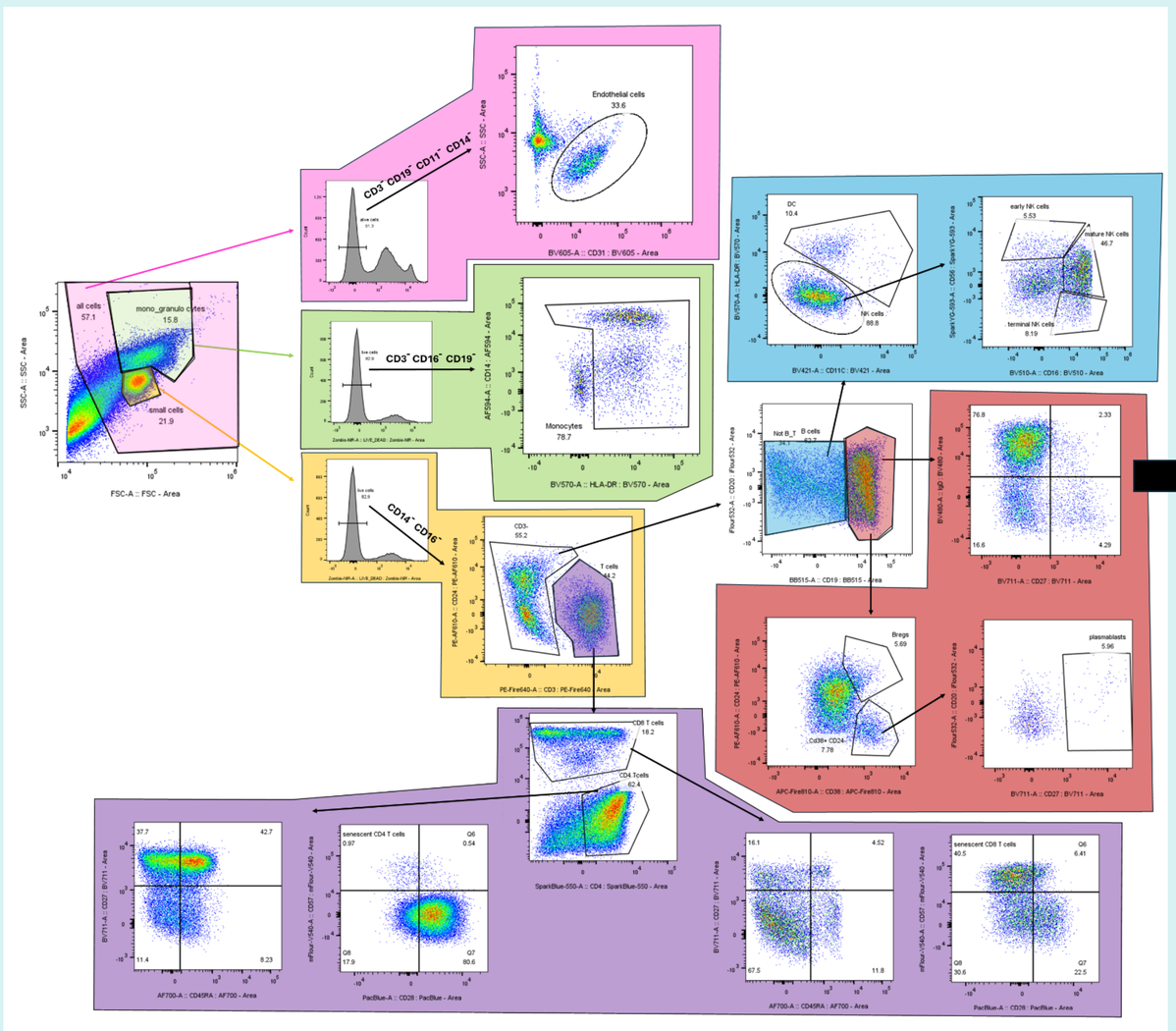
1 Institute of Inflammation and Ageing, University of Birmingham, UK

Introduction

Altered cellular metabolism is a key hallmark of ageing and may contribute to immunosenescence and sarcopenia. However, age-related metabolism shifts in key cellular subsets in the circulation and peripheral tissues are not well defined. Improving our understanding of cellular metabolism at a higher resolution may identify novel targets to limit age-associated cellular dysfunction. Therefore, with recent funds from the BLAST network, **we aim to validate a new technique, combining spectral flow cytometry with SCENITH to study cellular metabolic function at a high resolution scale.**

Spectral Flow Cytometry

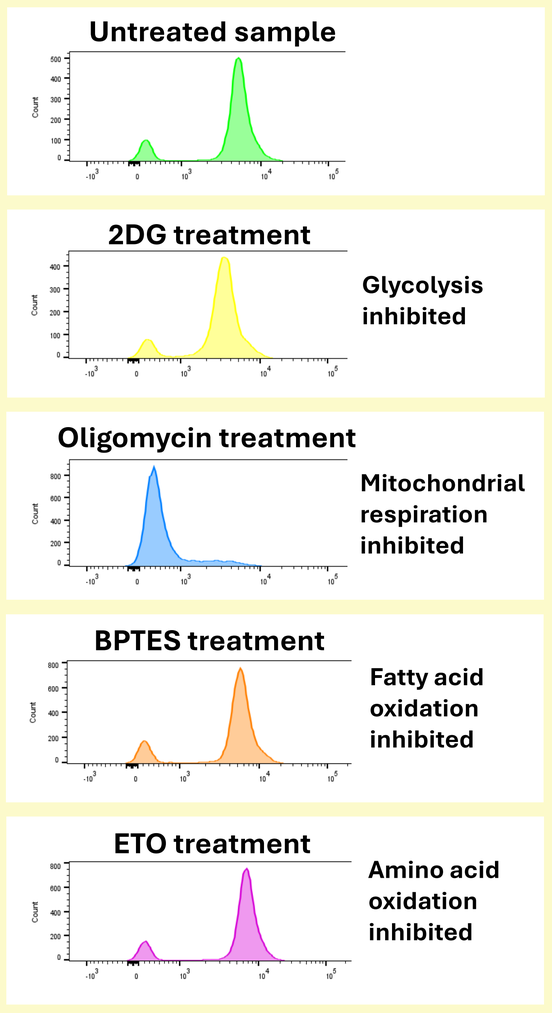
A novel 30-colour spectral flow cytometry panel has been developed that identifies multiple **T cell, B cell, NK cell, monocyte, stromal and endothelial cell subsets in a single sample of peripheral blood mononuclear cells**.



This will enable greater insights into age**-related changes across different circulating immune and stromal populations** across blood and tissues.

SCENITH

Single-cell Energetic metabolism by Profiling Translation Inhibition: (SCENITH) is a novel flow cytometry-based technique **examining metabolic function** through measuring the **incorporation of puromycin** into newly synthesised proteins [1].



Using four different inhibitors of cellular metabolism acting on different targets, we are able to determine:

glucose dependence

mitochondrial dependence

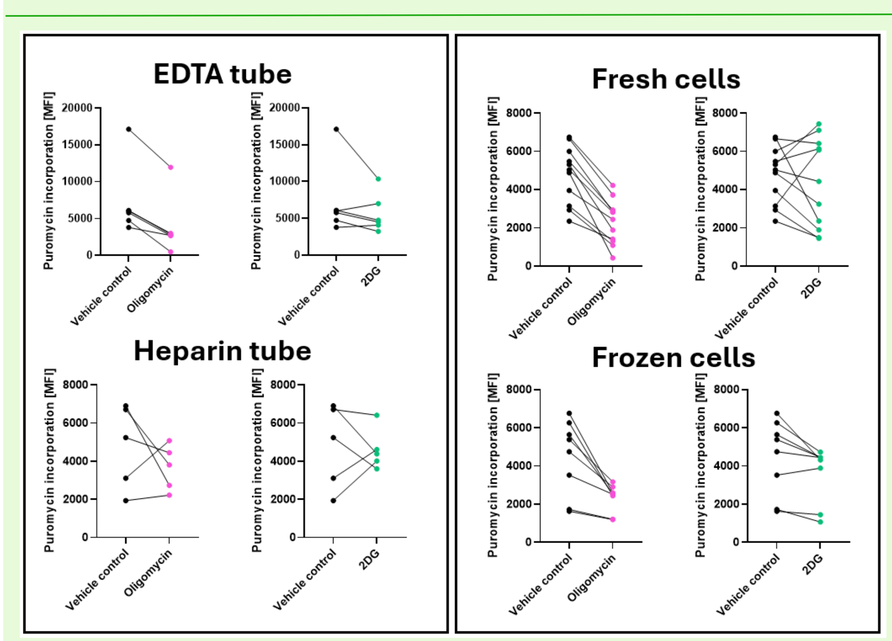
fatty acid oxidation + amino acid oxidation capacity

glycolytic capacity

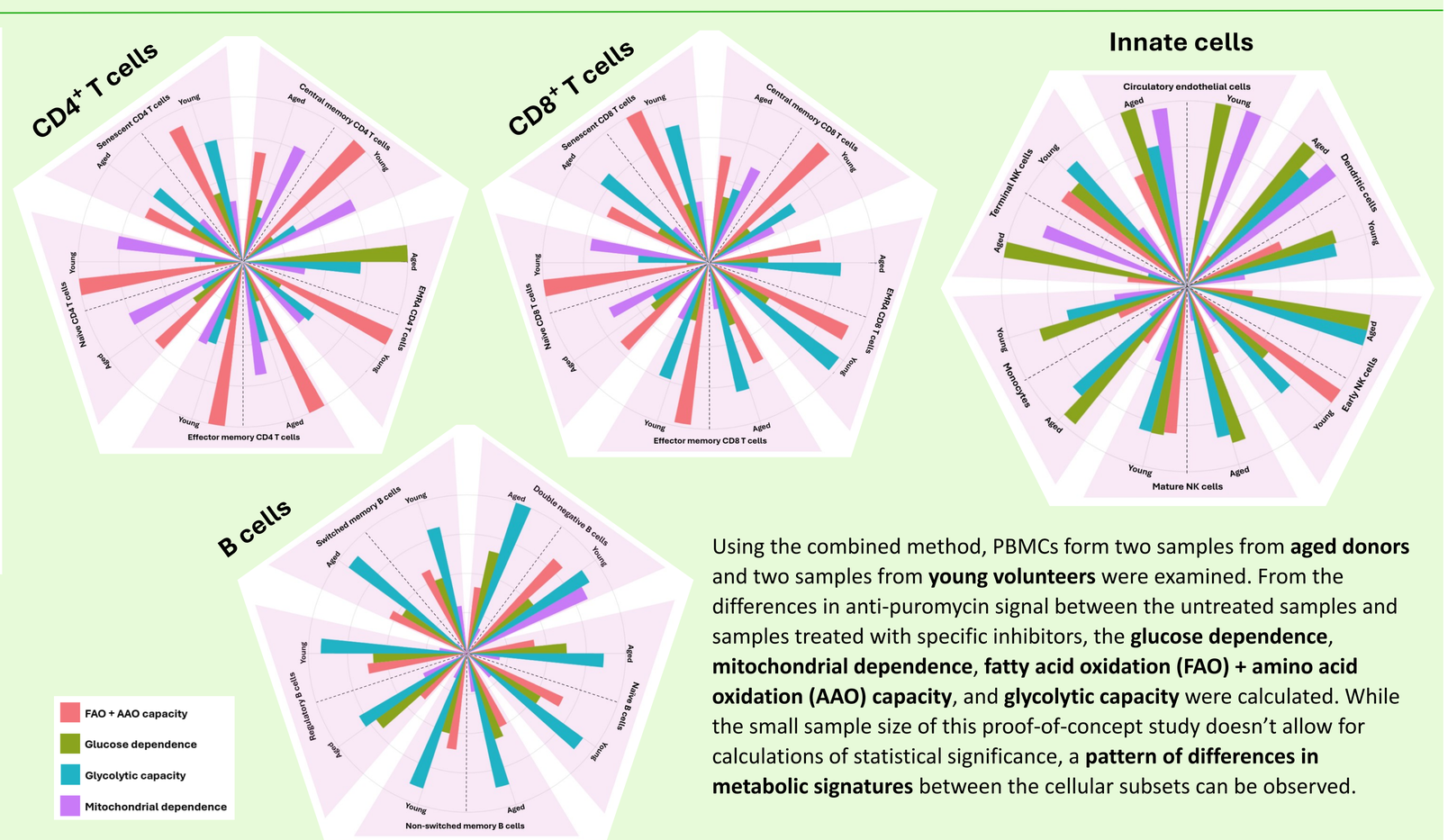
Unlike traditional methods used to study cellular metabolism, SCENITH provides **higher cellular resolution,** can represent the **wider metabolic landscape** of a given tissue/organ, and can be performed using **lower sample amounts.**

High resolution metabolic profiling

The combination of SCENITH and spectral flow cytometry in human PBMCs will provide an extremely **useful and translatable technique** that will significantly contribute to the field of ageing research.



The comparison of ETDA vs Heparin blood collection tubes has shown that the **EDTA anti-coagulant preserves cellular function** better as more of the cells collected form these tubes were responsive to oligomycin and 2DG treatment. Both freshly tested cells and cells tested after cryopreservation responded as expected to oligomycin treatment. 2DG treatment was more effective in the frozen cells, suggesting that **cryopreservation can lead to increased usage of glycolysis** as a source of energy production.



Using the combined method, PBMCs form two samples from **aged donors** and two samples from **young volunteers** were examined. From the differences in anti-puromycin signal between the untreated samples and samples treated with specific inhibitors, the **glucose dependence**, **mitochondrial dependence**, **fatty acid oxidation (FAO) + amino acid oxidation (AAO) capacity**, and **glycolytic capacity** were calculated. While the small sample size of this proof-of-concept study doesn't allow for calculations of statistical significance, a **pattern of differences in metabolic signatures** between the cellular subsets can be observed.

References

[1] SCENITH: A Flow Cytometry-Based Method to Functionally Profile Energy Metabolism with Single-Cell Resolution Argüello, Rafael J. et al. Cell Metabolism, Volume 32, Issue 6, 1063 - 1075.e7