Multiplexed Ion Beam Imaging For Characterization of the Tumor Microenvironment Across Tumor Types

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Abstract

Background: Cancer arises from tumor cells taking advantage of complex relationships between stromal, vascular, and immune cell subsets. To date the ability to characterize the cellular composition and spatial organization of the tumor microenvironment (TME) has been limited by the techniques available to image the necessary number of biomarkers for broad phenotyping at a subcellular resolution. Here we show the applicability of Multiplexed Ion Beam Imaging (MIBI) for cell phenotype identification and their spatial relationships across multiple tumor types.

Questions of the TME addressed in this study by MIBI are:
1. Spatial organization of tumor and immune cells
2. TME immune profile
3. Expression of immune checkpoints PD-1 and PD-L1 in the TME

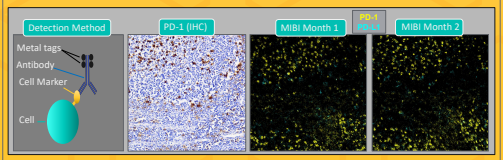
Methods: Formalin-Fixed Paraffin-Embedded (FFPE) samples from 25 tumor biopsies plus controls were imaged for their cellular composition and architecture using MIBI.
- Samples were stained with a panel of 15 antibodies, each labeled with a specific metal isotope.
- Tissue is scanned via secondary ion mass spectrometry to image the tissue for expression of the antibody targets.
- Multi-step processing, including machine-learning-based segmentation, was used to determine both the frequency of cell subsets and the distance between immune cells and tumor cells.

Results: Tumor-associated macrophages (TAMs) and tumor infiltrating lymphocytes (TILs) were observed in bladder, breast, gastric, lung, ovarian, and head and neck cancers. Nearest-neighbor immune:tumor distances revealed the level of mixing between tumor and immune cells. For example, ovarian serous carcinoma samples showed large numbers of infiltrating cytotoxic T cells and macrophages amongst tumor cells. However, the TMEs differed with one showing mixing of the populations and the second showing a compartmentalized organization. Further phenotyping showed a wide range of PD-1 and PD-L1 expression in the tumor samples.

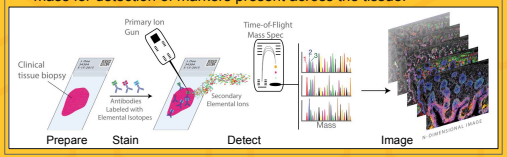
Conclusions: The function and phenotypes of cells can only be determined through the co-expression of multiple proteins. Multiplexed imaging by MIBI reveals the complex tumor immune landscape by enabling the characterization of the spatial relationship of immune and tumor cells and the expression of immunoregulatory proteins. This work demonstrates the possibilities of MIBI for future patient stratification through characterization of the TME.

Multiplexed Ion Beam Imaging (MIBI)

- FFPE samples are processed similarly to traditional IHC except staining is performed with a panel of isotopically-labeled antibodies.
- The panel was validated by comparing to single-plex IHC and included antibodies for tumor and immune cell subsets in addition to immunotherapy targets (PD-1, PD-L1).
- MIBI produces repeatable results without the need for instrument normalization.



- An ion beam rasters across the tissue liberating ions including the isotopes bound to the tissue via the antibodies.
- Time-of-flight mass spectrometry separates the labels based on mass for detection of markers present across the tissue.



Quantifying Cell Types and Their Spatial Relationships

- Tumors vary in the amount of immune infiltration and cell types present.
- Immune cells, if present, can be mixed among tumor cells or compartmentalized.
- Cell populations vary in their PD-1/PD-L1 functional status.
- These parameters are the focus of many studies and their association with patient outcomes.

Staining Panel For Phenotypic Analysis of the TME

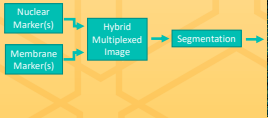
- Sensitive, high-resolution multiplexed imaging is needed to characterize the TME.
- Specifically, the panel for this study was designed to address:

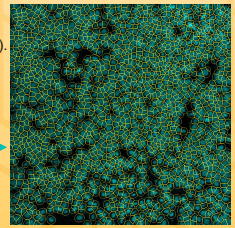
1. Tumor:immune cell spatial organization
2. Immune subsets present (immune profile)
3. Functional status of cell types (PD-1, PD-L1)

Target	Cell Expression
dsDNA	All cells
beta-tubulin	All cells
Keratin	Tumor cells, epithelial cells
CD3	T cells
CD4	T helper cells, some macrophages
CD8	Cytotoxic T cells
CD45	Immune cells
CD56	NK cells, neurons
CD68	Macrophages
FOXP3	Regulatory T cells
PD-1	T cells
PD-L1	Tumor cells, macrophages, APCs

Segmentation: From Complex Tissue To Single Cells

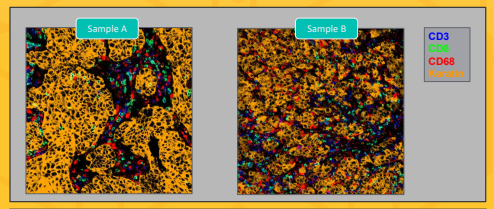
- Preserves spatial information allowing tumor:immune cell boundary and distance determination.
- Uses multiplexed information (nuclear and membrane channels).
- Uses segmentation results from earlier method as ground truth.

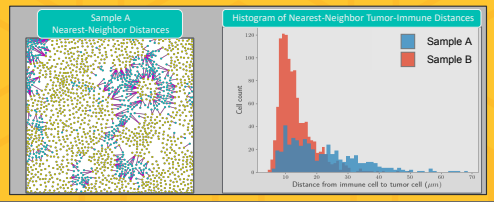




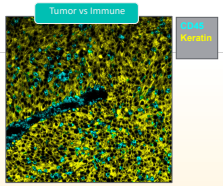
Visualizing Differences in TME Architecture

- The spatial distribution of immune cells and tumor cells differs between ovarian cancer samples.
- Sample A shows a compartmentalized organization versus Sample B that shows mixing of immune cells and tumor cells.
- The spatial information provided by MIBI enables nearest-neighbor quantification of distances between tumor cells and the nearest immune cells.

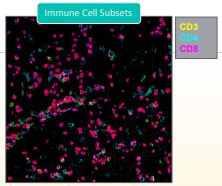




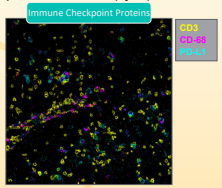
Immune Profiling: Immune Subsets of the TME



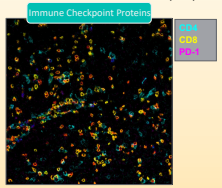
Urothelial cancer sample shows presence of TILs (cyan)



CD4 and CD3 colocalized (green)
CD8 and CD3 colocalized (red)



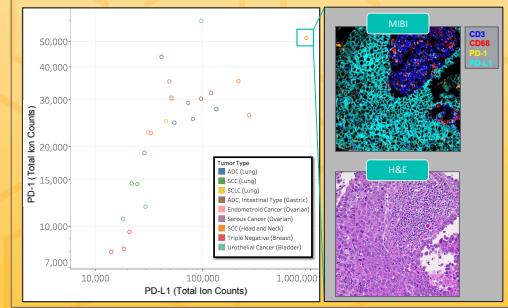
PD-L1 present on:
- $CD68^+$ macrophages (blue)



PD-1 present on:
- subset of $CD8^+$ T cells (red)
- subset of $CD4^+$ T cells (blue)

PD-1 and PD-L1 Expression Across Tumor Types

- On average, head and neck tumor (HNSCC) samples had the greatest PD-L1 expression and triple negative breast cancer samples had the lowest in this cohort.
- A HNSCC sample showed a compartmentalized architecture with PD-1 expression on T cells and high PD-L1 expression on tumor cells.



Conclusions

- MIBI offers many features advantageous for the analysis of complex tissues including the tumor microenvironment.
  - Large panels of antibodies specific to a wide range of cell types and functional states can be used to image a single tissue sample.
  - The tissue architecture is clearly defined at sub-cellular resolution enabling segmentation and single-cell analysis.
  - Sensitivity afforded from the low background of MIBI enables low expression markers such as PD-1 and FoxP3 to be readily detected.
- This study evaluating multiple tumor types shows the possibilities of MIBI for patient stratification and immune profiling of tumor samples under therapeutic forces.

Acknowledgements
We thank all patients who have donated samples for this investigation.