Society for Immunotherapy of Cancer (SITC) 2024, Poster Number 101

Enhancing immunofluorescence biomarker detection sensitivity through HDR imaging and Optimized Background Removal (OBR)

Daniel Jimenez-Sanchez[1], Abdullah Chaudhary[2], Xenia Meshik[1], Charles E. Jackson[1], Matthew H. Ingalls[1], John Schwarz[1], Douglas Werner[2], and Oliver Braubach[1]

1 Bruker Spatial Biology, St. Louis, MO, USA

2 Bruker Spatial Biology, San Jose, CA, USA

Introduction

The identification of protein biomarkers *in situ* holds great promise for advancing disease diagnoses and treatment options. To be considered for inclusion, protein markers must meet two main criteria: specificity, indicating they should be unique to the tissue or cell type being studied, and sensitivity, meaning that their levels should be sufficient to accurately detect changes in protein expression. This study assesses the sensitivity of protein markers in the CellScape™ Precise Spatial Proteomics platform using High Dynamic Range (HDR) image acquisition and Optimized Background Removal (OBR) techniques. Collectively, these techniques improve the detection of true signal from background noise, and thereby ensure that even subtle changes in protein expression levels can be reliably detected and quantified within samples as well as between samples.

Methods

Human FFPE tonsil sections were stained with the 15-plex VistaPlex™ Spatial Immune Profiling Assay Kit. Combinations of 3-4 markers were applied in rounds of iterative multiplex immunofluorescence staining and imaging with the CellScape platform, with unstained background images acquired before each round of staining. Coined "Optimized Background Removal", stained and unstained data were optimized at the pixel level to enhance the sensitivity of the signal. The resulting multi-layered whole slide image dataset was then processed using standard single-exposure vs. multi-exposure HDR methods and standard immunofluorescence performance metrics, including signal sensitivity and positive-to-negative signal ratios.

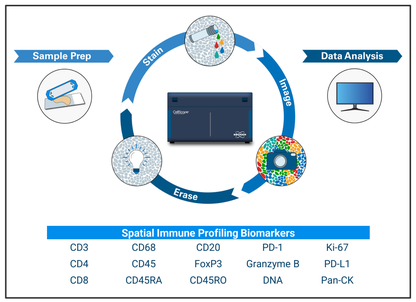

Figure 1. CellScape Precise Spatial Multiplexing workflow. The CellScape platform uses cycles of staining, high dynamic range (HDR) imaging, and non-destructive signal removal to detect biomarkers with spatial context and single-cell resolution. To facilitate downstream image analysis, CellScape generates a standard image file output (OME-TIFF) compatible with both commercially available software and custom image analysis pipelines. The VistaPlex Spatial Immune Profiling Kit enables spatial phenotyping of key immune populations and epithelial cells in human formaldehyde-fixed, paraffin-embedded (FFPE) tissues.

Multi-exposure HDR obviates single-exposure optimization

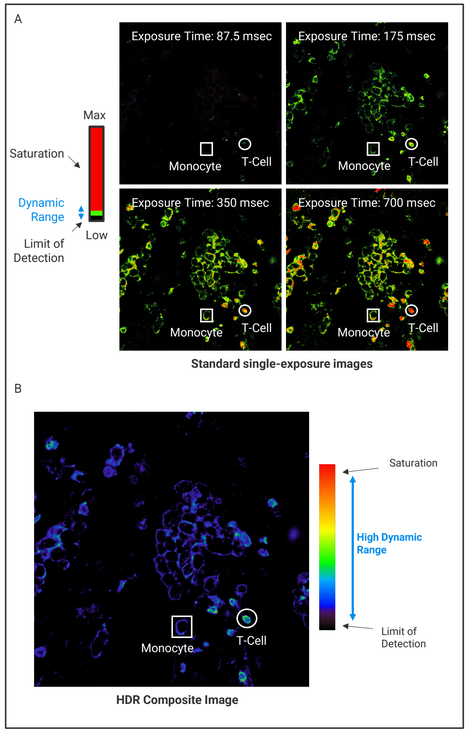

Figure 2. HDR image acquisition extends the range of detection and obviates exposure optimization. Combining a series of single exposures, each with their own dynamic ranges, HDR expands the total dynamic range by multiple orders of magnitude while also collecting the optimal single exposure time for each marker in the assay. On the top, single exposures of a lung carcinoma tissue sample with CD4 labeled with a fluorescent antibody, colored to indicate signal saturation. On the bottom, the composite image overlay after HDR imaging. Colored scale bars indicate signal levels and saturation.

Unstained image acquisition enables pixel-level precision in biomarker detection

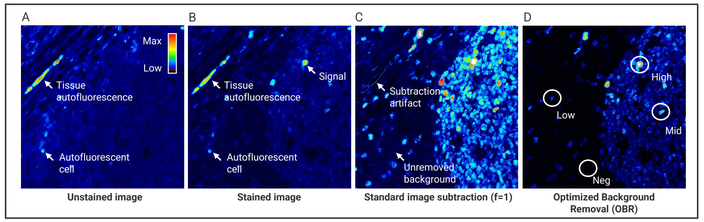

Figure 3. OBR mediates unstained and stained pixel data to enhance pixel-level biomarker detection. A-B. display unstained and stained images with visible signal and autofluorescence. C. displays non-optimized background removal where artifacts and unremoved background remain. Note that *f* is the background subtraction factor used. D. demonstrates OBR, effectively eliminating background noise and allowing precise pixel-level signal extraction at different expression levels, enabling more accurate signal quantification.

OBR removes background while catching low-expressing cells

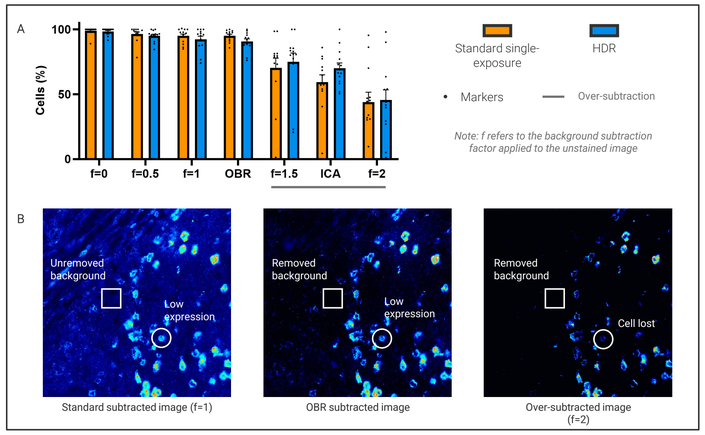

Figure 4. Comparison of cell detection percentages across various subtraction methods. A. ICA stands for Independent Component Analysis and *f* is the background subtraction factor used, where OBR achieves highest sensitivity without losing cells. B. Image comparison: standard subtraction (f=1) leaves background noise, OBR removes background while retaining low-expressing cells, and over-subtraction (f=2) results in cell loss.

The combination of HDR and OBR yields 3-fold sensitivity increase in biomarker detection

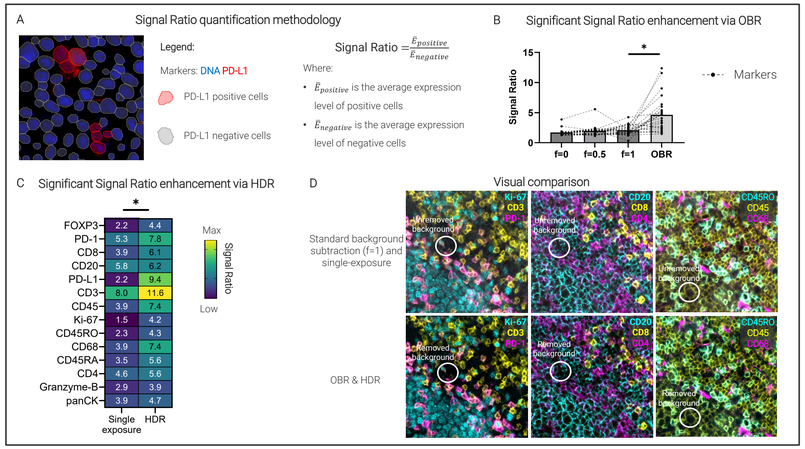

Figure 5. Impact of Optimized Background Removal and High Dynamic Range imaging on signal ratio enhancement. A. Signal ratio quantification methodology, showing how the ratio is calculated based on the expression of positive and negative cells. B. Bar graph showing that OBR significantly increases the signal ratio compared to other subtraction methods. C. Heatmap illustrating that HDR significantly enhances signal ratios across various biomarkers, particularly for low-expressing markers. D. Images comparing standard background subtraction (top row) with OBR and HDR (bottom row), where OBR + HDR removes background noise and improves signal clarity for key biomarkers.

Conclusions

- Demonstrated efficacy of Optimized Background Removal and HDR image acquisition techniques in improving detection sensitivity for tissue-based protein markers.
- Accurately detected protein markers in tissue samples of weakly-expressing biomarkers critical for immuno-oncology translational research.

Contact Us

Presenter: Daniel Jimenez-Sanchez
D.Jimenez_Sanchez@bruker.com

For more information, visit www.BrukerSpatialBiology.com

Use QR code to request more information or a quote

Innovation with Integrity