

A User-friendly Image Analysis Pipeline for Highly Multiplexed Imaging Data

Kenta Yokote¹, Claire Marceaux^{1,2}, Nina Tubau¹, Velimir Gayevski¹, Terry Speed¹, and Marie-Liesse Asselin-Labat^{1,2}

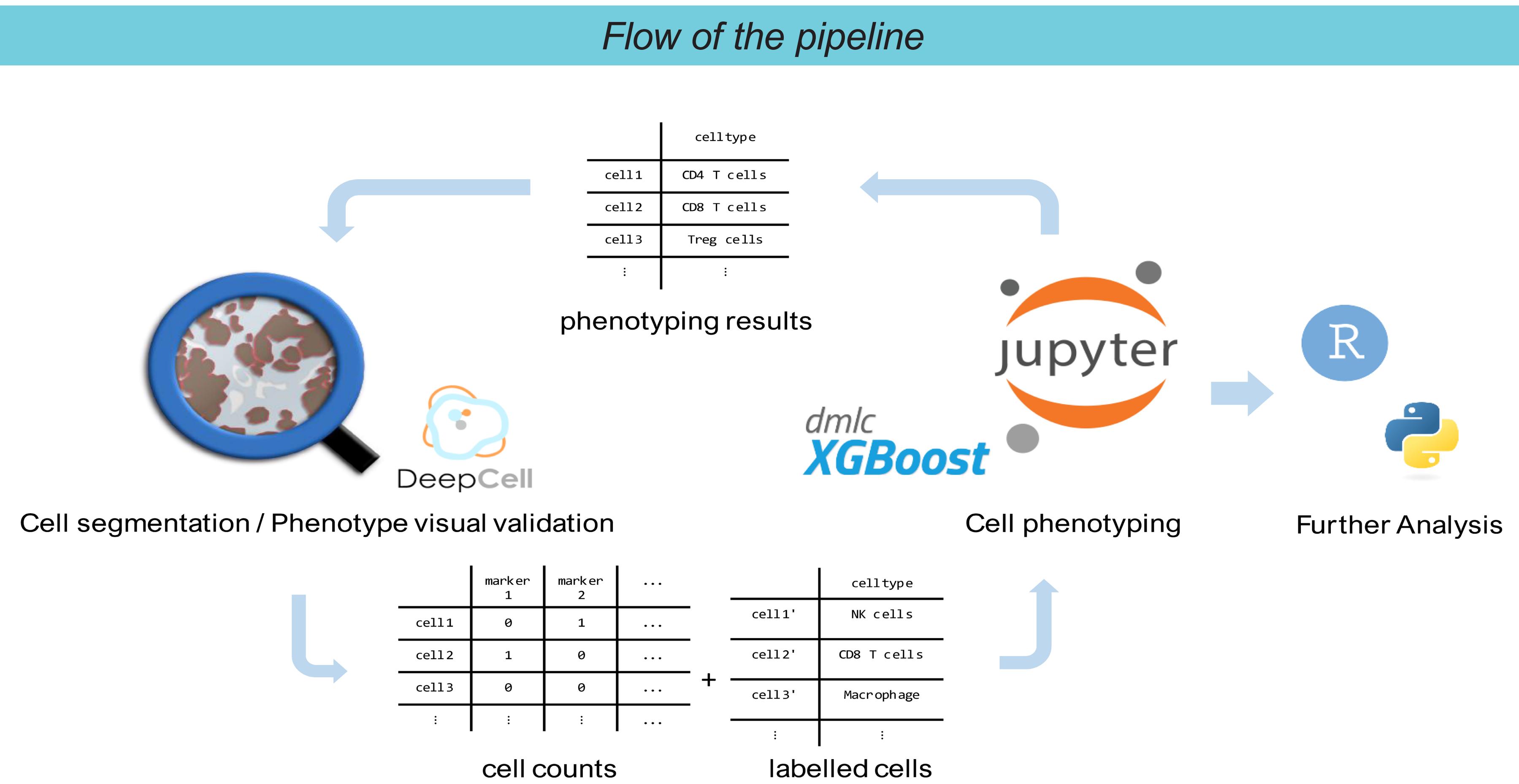
¹ Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3052, Australia

² Department of Medical Biology, The University of Melbourne, Parkville, Victoria 3010, Australia

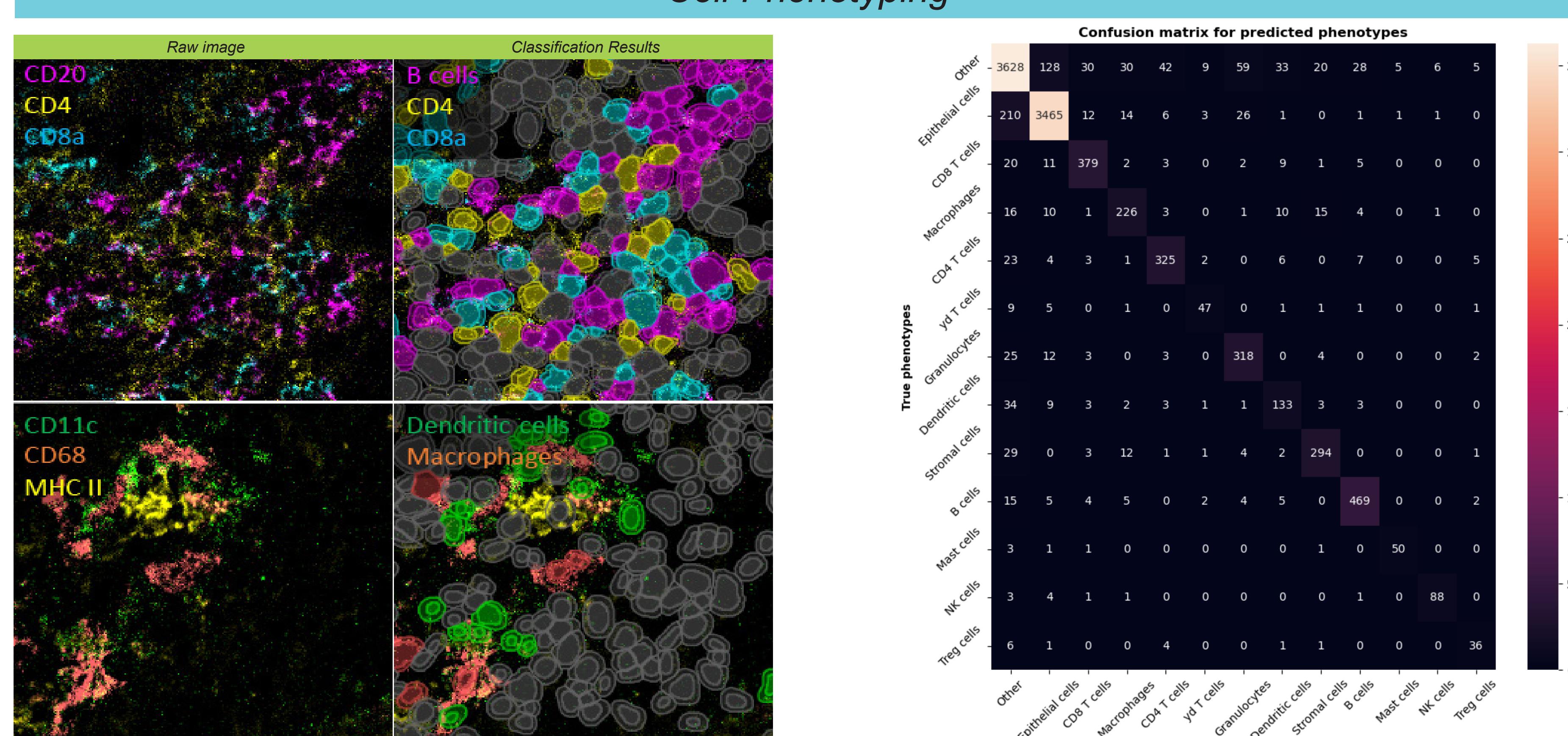
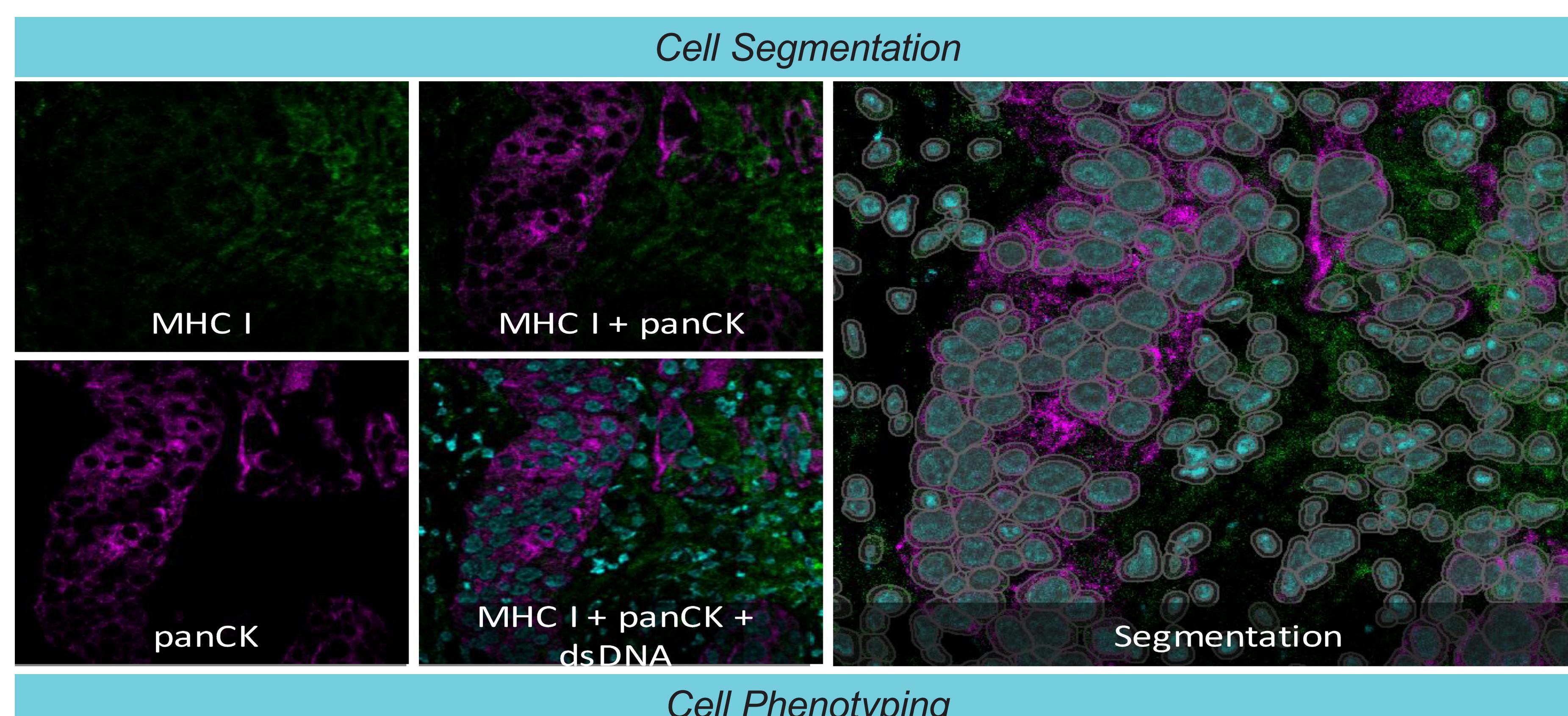
Introduction

- Image analysis tools often come in the form of a package in a programming language.
- This makes using the tools and visual validation of results cumbersome for a biologist without previous programming experience.

Methods



Results



Aim

- To integrate existing tools for cell segmentation and cell phenotyping into QuPath, minimising the need for the user to interact with code.

Cohort

- 90 formalin fixed paraffin embedded NSCLC samples.

Multiplex Immuno-staining

- Sections were stained with a bespoke antibody panel (38 metal conjugated antibodies) and scanned on the MIBIscope (Multiplexed Ion Beam Imaging).

Cell Segmentation

- The segmentation was performed with a QuPath extension of the DeepCell Mesmer model.
- The QuPath extension was developed using TensorFlow Java, Bytedeco OpenCV, and JavaFX.
- A graphical user interface (GUI) was developed for the extension to configure and run the segmentation.
- Each segmented cell has four compartments in which the intensity of each marker is measured: cell, cytoplasm, membrane, and nucleus.
- For every cell, three major summary statistics are calculated describing the intensity of each marker in each compartment: mean, standard deviation, percentiles ranging from 70th-90th.

Cell Phenotyping

- An extreme gradient boosted tree (XGBoost) model was trained with 8 images, ~50, 000 annotated cells.

Discussion

Cell Segmentation

- The segmentation can be easily performed without the need to interact with code.
- In addition to the original implementation of Mesmer, the GUI allows the user to select multiple membrane channels in order to better segment the cell boundary.
- The results can be easily visualised and parameters can be easily changed.

Cell Phenotyping

- The tools developed still allows for relative ease of visually validating the phenotyping results in QuPath.
- The next step will be to implement the phenotyping into QuPath for easier use.
- Due to the selection process of the 8 images on which the model was trained on, the model is more robust to non-biological variations such as batch-effects and noise compared to traditional thresholding methods and clustering methods.
- Achieved an accuracy of 0.8970, a balanced accuracy of 0.8399 and an F1 score (macro averaged) of 0.8325.

References

- Greenwald, N. F. et al. (2022) Whole-cell segmentation of tissue images with human-level performance using large-scale data annotation and deep learning. *Nature biotechnology*, 40(4), 555–565. <https://doi.org/10.1038/s41587-021-01094-0>
- Bankhead, P. et al. (2017) QuPath: Open source software for digital pathology image analysis. *Scientific Reports* <https://doi.org/10.1038/s41598-017-17204-5>
- Chen, T., & Guestrin, C. (2016). XGBoost: A Scalable Tree Boosting System. In Proceedings of the 22nd ACM SIGKDD International Conference on Knowledge Discovery and Data Mining (pp. 785–794). New York, NY, USA: ACM. <https://doi.org/10.1145/2939672.2939785>