

A Customized Approach to Spatial Transcriptomics and Proteomics

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The mission of the CU Anschutz Human Immune Monitoring Shared Resource (HIMSR) is to facilitate clinical and translational studies by dissecting immune-based mechanisms with diagnostic and therapeutic applications, including discovery of novel biomarkers in liquid and tissue biopsies. These studies contribute to a better understanding of the underlying mechanisms of immune evasion, identify prognostic biological measures for responses to treatment, and help develop rationale for future novel combinatorial treatments. To this end, HIMSR operates a state-of-the-art tissue imaging facility equipped with Akoya Biosciences' Vectra Phenoimager HT, IONpath's Multiplexed Ion Beam Imaging (MIBIScope), and Nanostring's GeoMX Digital Spatial Profiler. We specialize in developing customized assays on these platforms and have optimized a workflow for validation of new antibodies and panels. Antibodies that have not been previously validated in-house on these platforms are first tested in single stain, where the antibody is titrated in positive tissue to ensure low background, optimal signal-to-noise ratio, and complete staining morphology consistent with published data. Once optimized in single stain conditions, the antibodies are assigned fluors and the staining order is established utilizing knowledge gained from previously optimized panels. Next, full panel testing is completed in positive control tissue to ensure that all antibodies perform in multiplex similar to single stain conditions. Stripping between sequential staining cycles and spill-over into adjacent channels are assessed with fluorescence-minus-one (FMO) controls. Standard operating procedures are followed to ensure quality panel performance. Currently, HIMSR has optimized over 300 antibodies for use in over 320 6- and 8 marker panels on the Vectra Phenoimager HT, 8 40-parameter MIBIScope and 10 Nanostring GeoMX panels in human, mouse, and canine species. Here we present this optimized workflow and offer ABRF members insight into our highly rigorous processes.