



ADVANCED DATA ANALYSIS TECHNIQUE:

Spatial quantification of biomarkers within target issues with image analysis platform

SUMMARY

Tissue-based biomarker investigation is a key step in pre-clinical / clinical drug development as it can serve either identification of novel therapeutic target, assessment of a surrogate marker of drug efficacy as well as prediction of a candidate compound benefit. The application of quantitative image analysis has thus become an indispensable tool for in-depth tissue biomarker questions in this context. In this perspective, we highlight the use of ImaLink digital pathology analysis platform to unravel cell interplays during complex biological events.

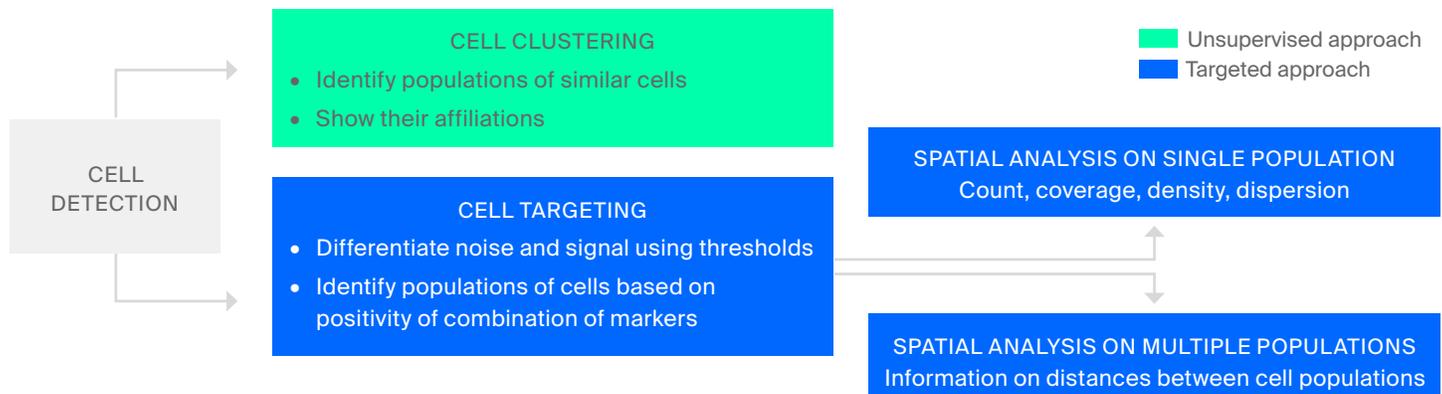
APPROACH

Here we described the analysis workflow (Fig.1) that allows the automation of cell segmentation and their classification to resolve the molecular architecture of the tissue.

The workflow that reports morphological and multiplexed expression data on a cell-by-cell basis across the tissue section, first includes the import of the TIFF images into QuPath to detect the cells and retrieve the information including the intensities for a simultaneous analysis of an unlimited number of fluorescent markers in any cellular compartment – nucleus, cytoplasm, and/or membrane. With the option to define specific

cells phenotypes according to markers positivity the cell targeting module is ideally suited for the characterization of distinct cell populations where multiple markers are required to characterize distinct immune and tumor cell populations within the tissue. Spatial relationship of these population can be further interrogated to understand the mechanism of tissue heterogeneity. Unsupervised machine learning analysis in the cell clustering module allows for the detection of clusters of cells with similar information without providing input parameters a priori. The method allows for the discovery of interesting patterns in data in the context of known biological knowledge.

FIGURE 1. Digital pathology data analysis workflow





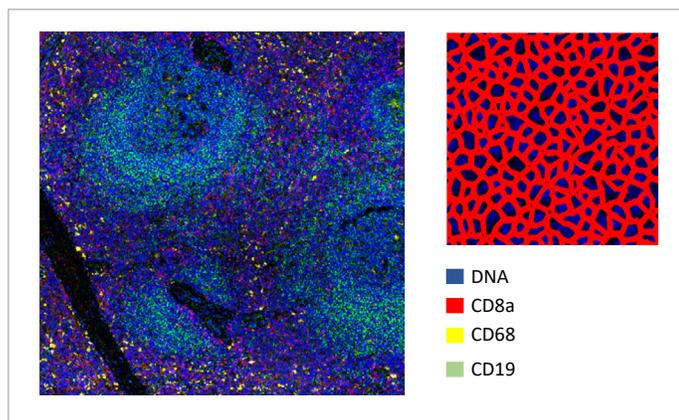
CASE ILLUSTRATION STUDY

In this cases study, we report an example of a tonsil tissue simultaneously interrogated with 33 protein markers using the Hyperion Imaging System from Fluidigm to examine the tissue complexity and cellular heterogeneity. A view of 4 markers out of the 33 used in this study is visualized in Fig.2.

Single cell segmentation

In order to define cells, segmentation algorithm was implemented in QuPath that took into account the presence of nuclear DNA-Iridium intercalator staining. Segmentation around the nuclei was expanded to simulate the cytoplasm, corresponding to individual cell areas, using a combination of threshold and watershed filters.

FIGURE 2. A- View of 4/33 markers of tonsil image and magnification of the automatic cell segmentation

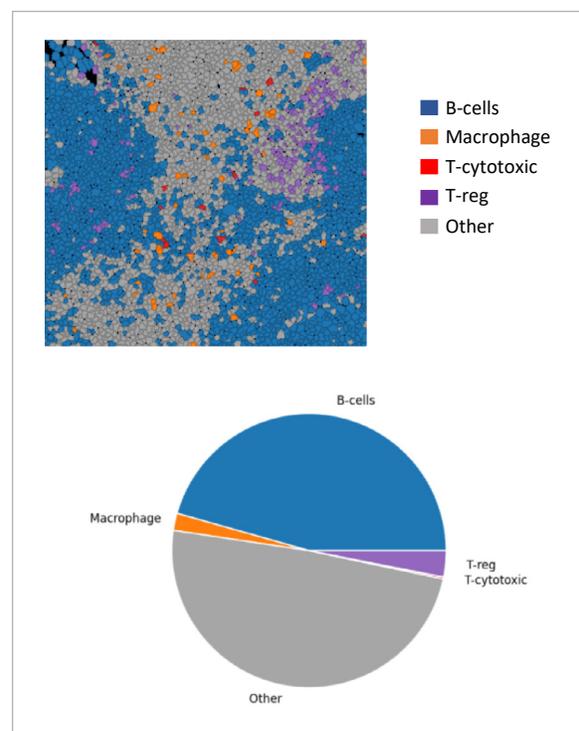


Cell targeting

Next, we interrogated the segmented image for the presence of specific markers. To identify positive signals, we combined automatic thresholding to decompose signal intensities together with manual curation by mixture models to identify positive and negative cutoffs for each individual channel. The cell-type calling results from the algorithm aligned with visual inspection and agreed with manual gating (Figures S2C–S2F).

In addition, the automated cell-type calling was insensitive to cell masks generated independently by two different investigators (Figures S2G and S2H). Estimation of the cell type abundance in the tonsil tissue is shown in Fig. 3 in which each detected immune cell population were given a color to facilitate the representation.

FIGURE 3. Visualization and quantification of cell population in tonsil based on protein marker positivity.





Spatial analysis

Once we have identified our populations, we can perform spatial analysis on a single population. This will provide us with information such as coverage, dispersion and count. Furthermore, we can analyze how multiple populations spatially interact with each other. Figure 6A and 6B show how far Macrophage, T-cytotoxic and T-reg cells stand from B-cells.

FIGURE 5. Coverage, dispersion and count of B-cells in Tonsil tissue.

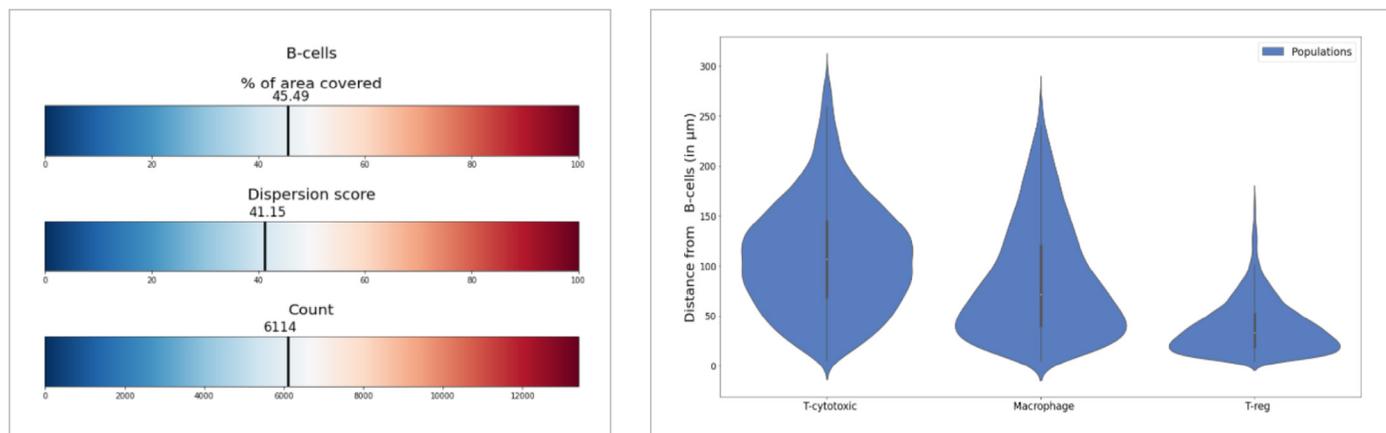
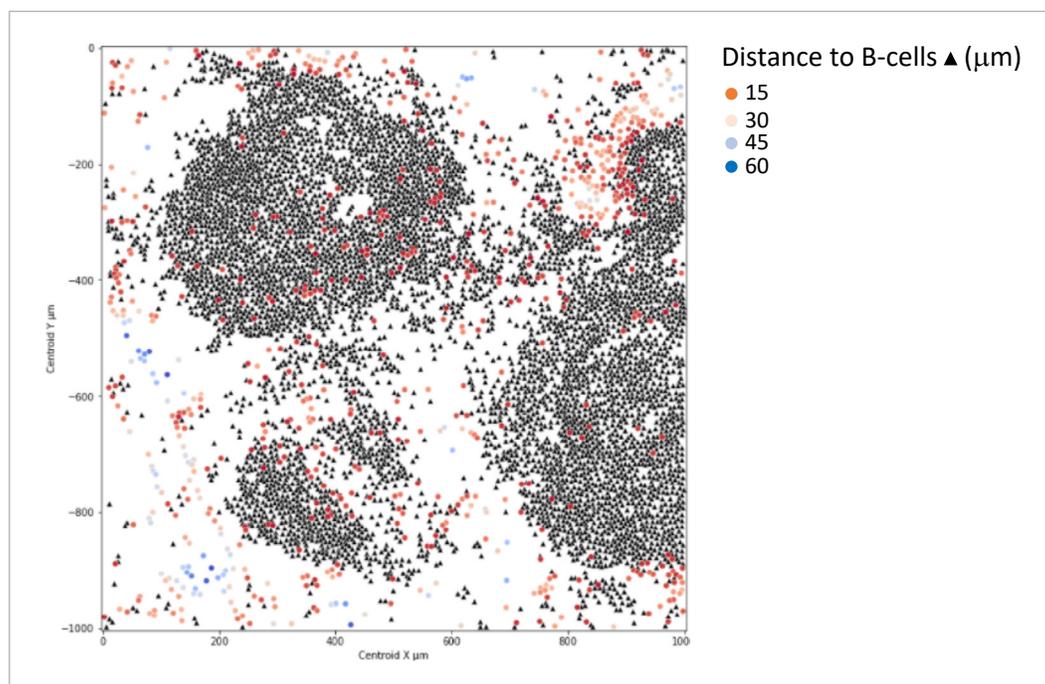


FIGURE 6. A- Macrophage, T-cytotoxic and T-reg (in red/blue) colored by how close they stand from B-cells (in black). B- B-cells distances from T-cytotoxic, Macrophage and T-reg.





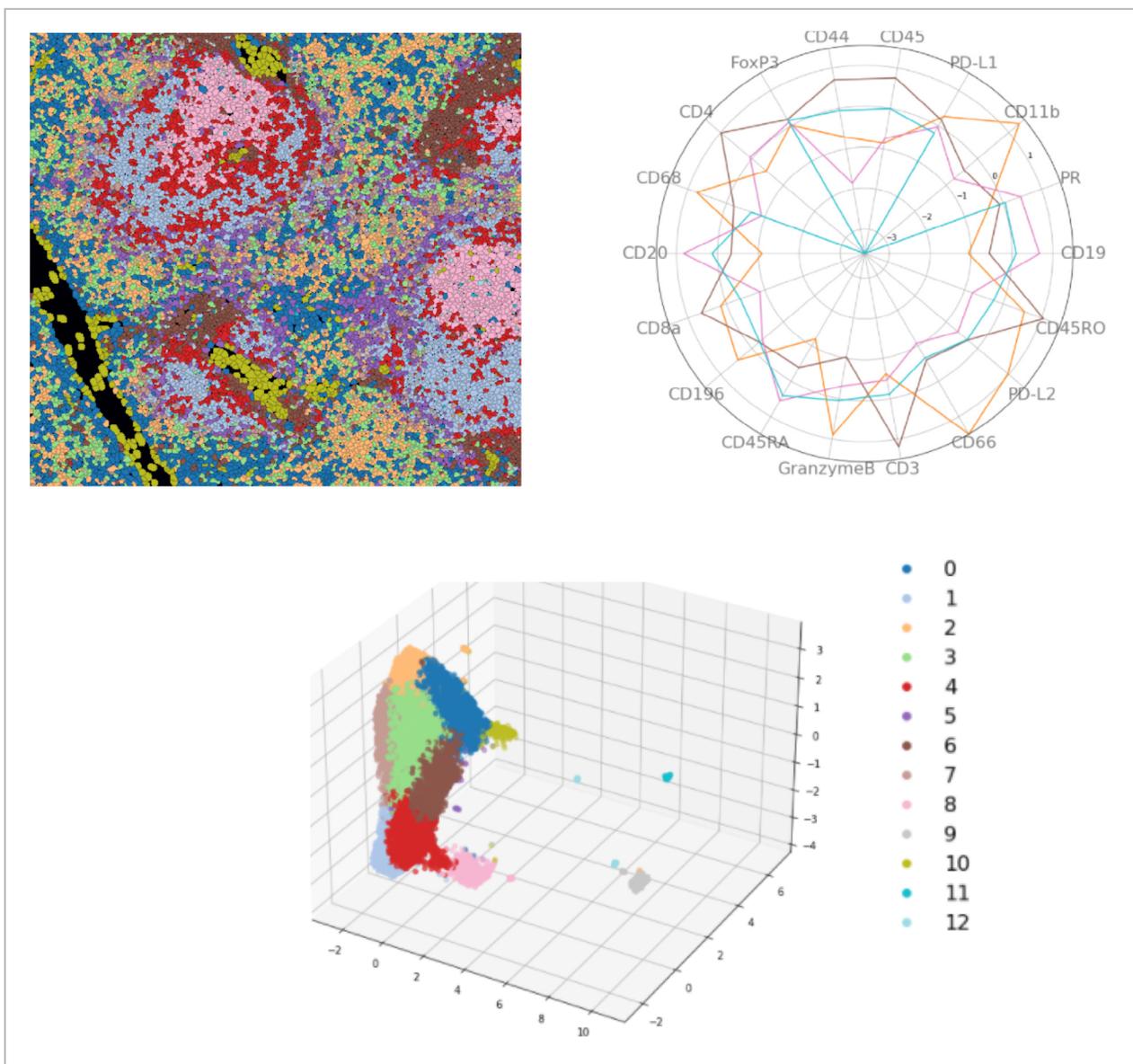
Cell clustering

After normalizing our data, we use a PhenoGraph algorithm to create clusters of cells. Once the cells have been clustered, as seen in Figure 3A, we can easily see which cells have similar phenotypes. We can then look at the representation of each cluster, as in Figure 3B; when looking at the brown line, representing cluster 6, we see that it contains high intensities for marker CD4, CD8a, CD3, CD45RO, CD44 and CD45. The UMAP seen in Figure 3B helps determine which clusters are most similar to each other. In this example we can see that cluster 8 is similar to cluster 12 while being very different to cluster 2.

FIGURE 3. A- Cells in tonsil tissue colored based on their clusters from PhenoGraph.

B- Representation of cluster 2,6,8 and 12 based on mean markers' intensities.

C- UMAP representing each cell on a 3d plot based on markers' intensities, each cell is colored based on clusters





APPLICATIONS

Given an original image, which could represent all sorts of different tissues, applying our digital pathology analysis will return a large amount of data. A typical application of this data is to focus on a specific population, looking at its density and how other populations of cells spatially interact with our population. Having images from different contexts, such as different progression of a disease or with vs without treatment, is very informative as we can look at how populations' densities changed and how spatiality was affected.

These observations will be very useful in drug development by identifying:

New potential drug targets

New efficacy biomarkers/
predictive biomarkers

Impact of drug on tissue

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