Background Tissue consists of heterogeneous cell types, each with diverse functions and functional states, arranged spatially in a way that impacts patient health status. Resolving this complexity at the subcellular level has historically been challenged by fluorescence overlap, autofluorescence, a limited number of targets that can be simultaneously assessed, and low throughput. Orion technology breaks these barriers, providing rapid, straightforward, highly multiplexed whole slide tissue analysis. The recently announced Orion platform rapidly generates high resolution 21-channel images to enable comprehensive phenotypic profiling and characterization of tissue architecture including micro-anatomy, analysis of tumor heterogeneity and the complex tissue microenvironment.

Methods FFPE tissue samples are stained with TissuePlex™ reagents and whole slides are imaged on the Orion Instrument. Resulting 21-channel datasets are reviewed by a pathologist to verify staining specificity. The TissuePlex core panel includes antibodies against CD45, CD4, FoxP3, CD8A, CD11b, LAG-3, PD-L1, CD11c, CD68, PD-1, Ki-67, CD3d, and CD20. Single marker IHC is used as the gold standard to evaluate TissuePlex reagents and instrument spectral isolation performance is evaluated using fluorescent microspheres.

Results We will present the novel Orion spatial biology platform, provide a technology and workflow overview, demonstrate instrument validation results, and present sample datasets.

Conclusions The Orion platform enables rapid and deep phenotypic analysis of tissue samples for high resolution, whole slide sample analysis.

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Optimization of an ultrasensitive, quantitative immunoassay for detection of CD20 in non-Hodgkin’s lymphoma (NHL) FFPE samples

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Background CD20, a membrane B cell marker, is expressed on the majority of mature B cell neoplasms, including diffuse large B cell lymphoma and follicular lymphoma. Importantly, CD20 is the target of rituximab as well as autologous T cell and BiTE® therapies in clinical development. Studies show that one mechanism of resistance to rituximab-containing therapies is downregulation of CD20.1 2 Development of an assay that provides highly sensitive and accurate detection of CD20 levels in the tissue context may help to assess whether there is a minimum CD20 threshold associated with response to rituximab or other CD20-targeted therapies. Here, we describe the development of a novel Quanticell™ assay for sensitive and quantitative detection of CD20 expression in formalin-fixed paraffin-embedded (FFPE) biopsy samples from NHL patients.

Methods A CD20 (Abcam, clone SP32) Quanticell-based assay, which utilizes Konica Minolta’s novel fluorescent phosphor-integrated dots (PIDs)3 was optimized on a panel of B lymphoma cell lines. Flow cytometry was performed to benchmark assay performance. Next, a human B lymphoma tissue microarray (TMA, n=39 cores) was stained using DAB-IHC to evaluate CD20 expression. Tumor cores (n=10) showing CD20highCD19high expression by DAB-IHC and immunofluorescence (IF)-IHC were selected for further evaluation. Human tonsil tissue was used to assess CD20 assay performance as a Quanticell singleplex or duplexed with CD19 IF-IHC. The TMA was stained with CD20 Quanticell plus CD19-AF488 to measure CD20 expression on a per cell basis. To assess sensitivity of CD20 Quanticell detection, a CD19 negative non-B cell core was analyzed. CD20 expression determined by Quanticell was compared to results generated with a commercially available method enabling digital profiling of CD20 protein in FFPE sections.

Results Analytical comparison between the Quanticell assay and flow cytometry on cell lines showed strong concordance between the two methods (CD20 Quanticell score versus CD20 receptor number). The Quanticell method demonstrated a broader dynamic range in CD20 expression in the TMA samples compared to DAB-IHC. Both the Quanticell and digital protein detection assays appropriately clustered cores into CD20low and CD20high categories. Notably, the CD20 Quanticell assay demonstrated the ability to measure CD20 expression accurately and precisely over a broader dynamic range when compared to the digital method.

Conclusions Relative to DAB IHC, the novel CD20 Quanticell assay provides significantly enhanced detection and quantification of CD20 in FFPE tissue samples. This technology may be useful to assess whether there are critical antigen densities associated with response to CD20-targeting therapies.

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