Segmentation and Classification of Single Cells using Multiplexed Ion Beam Imaging (MIBI)

Jay G. Tarolli[1], Rachel Finck[1], Murat Aksoy[1], Yari Sigal[1], Noah Newgren[1], Jessica Finn[1], Jason Ptacek[1]

[1]IONpath, Menlo Park, CA

Introduction

When studying the tumor microenvironment, knowing not only the types of immune cells present but also the spatial distribution and relationship of these immune cells to other immune and tumor cells provides crucial information. In the past, techniques used to analyze these spatial relationships have been limited by the number of biomarkers that could be simultaneously measured. Recently, with the development of multiplexed ion beam imaging (MIBI), 40+ biomarkers can be simultaneously measured in a single scan. By probing with an ion beam, tissue sections can be imaged at a spatial resolution on the same order of magnitude as light based techniques, providing subcellular resolution. This combination of multiplexed biomarker measurements and subcellular spatial resolution enables segmentation of the image into individual cells, making possible subsequent cell type classification and quantification.

Antibody Labeling and Tissue Staining

MIBI is the combination of metal tag labeling of antibodies which target proteins of interest in a tissue sample with subsequent ToF-SIMS imaging. Isotopically pure metal atoms are conjugated to an antibody which will selectively bind to a protein of interest in the sample. A mixture of 27 different antibody/metal tag pairs was used to stain sections from a variety of tissue types, including tonsil, lung adenocarcinoma, lung squamous cell carcinoma, lymphoma, and melanoma.

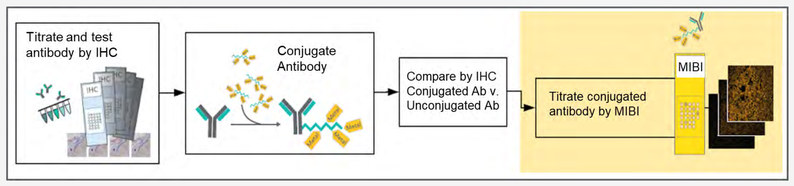

Figure 1. Antibody conjugation and validation workflow. Performance of antibodies is first assessed by IHC before conjugation to metal labels. Conjugated antibody performance is then measured with MIBI for comparison to IHC.

MIBI Imaging with Time-of-Flight Secondary Ion Mass Spectrometry

A specifically designed time-of-flight secondary ion mass spectrometry (ToF-SIMS) instrument was built to image formalin-fixed paraffin embedded (FFPE) tissue sections stained with metal tagged antibodies. The MIBI instrument utilizes a high current, tightly focused primary ion gun to raster the tissue surface. An electrostatic analyzer following the extraction optics preferentially filters diatomic and polyatomic sputtered species to increase the ratio of metal tag to organic fragments and other background signal. Using a ToF mass analyzer enables the parallel detection of 40+ metal tags in a single scan.

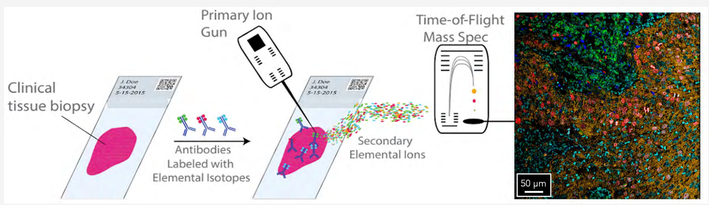

Figure 2. Diagram of the tissue staining procedure and subsequent imaging using ToF-SIMS. The image is of a lung squamous cell carcinoma tissue section showing the distribution of dsDNA (cyan), CD45 (green), CD68 (blue), keratin (orange), and Ki-67 (red).

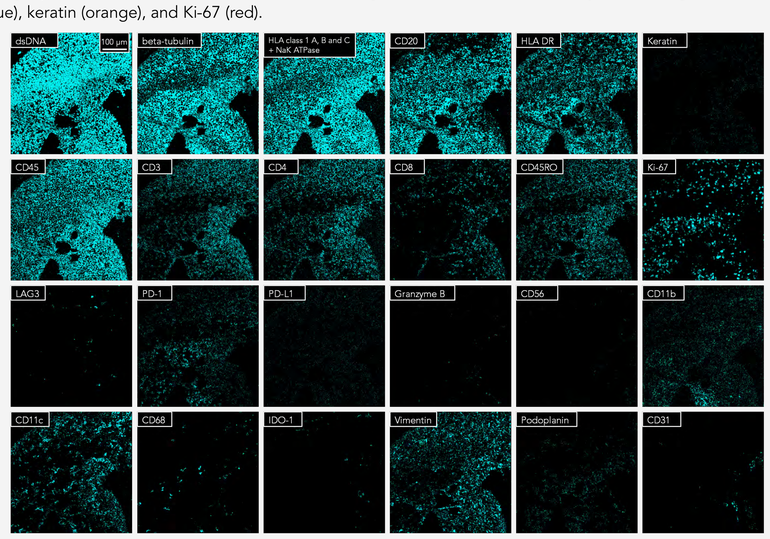

Figure 3. Single channel images from a tonsil tissue section. All images are 1024 x 1024 pixels of a 500 µm x 500 µm area.

Single Cell Segmentation

Subcellular spatial resolution can be leveraged to segment a MIBI image into individual cells instances. A convolutional neural network was trained to predict cell locations utilizing the multiplexed nature of MIBI data. Using the predicted locations as seed points, boundaries between cells were then defined using watershed segmentation.

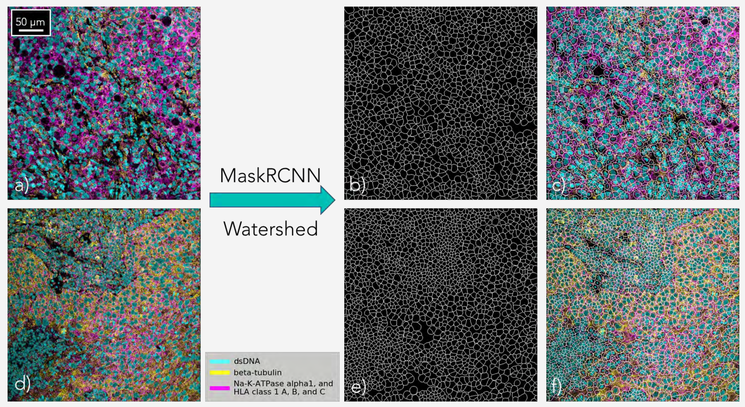

Figure 4. a) MIBI image of a lung adenocarcinoma tissue section representing the input data for instance segmentation with MaskRCNN; b) boundary image showing instance boundaries as predicted from MaskRCNN followed by watershed segmentation; c) boundary image from (b) overlaid on MIBI image from (a); d-f) a second example with the same information as (a-c) but for a lung squamous cell carcinoma tissue section. All images are 1024 x 1024 pixels of a 500 µm x 500 µm area.

Cell Phenotyping and Marker Co-expression

With cell instance information, marker intensities from all channels in the panel can be measured to perform single cell phenotyping, or classification. Since membranous biomarkers may be distributed across cell boundaries, and will thus potentially be measured in neighboring instances, a quadrant averaging method is utilized to calculate a cell score which gives preference to counts that are evenly distributed geometrically throughout a cell. Expression of checkpoint markers targeted by new cancer therapies, such as PD-L1 and LAG3, on particular cell types can then be measured.

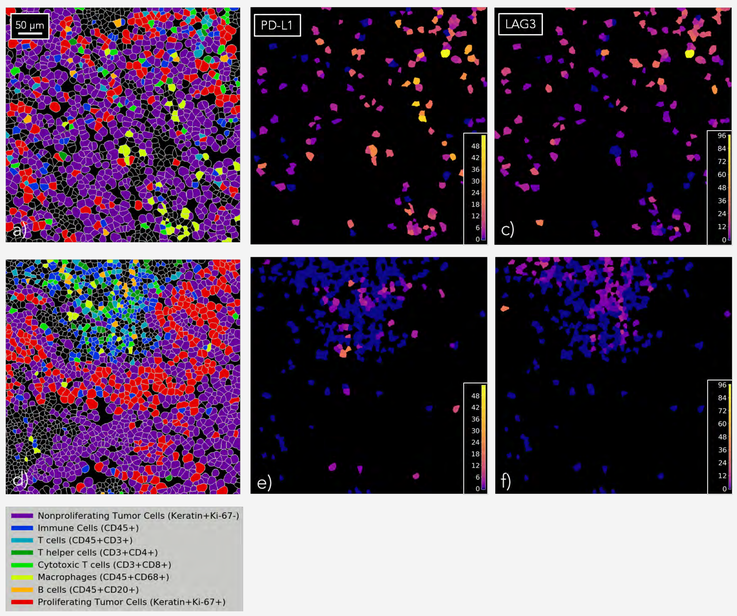

Figure 5. a) Lung adenocarcinoma image from Figure 4a classified into cell types; b) integrated PD-L1 expression within cells classified as one of the immune cell types; c) integrated LAG3 expression within cells classified as one of the immune cell types; d-f) cell classifications and PD-L1 and LAG3 expression for lung squamous cell carcinoma image from Figure 4d.

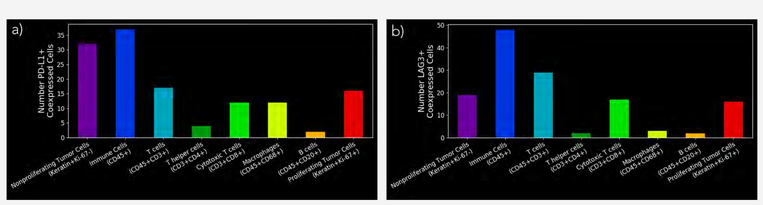

Figure 6. Charts showing the number of tumor and immune cells which are (a) PD-L1 positive and (b) LAG3 positive for the lung adenocarcinoma sample.

Distance Profiling of Classified Images

In addition to quantifying co-expression of markers, the spatial information available in MIBI can be used to measure the distances between cells of different phenotypes.

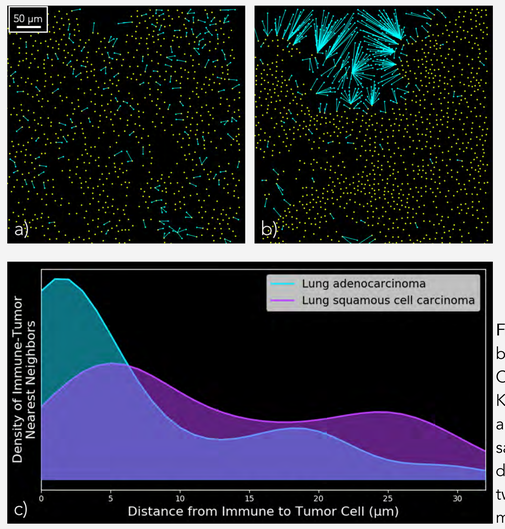

Figure 7. Nearest neighbor assignments between general immune cells (cyan, CD45+) and the closest tumor cell (yellow, Keratin+) for the (a) lung adenocarcinoma and (b) lung squamous cell carcinoma samples; c) plot showing the distance density of nearest neighbor cell pairs between immune cells and the nearest tumor cell within the two samples.

The distances between tumor-infiltrating immune cells and tumor cells have prognostic value in certain cancers. Within a sample, the multiplexed nature of MIBI data can be leveraged to distinguish between proliferating (Ki-67+) and nonproliferating (Ki-67-) tumor cells (Keratin+) and to determine the spatial distribution with respect to immune cells.

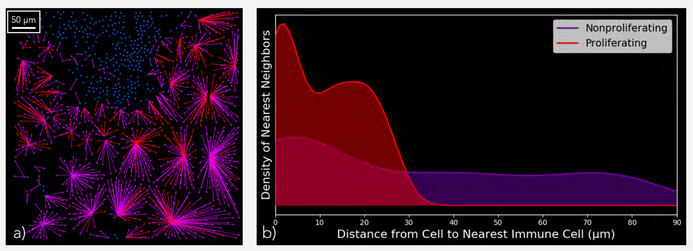

Figure 8. a) Nearest neighbor assignments between proliferating tumor cells (red, Keratin+Ki-67+) and nonproliferating tumor cells (purple, Keratin+Ki-67-) and the nearest immune cell (blue, CD45+) within the lung squamous cell carcinoma sample; b) plot showing the distance density of the nearest neighbor pairs shown in (a).

Larger features present in tissue can be determined from either signal intensity or cell classifications to denote the boundaries of vasculature (magenta) and tumor (green) regions.

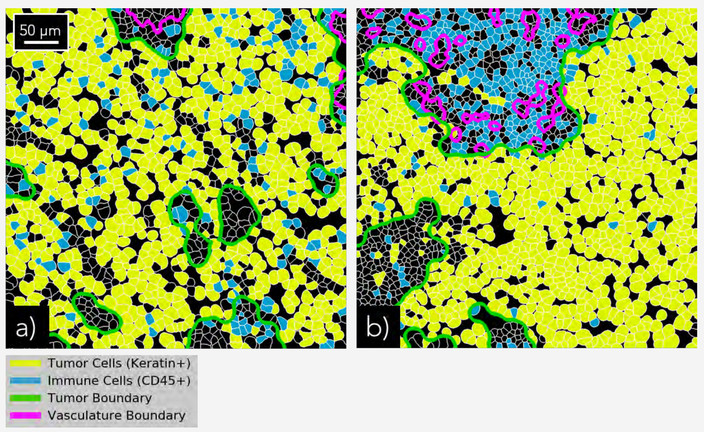

Figure 9. Images showing general immune cells (cyan, CD45+) and tumor cells (yellow, Keratin+) for the a) lung adenocarcinoma and b) lung squamous cell carcinoma samples with tumor region boundaries (green) and

Conclusions

MIBI provides highly multiplexed biomarker information at subcellular resolution through the combination of metal antibody labeling and ToF-SIMS imaging. The unique combination allows for single cell segmentation and phenotyping to characterize the cellular makeup and spatial distribution within the tumor microenvironment. Expression of PD-L1 and LAG3 was characterized within immune cells and across various tumor and immune cell types. Tumor and blood vessel boundaries were determined and distances between tumor cells, including proliferating and nonproliferating, and immune cells were measured and characterized.