Background As the fourth most common cause of death in developed countries, pancreatic cancer remains a deadly disease due to difficulties hindering its early diagnosis, giving way to metastasis of the tumor, resulting in poor prognosis. Pancreatic invasive ductal adenocarcinoma (PDAC) is the most common of pancreatic neoplasms and treatment options are few, with poor overall survival. While it is clear that immunotherapy has revolutionized the treatment of solid tumors by leading to cures where none existed a decade ago, optimally selecting patients as candidates for immunotherapy-chemotherapy combinations remains a critical challenge.

The complexities of the tumor microenvironment (TME) have been implicated in the failure of treatments options. The TME of PDAC is especially rich with multiple interactions between pancreatic epithelial/cancer cells, stromal cells, immune cells, and the extracellular matrix (ECM). PDACs are characterized by a complex ECM of desmoplastic reaction consisting of an extensive and dense fibrotic stroma that surrounds and infiltrates clusters of malignant epithelial cells, together with the loss of basement membrane integrity and an abnormal vasculature. As critical players in the development of PDACs, cancer-associated fibroblasts (CAFs) are the predominant cell type within the tumor stroma; they exhibit considerable heterogeneity that can have both tumor-promoting and tumor-repressing functions. Given that these effects are potentially affected by the tumor-stromal crosstalk and the spatial context of other cell types, a deeper understanding with respect to the different cell types present and their relationship to each other will help uncover the much-needed therapeutic approaches for this disease.

Methods Herein, we demonstrate a spatial phenotyping workflow combining complementary methods that can unravel the complexity of the PDAC TME, in particular the important spatial relationships of different T cells subsets and CAFs. We highlight the utility, robustness, and ability to derive meaningful biological and actionable insights in PDAC samples using the InSituPlex® (ISP), Imaging Mass Cytometry® (IMC) spatial technologies coupled to Oncotopix Discovery® A.I.-based multiplex image analysis.

Results This workflow utilizes a 4-plex whole-slide ISP assay with image analysis to define regions of interest for a comprehensive 40-plex IMC imaging and analysis. Both assays were analyzed using a workflow that includes tissue region and cellular segmentations followed by cellular phenotyping and spatial analyses.

Conclusions Our novel tissue imaging workflow affords the comprehensive and multiparametric in situ exploration of the TME at the single cell level, but importantly alludes to a better understanding of PDAC tumor immunology and potential optimization of immunotherapy protocols for this disease.