

# Imaging Mass Cytometry: a new approach to study the liver microenvironment

Patrik Stoller BMA 17-20A

College of Higher Education  
Educational Program for Biomedical Scientists

Visceral Surgery and Medicine, Inselspital Bern, University Hospital and University of Bern, 3010 Bern, Switzerland

## 1. Abstract

The aim of this thesis is to perform a data analysis for Imaging Mass Cytometry (IMC) raw data with the Histology Topography Cytometry Analysis Toolbox (histoCAT). Due to the Sars-COV-2 pandemic, this was performed with a data set from the Bodenmiller laboratory. It consisted of a human breast cancer tissue sample with 8 regions of interest (ROI). In this sample dataset, cell segmentation was successfully applied, therefore, it was possible to analyze the single cells and also neighborhood analysis could be performed. Three neighborhoods and 12 subsets of cell types could be detected and identified. This thesis shows the importance of data analysis for a successful IMC project.

## 2. Introduction

The data generated from by IMC can be analyzed with the open source software histoCAT, first published in 2017 by the Bodenmiller Laboratory in Zürich. [1] To be able to analyze the data with histoCAT it is important that also different markers for cell segmentation are included so that the software can identify each single cell and then analyze the data correctly.

As histoCAT allows the analysis of each marker within a run separately or all used markers at once, it is possible to analyze the microenvironment of a tissue section with images or statistically. [1]

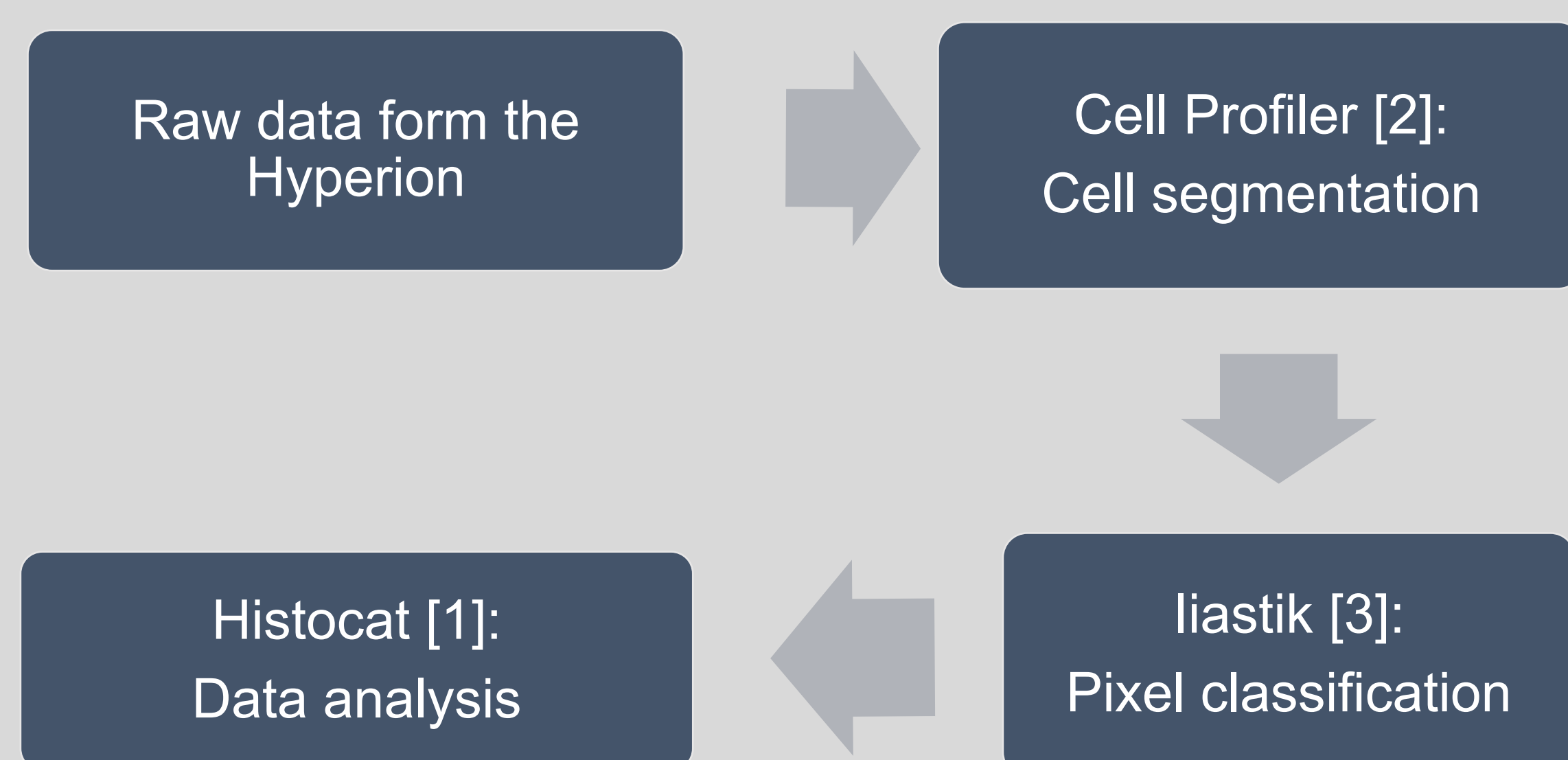
## 3. Aims/ Leading Question

One aim of the thesis is being able to analyze IMC data by using the open source software histoCAT. For the validation of this aim we questioned:

1. Is it possible to perform a cell segmentation with the tissue acquired with the Hyperion correctly by using the cell segmentation pipeline provided by the Bodenmiller lab from the University of Zürich?
2. Can we analyze the cell populations and microenvironment of the tissue by using the neighborhood analysis tool in histoCAT?

## 4. Material and Methods

In order to prepare the raw data correctly, a segmentation pipeline developed by the Bodenmiller laboratory in Zürich has been used. [4] After the preparation, the data was analyzed by using the open source software histoCAT. [1] The steps of the preparation are visualized below.



## 6. Discussion

1. According to chapter 4, we can report that cell segmentation cannot be done by using histoCAT alone. This data preparation is a crucial preprocessing step before the analysis, as the analysis in histoCAT cannot be done until this is accomplished. Cell segmentation attributes the metal signals detected by to Hyperion to individual cells, thus allowing for a single cell analysis. For the cell segmentation the use of multiple programs is necessary, according to the cell segmentation pipeline by the Bodenmiller laboratory. In the heatmap figure 5.1, we show that the cluster merging into the 12 populations from the original 23 Phenograph clusters was performed well. In the heatmap we show that the expected markers used for the identification of the clusters are highly expressed in the according cell type cluster and not or to a lesser extent in the other clusters. Therefore, we can assume that the clustering was performed well.

2. After the successful identification of the cell subsets in the data, we proceeded to the cellular landscape analysis. As output for the performed neighborhood analysis with histoCAT, we produced the neighborhoods for the proliferative tumor cells in figure 5.2. In the eight analyzed ROI we could identify three different neighborhoods. Those three neighborhoods are distinguished by similar detected interactions and avoidance. We can see in the heatmap in figure 5.2, that the proliferative tumor cells mostly interact with each other, the tumor cells (epithelial) and cytotoxic T-cells. Based on these interactions, we learn that in this tumor slice, the microenvironment is less distinctive, and this tumor interacts mostly with itself. Furthermore, it is infiltrated with cytotoxic CD8 T cells that could either be tolerogenic, or cytotoxic if they are activated in this patient sample. In conclusion, the second question for the data analysis is fulfilled due to the proper detection of the three neighborhoods and 12 cell populations with histoCAT for one patient sample. Further analysis would require more than one patient sample and clinical data.

## References:

- [1] Schapiro, D., Jackson, H. W., Raghuraman, S., Fischer, J. R., Zanotelli, V. R. T., Schulz, D., Bodenmiller, B. (2017). histoCAT: analysis of cell phenotypes and interactions in multiplex image cytometry data. *Nat Methods*, 14(9), 873-876. doi:10.1038/nmeth.4391
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- [3] Berg, S., Kutra, D., Kroeger, T., Straehle, C. N., Kausler, B. X., Haubold, C., Kreshuk, A. (2019). ilastik: interactive machine learning for (bio)image analysis. *Nat Methods*, 16(12), 1226-1232. doi:10.1038/s41592-019-0582-9
- [4] Jackson, H. W., Fischer, J. R., Zanotelli, V. R. T., Ali, H. R., Mechera, R., Soysal, S. D., Bodenmiller, B. (2020). The single-cell pathology landscape of breast cancer. *Nature*, 578(7796), 615-620. doi:10.1038/s41586-019-1876-x

## Figures

5.1 Stoller, P. (2020). Heatmap of the 12 subsets on the y axis and the antibody targets they express on the x axis. In red are all the antibodies colored which are expressed in this sub cluster and in blue all the markers which are not expressed in the cluster. Bern: Department for Biomedical Research

5.2 Stoller, P. (2020). Neighborhoods for proliferating tumor cells. On the left and right of the heatmap are dendrograms that group the 8 different Samples and the interactions with proliferating tumor cells. Bern: Department for Biomedical Research

## 5. Results

As no data could be generated by using our panel, an already published dataset from an IMC analysis of breast cancer samples was used. [1]

In a first step, the data was clustered from originally 23 into 12 subset cell clusters in Histocat. In figure 5.1 the 12 merged cluster and their expression pattern are shown as a heatmap.

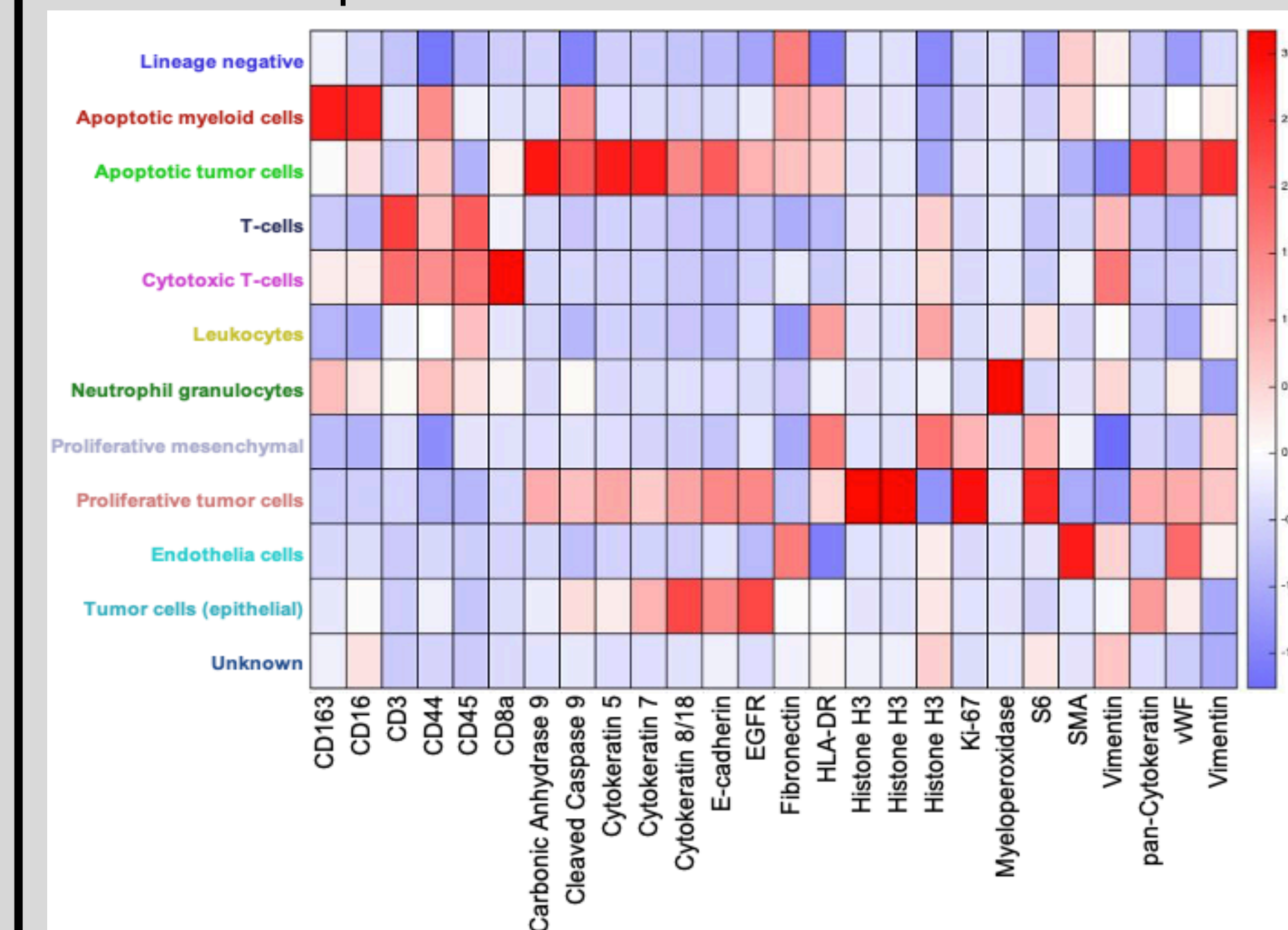


Figure 5.1 Heatmap of the 12 subsets on the y axis and the antibody targets they express on the x axis. In red are all the antibodies colored which are expressed in this sub cluster and in blue all the markers which are not expressed in the cluster. (Stoller, 2020)

After the cell identification, it was possible to run the neighborhood analysis in order to analyze the microenvironment of the tissue. As the data is a cancer sample, we focused the analyzation on the proliferative tumor cell. The results are shown in figure 5.2. In red the significant interactions of the clusters according to the x axis and the legend are shown and in blue the significant avoidances. White means histoCAT could not detect any significant interactions.

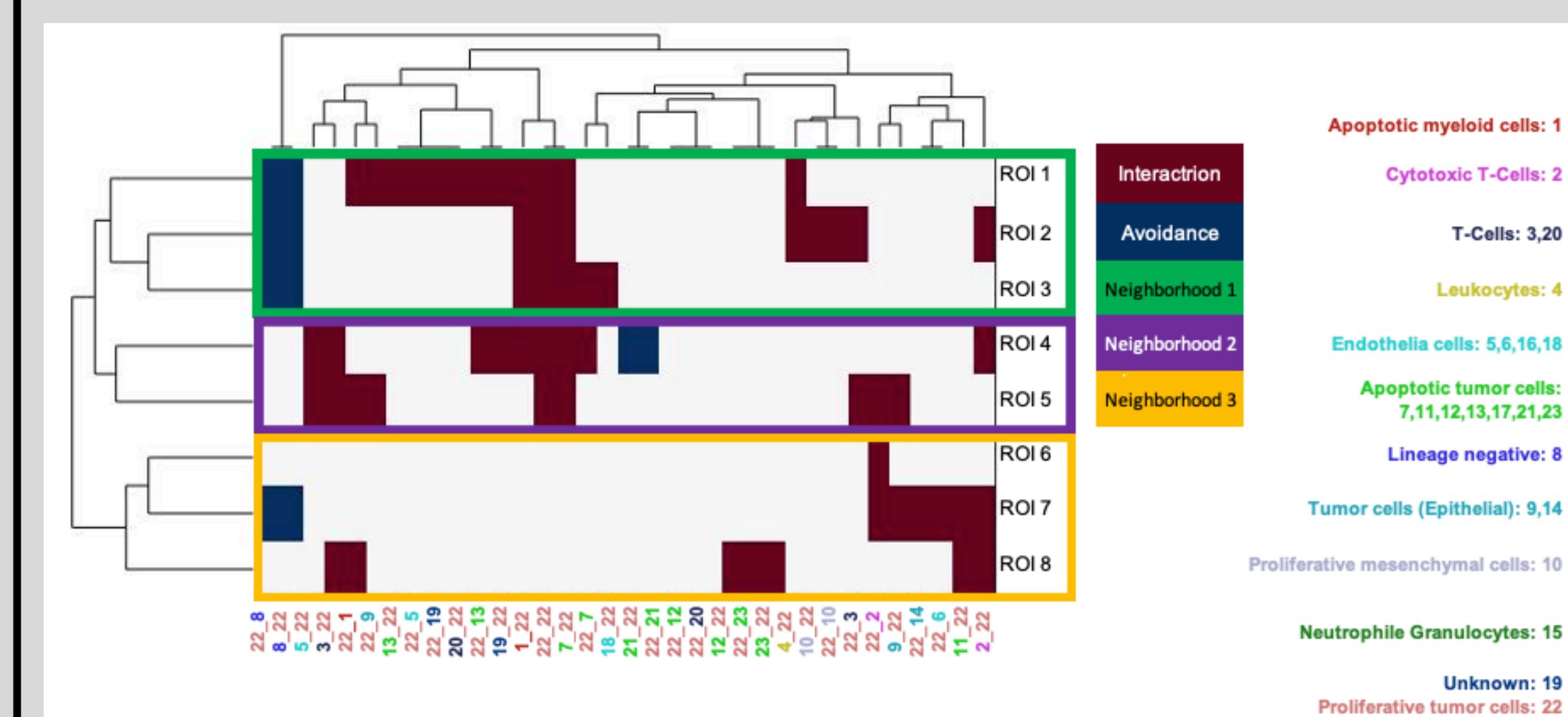


Figure 5.2 neighborhoods for proliferating tumor cells. On the left and right of the heatmap are dendrograms that group the 8 different Samples and the interactions with proliferating tumor cells (Stoller, 2020)