ImaBiotech
Revealing Biological Secrets

APPLICATION NOTE

SPATIAL QUANTIFICATION OF BIOMARKERS WITHIN TARGET TISSUES WITH IMAGE ANALYSIS PLATFORM

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 interrogate 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.

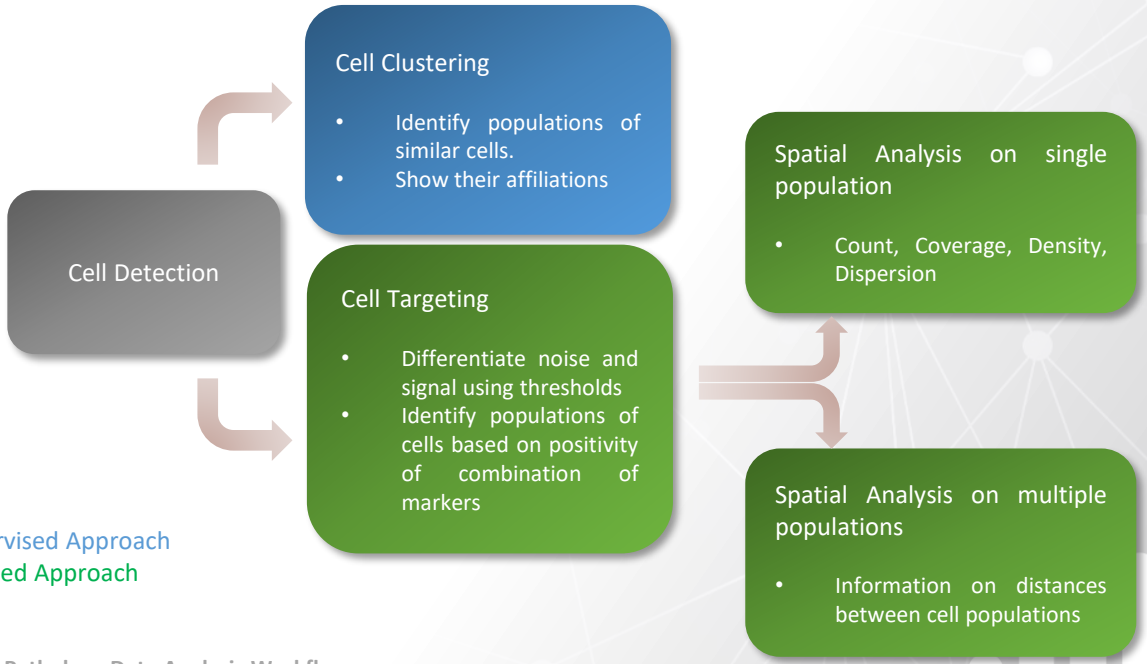


Figure 1: Digital Pathology Data Analysis Workflow

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

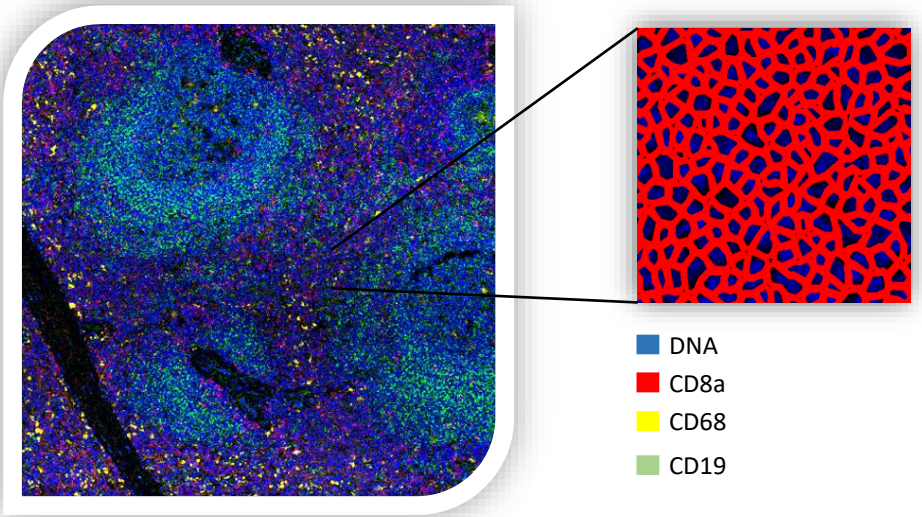


Figure 2: A- View of 4/33 markers of tonsil image and magnification of the automatic cell segmentation

Cell Targeting

Next we interrogated the segmented image for the presence of specific markers. To identify positive signals, we combined manual thresholding to decompose signal intensities together with automatic curation by mixture models to identify positive and negative cutoffs for each individual channel. The cell type calling further resulted from the algorithm aligned with visual inspection and agreed with manual gating. Quantification of the cell type abundance in the tonsil tissue is showed in Fig. 3 in which each detected immune cell population were given a colour to facilitate the representation.

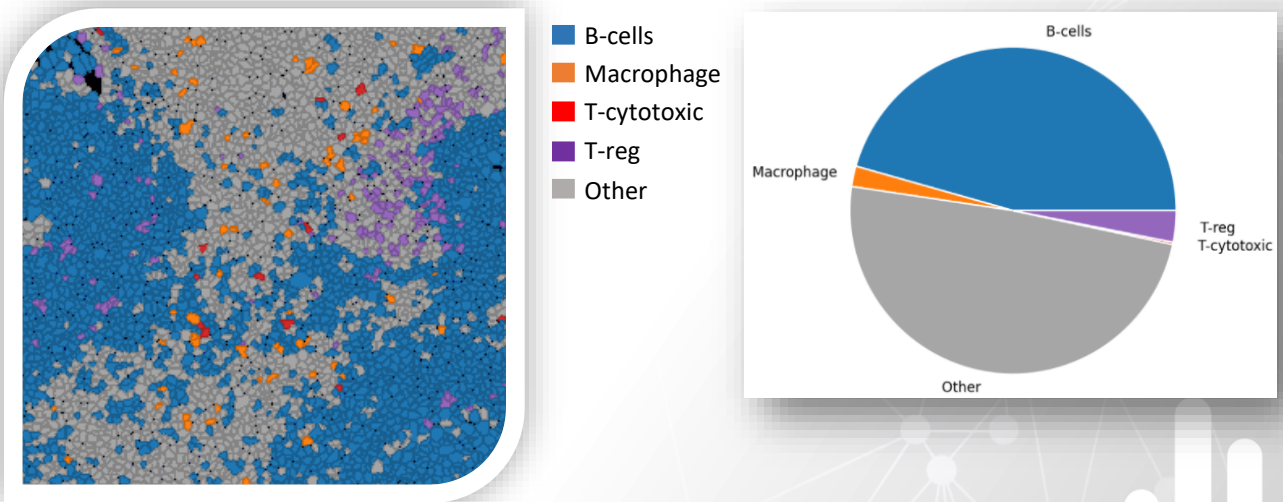


Figure 3: Visualization and quantification of cell populations in tonsil based on protein marker positivity.

Spatial Analysis

In order to identify proximity and relative spatial distribution of cells across the tonsil tissue, we first applied basic morphometry algorithms on *single population* independently (Fig.4A) and analyzed how *multiple cell populations* spatially interact with each other by computing the number of cells within a certain distance of another cell (Fig.4B). Distance is set to 15 microns. Macrophages, Tcytotoxic and Tregs positive cells within 15 microns of a B cell are labeled in orange and Macrophages, Tcytotoxic and Tregs positive cells within 60 microns from B cells are labeled blue. A corresponding violin plot is automatically generated and represents unique immune cell type distance to B cells (Fig. 4C) to show how important cell-cell interactions are within a disease or pathology context.

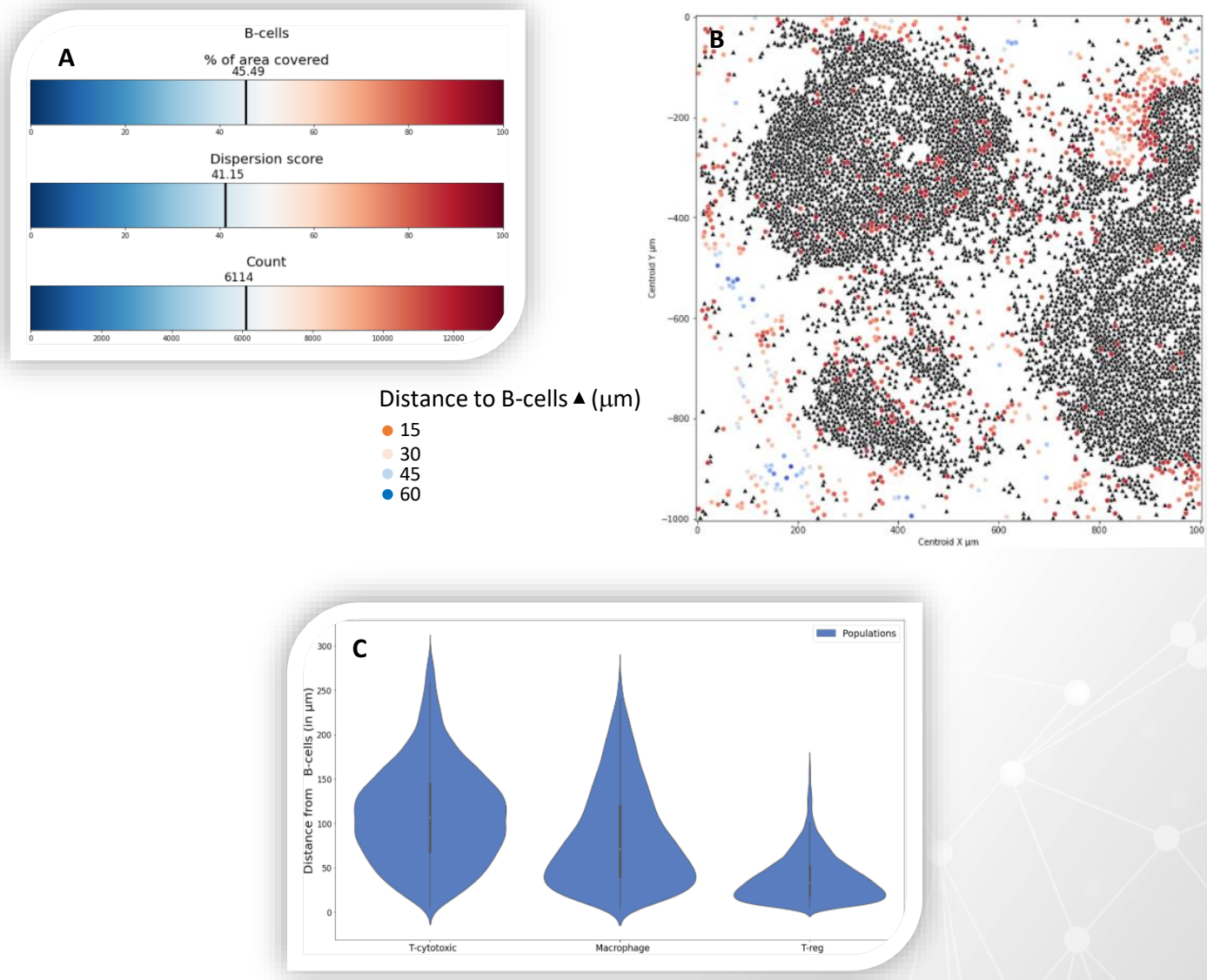


Figure 4: A- Coverage, Dispersion and Count metric for the B-cells population. B-cells distances from T-cytotoxic, Macrophage and T-reg. C- Colored Macrophage, T-cytotoxic and T-Reg cells according to their distance to B-cells,

Cell Clustering

To reveal cell similarities in the tonsil tissue without *a priori,* the unsupervised analysis module started with preprocessing steps that included the log transformation to reduce the skewness of the original data followed by a standardization. We then applied the Python implementation of the PhenoGraph algorithm to create and extract clusters from our cells. Clustering results from the tonsil tissue can be visualized (Fig. 5A). Each colour represents a cluster of cells showing phenotypic similarities.

The spider map (Fig. 5B) indicating the intensity of the signal for each relevant markers in each cluster allowed the detailed content comparison between each cluster. The projected results in a 3D space resulted in spatial phenotyping that showed the different cells in the same cluster attributed one colour (Fig. 5C).

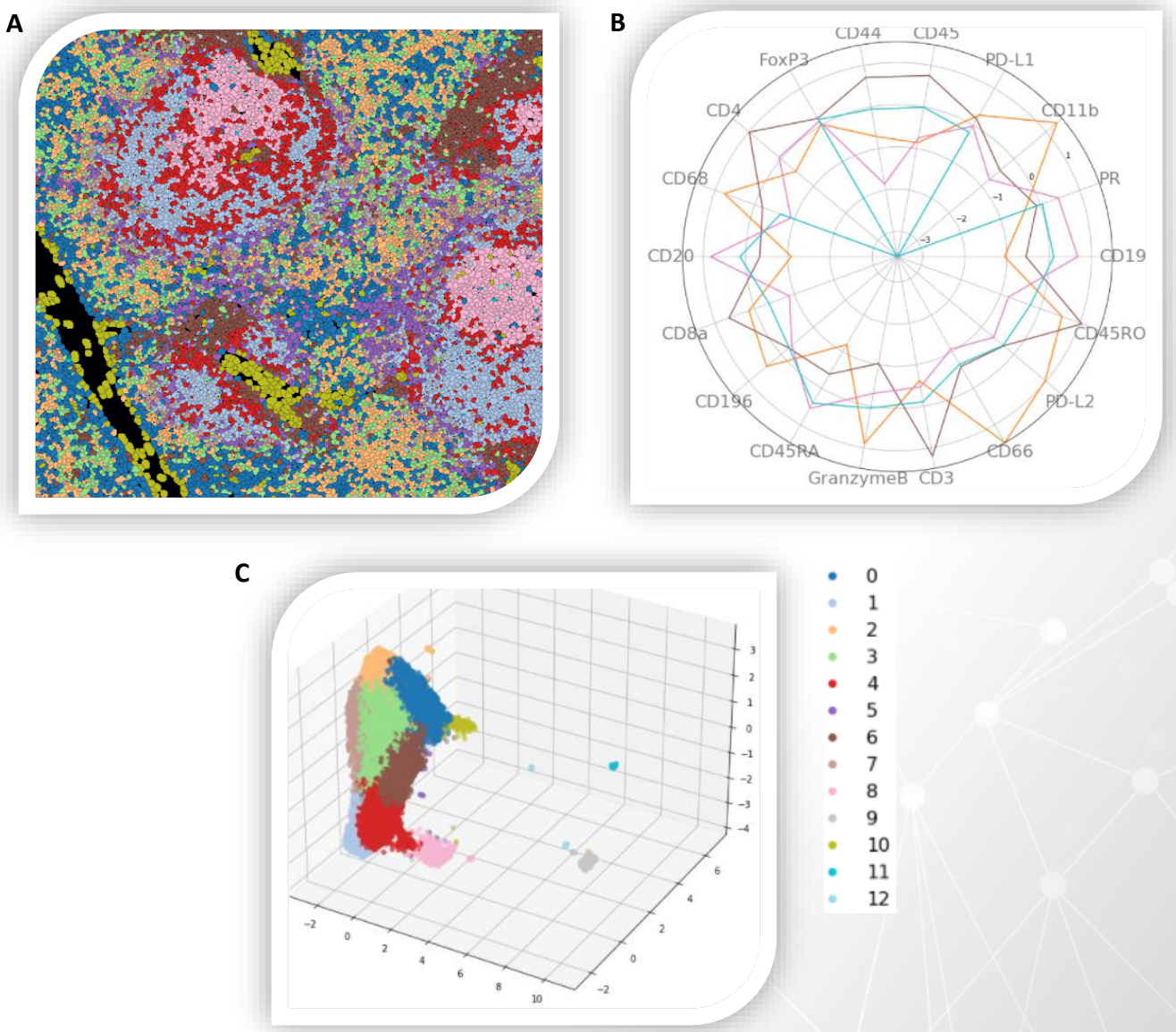


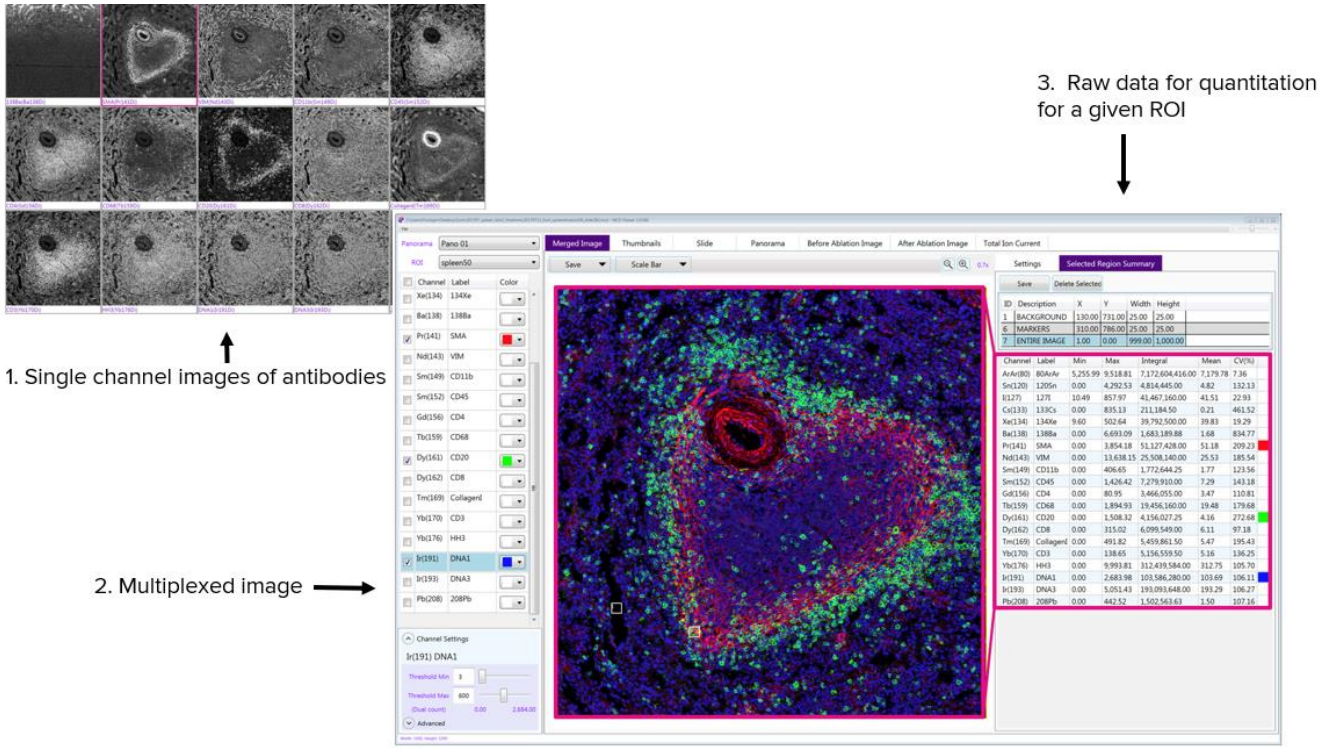
Figure 5: A- Cells in tonsil tissue colored based on their clusters from PhenoGraph. B- Spider Representation of cluster 2,6,8 and 12 based on mean markers' intensities. C- 3D projection of the result of UMAP based on markers' intensities, each cell cluster is given a colored based on clusters. There are 12 clusters.

APPLICATIONS

What role do cellular interactions play in promoting or suppressing disease? How do the same cell types behave differently based on their cellular microenvironments in areas such as oncology, immunology, and neurology?

These conceptual phenotypes based on adjacencies between cell types are providing new insights into inflammatory process and tumour progression to reveal:

- Novel drug targets
- Novel efficacy biomarkers/predictive biomarkers
- Novel Impact of drug on tissue



For more information visit our website www.imabiotech.com
Or contact us at contacts@imabiotech.com

ImaBiotech France
152 rue Dr Yersin 59120
FRANCE

ImaBiotech USA
44 Manning Road Billerica
MA 01821
USA