SPATIAL WHOLE TRANSCRIPTOME PROFILING OF THE TUMOR MICROENVIRONMENT IN ARCHIVED AND FRESHLY-MOUNTED FFPE TISSUES

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Background
The tumor microenvironment (TME) is composed of highly heterogeneous cell types that dynamically interact with each other. The constant interaction between a tumor and its microenvironment plays a critical role in how the cancer develops, progresses, and responds to therapies. Traditional tissue-based studies of the TME can be limited to a small number of target analytes, which can limit biological insights. In this work, we used the Visium CytAssist instrument and Visium Spatial Solutions from 10x Genomics which enable whole transcriptome gene expression and immunofluorescence-based protein expression profiling from archival and freshly preserved FFPE cancer tissues. Our data provided a more comprehensive understanding of cellular behavior in and around tumors yielding new insights into disease progression and therapeutic response.

Methods
Tumor FFPE tissues (breast, liver, lung, and ovarian cancer) were spatially profiled using the Visium CytAssist instrument and Visium Spatial Solutions. Tissues were mounted on glass slides, H&E or IF stained, and imaged to select the target region for whole transcriptome analysis. Following decrosslinking and probe hybridization, the samples were prepared for probe transfer to spatially barcoded Visium slides with 6.5 x 6.5 or 11 x 11 (mm x mm) capture areas. The captured probes were used in a downstream genomics workflow to generate sequencing-ready libraries.

Results
We applied this method to measure gene expression within the TME of archived human lung and ovarian cancer FFPE samples. Normalized gene expression levels superimposed on the H&E images demonstrated similar clustering patterns delineating the tumor and stromal region. In the lung cancer sample, canonical gene makers, TP63 and KRT5, confirmed the general phenotype of non-small cell carcinoma. In addition, other keratin and mucin genes revealed intra-tumor heterogeneity. In the same lung cancer sample, immune response genes were expressed in the stromal region, adjacent to the tumor bed, identifying the presence of immune cell subsets such as activated T, B, and regulatory dendritic cells. Freshly prepared breast and liver cancer samples were screened with fluorescently labeled antibodies in addition to transcriptomic profiling. In particular, PCNA and Vimentin showed the precision and accuracy of probe transfer using the Visium for FFPE spatial solution.

Conclusions
The data highlights that Visium CytAssist can retrieve transcriptome information from archived and freshly prepared FFPE sections in a spatial context. Visium CytAssist provides a more comprehensive understanding of clinical tissue samples and provides novel insights into architectural and cellular heterogeneity across multiple diseases.