Automated Gating and Antigen-Specific T cell Subset Discovery using FAUST and COMPASS on ICS data

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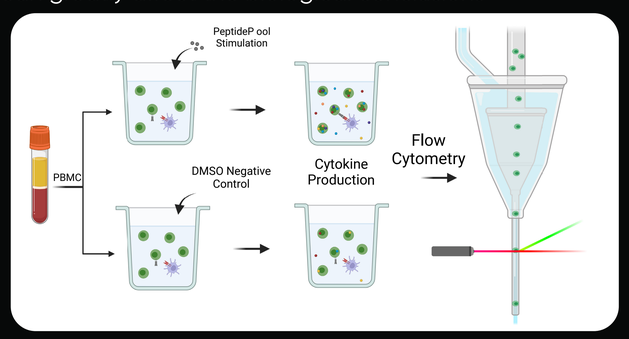
INTRODUCTION

We reanalysed a published Intracellular Cytokine Staining (ICS) dataset[1] in order to:

- assess the robustness of biological conclusions to independent reanalysis
- contrast the inferences derived from computational approaches to those derived from manual analysis

Some challenges associated with ICS analysis include:

- Technical and biological artifact result in high background-levels of fluorescent signal when measuring cytokine levels
- Antigen-specific cell populations can be rare (e.g. 0.001% of all CD4+ T cells)
- Due to the above two points, slight variations in data analysis can greatly affect the biological conclusions



REANALYSIS STRATEGY

Compensation and Transformation:

- Manual analysis and reanalysis were performed using the same compensation matrices.
- Manual analysis used biexponential transformation, whereas reanalysis was performed using the inverse hyperbolic sine function.

Identification of Lymphocytes:

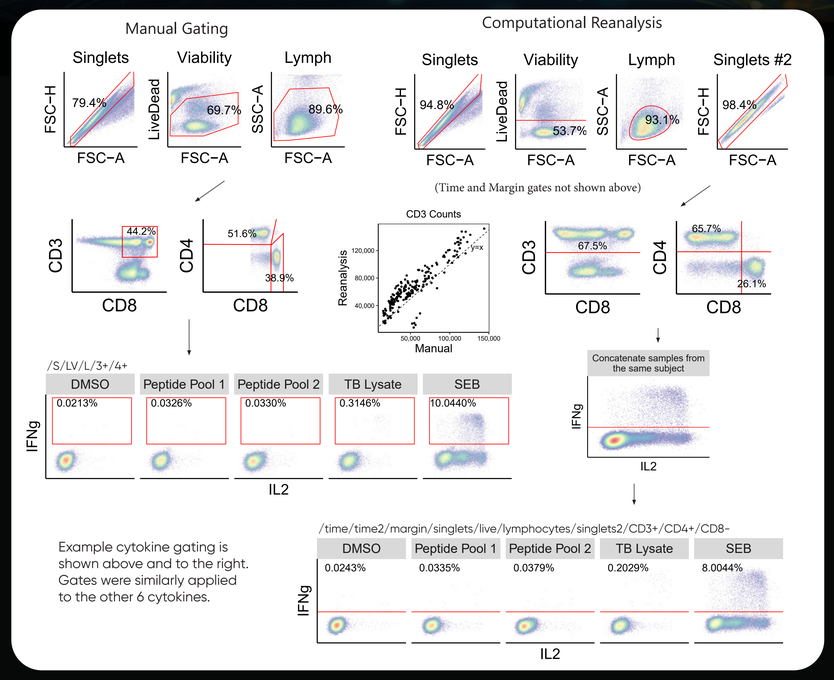
- For reanalysis, events with abnormal acquisiton rates along the time channel and events at the limit of detection for all remaining channels were excluded. Following this, live singlet lymphocytes were computationally gated, followed by gate adjustment using internal tools. Example gating plots are shown in the second column of this poster.

FAUST:

- FAUST (Full Annotation Using Shape-constrained Trees)[2,3] was applied to the remaining protein markers with the subject set as the experimental unit. This choice was informed by the experimental design: biosamples from each subject were assayed by flow cytometry under five stimulation conditions, and SEB stimulation is known to induce cytokine expression on T cells[4]. The adaptive thresholds produced by FAUST for the protein markers were then applied to each sample to identify CD4+ and CD8+ T cell subsets expressing each measured cytokine.
- We observed that the manual CD3 gate excluded an average of ~25% of CD3+ events (which appeared below the default visible range in the manual analysis software).

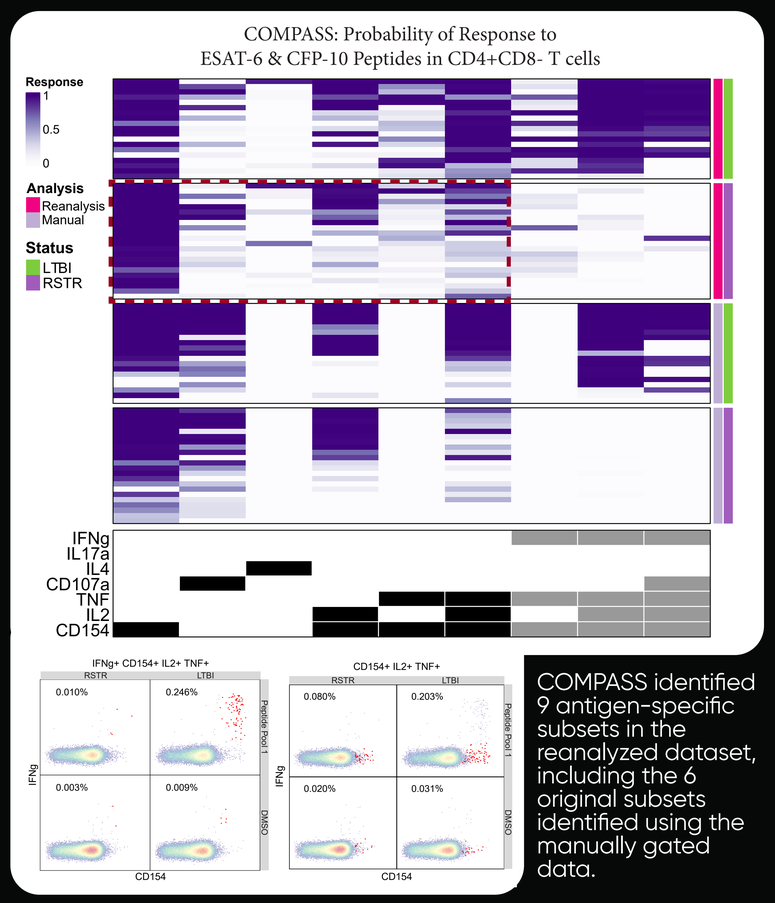
COMPARATIVE ANALYSIS

After gating the data, we identified antigen-specific T cell subsets by running COMPASS (Combinatorial Polyfunctionality analysis of Antigen-Specific T-cell Subsets)[5,6] on the CD4+CD8- T cells, using the cell counts obtained via FAUST for each combination of cytokine positivity.



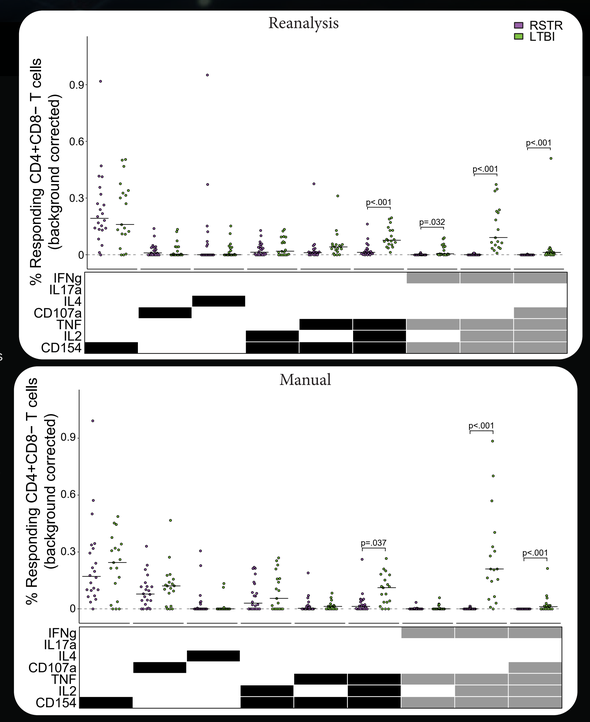
Example cytokine gating is shown above and to the right. Gates were similarly applied to the other 6 cytokines.

In the heatmap below, dark purple shading indicates a high probability of antigen-specific response according to COMPASS. Rows represent subjects and columns indicate a specific combination of cytokine positivity.



COMPASS identified 9 antigen-specific subsets in the reanalyzed dataset, including the 6 original subsets identified using the manually gated data.

The background-corrected magnitude of response for each of the COMPASS subsets is shown in the third column of the poster. The three subsets which were observed to have a greater magnitude of response in the LTBI group in the manual analysis are also observed to have greater response in the LTBI group for the reanalysis. In the reanalysis, an additional IFNg+TNF+CD154+ subset was newly observed to have greater response in the LTBI group. In general, an elevated response for a particular arm and subset in the manual analysis corresponds to an elevated response in the reanalysis, though some subsets have overall lower magnitudes in the reanalysis compared to manual analysis (e.g. CD107a+, CD154+IL2+, and CD154+IL2+TNF+IFNg+).



Statistical testing was performed using the Mann–Whitney U test, with correction for multiple hypothesis testing using Bonferroni's method, and two-tailed P values are depicted. One high outlier point is not displayed in the second column from the right in both plots.

The results of the reanalysis recapitulate the primary manuscript's findings insofar that RSTRs (individuals who persistently test negative to TST and QFN tests despite high levels of exposure to Mycobacterium tuberculosis) possess non-IFNg T cell responses to Mtb-specific protein peptides (ESAT-6 and CFP-10), which provides immunologic evidence of exposure to Mtb.

These responses (in the reanalyzed dataset) are indicated by a red dashed line in the heatmap. They are distinguished from IFNg+ responses (grey subsets), which are not expected to be present among QFN- individuals (because QFN is itself a test for IFNg response).

In the other study arm, LTBI (latent tuberculosis infection), individuals possess both IFNg+ and IFNg- responses to TB antigens. This result was observed in both the original and reanalyzed COMPASS runs.

SUMMARY OF FINDINGS

1. Biological inferences derived from independent computational analysis are consistent with published manual analysis
2. Reanalysis can surface issues with manual analysis
3. If biological inferences are preserved across independent analysis approaches, they are strengthened. Computational cytometry approaches, like those applied to this ICS dataset, provide an increasingly efficient mechanism to conduct independent, complimentary analysis to the standard manual approach.

REFERENCES

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