Background
Tertiary lymphoid structures (TLS) have been observed in a variety of solid tumors and growing evidence has shown that TLS can be promising prognostic indicators of positive outcomes for patients with solid tumors including colorectal cancer (CRC).1 Large-scale retrospective analysis shows patients with mature TLS in particular respond to PD-1/PD-L1 antibody treatment with improved objective response, progression-free and overall survival.2 Since not all patients respond to immune checkpoint blockade (ICB) therapy, identifying patients with TLS can be clinically relevant as it enables selection of patients likely to respond. In this study, we demonstrate a tissue-based phenotyping workflow combining two complementary multiplex immunofluorescence (mIF) platforms to enable identification of TLS, characterization of TLS maturation stage and spatial phenotyping of tumor infiltrating lymphocytes (TIL) on whole slide scan.

Methods
In this study, 40 CRC patients were first stained with an in-house developed PhenoImager™ mIF panel detecting CD20, CD21, CD23, CD3 and Cytokeratin. Whole slide scan was acquired using PhenoImager HT, followed by biomarker classification and TLS identification using custom analytics algorithm generated with Indica HALO® platform.

An adjacent section of tumor sample was then stained and analyzed with a 17-plex MultiOmyx™ mIF panel. Using the MultiOmyx assay in combination with proprietary deep-learning-based image analysis (NeoLYTX), we further characterized the TLS maturation stage and interrogated the correlation of the TLS presence with subtypes of TIL expression in the CRC samples.

Results
PhenoImager 5-plex TLS panel combined with Halo custom analysis successfully identified TLS in the tumor microenvironment (TME) of CRC samples and led to the strategic selection of regions of interest (ROI) for further characterization by high-plex assay. The TLS detection was found to be concordant between both mIF platforms in the CRC samples. MultiOmyx 17-plex analysis was able to provide a detailed picture of TLS and enabled further classification of TLS into different maturation stages based on biomarker expression and spatial organization of immune cells in the CRC samples.

Conclusions
Combination of PhenoImager and MultiOmyx IF provides a complementary and powerful solution to study cellular composition within the TME. PhenoImager assay characterizes the immunophenotypes and visualizes the spatial distribution of TIL at single-cell resolution on whole slides. High dimensional analysis by MultiOmyx can provide greater understanding of the immune contexture within the TME and deeper insights into the correlations between biomarkers. This combined approach may have broad application and provides novel insights into the complex TME.

REFERENCES