Cell Analysis

Advancements in multiplexed spatial phenotyping of CNS tissue using Primary Antibody Conjugates

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Poster #LBA38

Abstract

Spatial omics is an expanding research area focused on integrating spatial knowledge of tissue with transcriptomics (RNA) and proteomics (protein). The intrinsic tissue complexity of biological structure is an important aspect of brain and cancer immunotherapy research, requiring accurate target classification. Simultaneous translational profiling of 4+ targets on a single sample presents challenges that include panel design, staining protocols, and data analysis. Furthermore, designing a reliable multi-target biomarker panel in the brain introduces complexities such as protein abundance and localization, fluorophore compatibility, diverse cell types, and data characterization. While recent advances in cyclic staining and target detection with automated fluidics enable a growing plexity of diverse target types, these approaches suffer from low simultaneous target throughput. Here, we test and compare a streamlined process for CNS and other tissue types that enables detection of high multiplexed panels, completed within a couple of hours. With this new methodology, we explore detection of a range of protein markers across CNS and other tissue types used in biological research applications with our uniform technique of labelling.

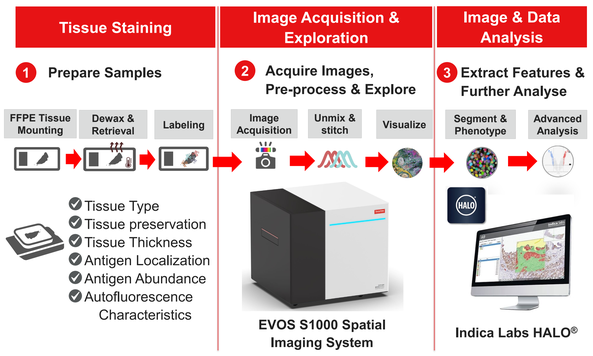
Introduction

Spatial Biology: Analyzing spatial relationships within organs and between cells on tissue to investigate organization of biomolecules, cellular structures, cellular communication, and how their arrangement influences biological function and behavior.

Ability to analyze single cells in spatial context provides a variety of information
- Cellular identity in time and space
- Cell types, cell states and cell functions
- Cell-cell interactions
- Cellular neighborhoods
- Tissue microenvironments and architecture

Workflow

Immunology targets labeled with antibody-based detection, to characterize how cells interact across complex tissues imaged on a high-resolution spatial slide scanner.



Cellular Phenotyping Characterization

Visualize and quantify cellular traits of innate and adaptive immune cell populations.

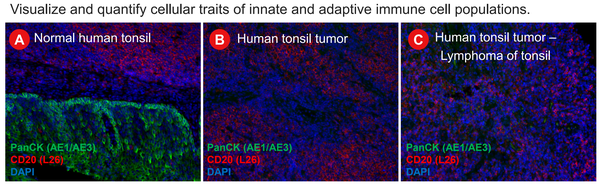
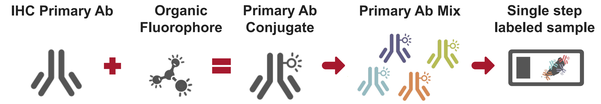


Figure 1. Different types of FFPE Human Tonsil labeled using our IHC validated primary antibody conjugates. (A) Normal Human Tonsil consist of distinct tissue structure with a clear separation between the epithelial layer and B cells. (B) Human tonsil tumor exhibits a loss of structure organization and the epithelial layer. (C) Lymphoma of human tonsil is characterized by a complete absence of normal tissue structure and a disarray of B cells.

Antibodies and fluorophores for spatial biology

Primary Antibody Conjugate mix

Convenient labeling method offered in a variety of fluorophores, including Alexa Fluor and Alexa Fluor PLUS dyes conjugated to highly validated IHC antibody clones for single-step labeling.



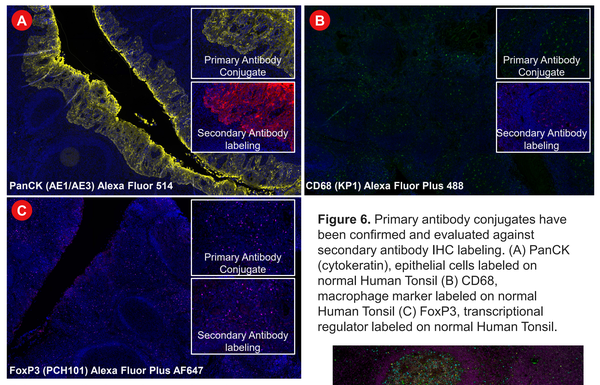
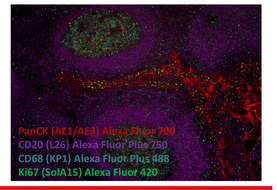


Figure 6. Primary antibody conjugates have been confirmed and evaluated against secondary antibody IHC labeling. (A) PanCK (cytokeratin), epithelial cells labeled on normal Human Tonsil (B) CD68, macrophage marker labeled on normal Human Tonsil (C) FoxP3, transcriptional regulator labeled on normal Human Tonsil.

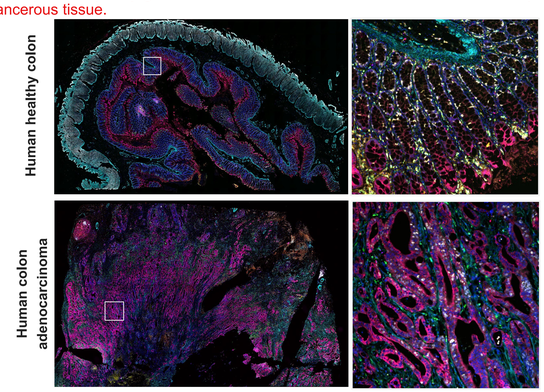
Primary antibody conjugates have been validated across various tissue types, including normal and certain cancerous human tissues: spleen, appendix, duodenum, tonsil, thymus, cerebellum, liver, colon etc…



Right Figure 7. Multiple primary antibody conjugates can be combined in a single mixture for multiplexing on a single sample. Human Tonsil

Multiplex staining with Primary antibody conjugates`

Primary antibody conjugates can be used to discern differences between healthy and cancerous tissue.



Phenotyping of the Central Nervous System

Human brain cancer grades

Brain tumor grading system features four distinct grades and provides an understanding of how the tumor is growing. The following tumor characteristics are the focus of assessing brain tumors: size and location, type of cells affected, and the spread of the cancer.

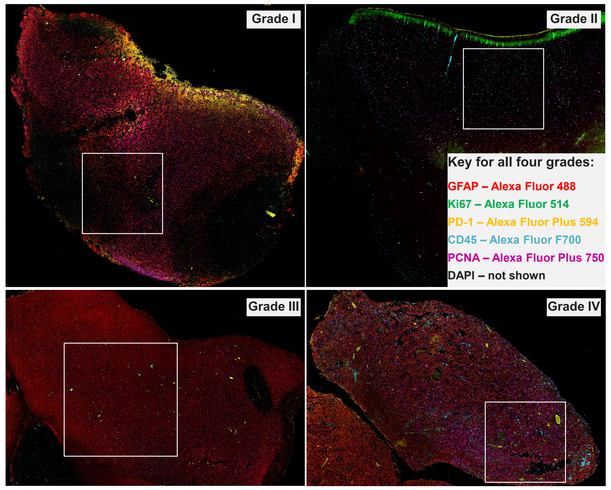


Figure 8. Representative cases from each of the four grades of brain cancer. (A) Grade I – Pilocytic Astrocytoma. (B) Grade II – Low-grade Astrocytoma. (C) Grade III – Anaplastic Astrocytoma. (D) Grade IV – Glioblastoma. White boxes are zoom in for figure 9.

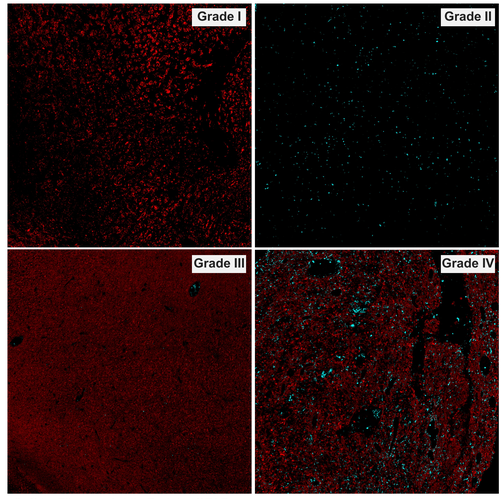
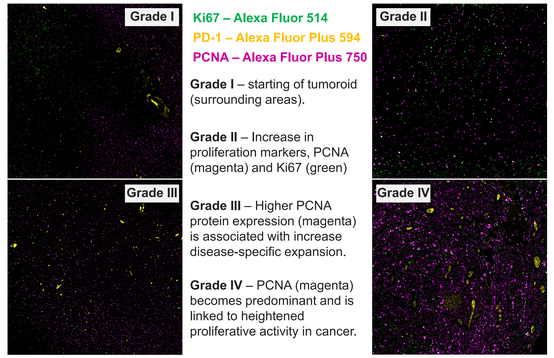


Figure 9. Glial Fibrillary Acidic Protein (GFAP) expression levels vary across different grades of glioblastoma. GFAP identifies malignancies of glial origin (red). CD45 expression levels correlates with higher incidence of metastasis and high grade of glioblastoma (teal).

Overview of proliferation and invasion

Proliferation associates with an increase in brain grade stages

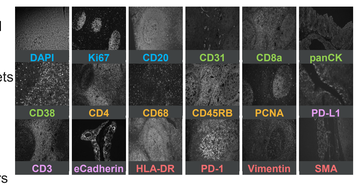


Primary antibody labeling advantages

Challenge:	Primary antibody approach:
Time to multiplex sample	Highthroughput – 8 plex within one hour
Efficiancy of workflow	Ready to use conjugate
Target degradation due to stripping cycles	Decrease in number of stripping steps to obtain high pexibility
Signal to noise ratio	Efficient low signal amplifciation
Workflow optimzation	Similar to known primary workflow

Steps in determining fluorophore selection

1. Identify main target of interest
2. Determine antigen expression level
3. Save brightest fluorophores for dimmest markers
   - Use on the most important targets
   - Use on worst resolved targets
   - Low/unknown expression
   - Poor access to antigen
4. Minimize spillover using known expression patterns
   - Space out co-expressed markers
   - Mutually exclusive markers in adjacent channels
5. Plan for autofluorescence
6. Avoid using dim or low expressing targets in channels with wide spectrums
   - The ability to resolve populations is a function of autofluorescence, background, and co-expressing markers.



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