**Comparison of Spatial transcriptomic platforms in lung adenocarcinoma tissue samples**

**Background:**

Single cell spatial transcriptome (scST) technologies are evolving rapidly as potential tools that can help to unveil the tumor biology of lung cancer and allows to investigate a large number of targets, gene signaling pathways and cell-cell interactions which are important to understand the lung cancer tumor microenvironment, however the performance of these platforms and comparison with morphological and biological data have not been previously performed.

**Experimental design**: We used formalin fixed paraffin embedded (FFPE) surgical resected lung adenocarcinoma placed in a tissue microarray. Serial sections of 5um were processed with CosMx, MERFISH and 10X Xenium commercial scST platforms using CosMx universal 1000plex, MERFISH 500plex immuno-oncology and 10X Xenium 289plex lung + 50 custom designed gene panels. We correlated gene expression information with orthogonal ST assay using the GeoMx Digital spatial profiler (DSP). Pathology review of the resulting phenotyping annotations produced against mIF and H&E sections was carried out in parallel. We then evaluated both relative technical and biological performance from a pathology and bioinformatics standpoint as they are essential for downstream analysis and phenotyping.

**Results**

All three platforms were highly concorded (R > 0.62, p < 0.0001) with matched orthogonal RNA spatial analysis using the GeoMx DSP, however performance characteristics varied among them including of transcript counts per cell, gene counts per cell, cell area size per cell, cell segmentation false discovery rates and morphological concordance with pathology annotations, which resulted in different phenotyping characteristics. Higher counts of negative control probes increased the false discovery rates of CosMx platform compared to 10X Xenium. Correlation of shared gene expressions between CosMx and Xenium data was better. These findings highlight the need for reproducibility when considering spatial RNA analysis.

**Conclusion**

ScST is an area that is under rapid development. Our work provides information of the advantages and limitations of these platforms that can be considered to develop workflows for downstream phenotyping and other methods for scST gene expression analysis in lung cancer.