## Deep ultrahigh-plex spatial phenotyping of human cancer tissues

## Imaging

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Spatial phenotyping is poised to revolutionize cancer research and treatment. Technologies like the PhenocyclerTM-Fusion, which enable high-throughput detection of dozens of protein biomarkers in situ, have already transformed immuno-oncology and are expected to play formative roles in future research. To further support the implementation of the PhenocyclerTM technology in diverse research agendas, we present a comprehensive antibody panel that is aimed at in-depth identification of immune cell lineages, activation states, immune checkpoints and tissue structure. Deployment of this panel is possible with high sample throughput on the PhenocyclerTM Fusion platform, which is a fully-integrated spatial biology solution that provides cyclical multiplexed imaging at unprecedented resolution and rapid turn-around time. The key features of this novel spatial biology system include: 1) true single-cell and sub-cellular resolution, 2) unparalleled biomarker imaging depth on whole FFPE samples, 3) wideranging compatibility with whole slide FFPE tissues, 4) panel customization and flexibility via dedicated barcodes, 5) uncompromised access to whole tissues, and 6) sophisticated single-cell analytical metrics. In this study, we generate a comprehensive account of the spatial biology in different human FFPE tissues. Single-cell spatial phenotyping coupled with deep bioinformatic analyses reveals new quantitative phenotypic and spatial information that will be essential to gain further insights into distinct cellular neighborhoods and their role in regulating immune and metabolic functions of certain cancers.