Background: Multiplexed ion beam imaging (MIBI) combines time-of-flight secondary ion mass spectrometry (ToF-SIMS) with metal labeled antibodies to image 40+ proteins in a single ion at subcellular spatial resolution. Here, we show that the recently released MIBIscope provides improved sensitivity for detecting immune checkpoint markers and offers greater throughput at higher resolution than the alpha instrument.

Results: Replicate regions of interest (ROI) were collected on both instruments with similarly sized ROIs acquired in 17 minutes with the MIBIscope compared to 280 minutes with the alpha instrument. Fourier Ring Correlation (FRC) showed the resolution to be greater on the MIBIscope as compared to the alpha instrument with FRC also demonstrating uniform resolution across ROI 2.5X greater in size. Even with the 10X greater speed of the MIBIscope, the signal of the 25 markers across replicates ROIs was increased (p<0.05) and showed similar expression patterns to those observed on the alpha instrument. This resulted in greater sensitivity to markers with low expression, such as checkpoint markers. Eleven cell populations were classified across the ROIs utilizing two methods, with both methods showing a similar frequency of tumor cells and B, T, and myeloid cell subsets between instruments. Segmentation enabled the number of cells within a population to be calculated, but defining boundaries is laborious and signal from neighboring cells can result in misclassification. Performing classification at the pixel level, without segmentation, enabled the fraction of the tissue that is tumor or any other cell type to be rapidly determined.

Conclusions: The MIBIscope enables the phenotypic characterization of tumor and non-tumor microenvironments. Co-expression of markers can be used to classify tumor and immune populations and to quantify the expression of markers associated with immune suppression. The increased sensitivity and throughput of the MIBIscope, in combination with the 40-parameter capability and subcellular resolution, provides a platform uniquely suited to understanding the complex tumor immune landscape.

Methods: Multiplexed Ion Beam Imaging (MIBI) Technology

Traditional imaging platforms are limited in their ability to identify both the cell types present in the tumor microenvironment and the spatial relationship between immune and cancerous cells. To address this, MIBI has been developed to image up to 40 markers at single cell resolution.

Instrument Improvements Result in Increased Speed, Signal, and Sensitivity

- Improvements to the primary ion gun have led to increased instrument stability and throughput
- Fourier Ring Correlation (FRC), a method of measuring effective resolution using spatial frequencies, was used to assess the resolution of images at various settings across instruments
- 25-gene images acquired from serial sections of NSCLC samples on both instruments show similar staining patterns for all markers
- Markers are consistent (F < 0.05) across instruments with a bias of increased signal measured with the MIBIscope as expected based on the improvements in hardware

Conclusions

- The MIBIscope has improved throughput, sensitivity, and resolution to enumerate immune cells within complex tissue environments including the tumor microenvironment
- Comparison of NSCLC samples across instruments shows similarities in marker quantification and resultant cell classification
- The increased sensitivity of the MIBIscope aids in detecting immunoregulatory proteins expressed at low levels in some samples
- The resolution of the MIBIscope enables determination of cell populations expressing these markers of interest, either through cell segmentation or by High Definition Pixel Phenotyping