Implementation and cross-validation of a Lunaphore COMET hyperplex assay within a global CRO infrastructure

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INTRODUCTION

Hyperplex immunohistochemistry (IHC) has emerged as a powerful approach for spatial proteomic profiling of the tumor microenvironment, enabling simultaneous assessment of multiple immune and stromal biomarkers within a single tissue section. As assay complexity increases, maintaining analytical quality, reproducibility, and robustness becomes increasingly challenging. Furthermore, the generation of ultra-large whole-slide images and the integration of numerous spatially related analytes demand scalable analytical workflows, particularly in multi-site laboratory environments.

To address these challenges, we developed and evaluated a cross-site validation workflow for the 13-plex SPYRE Immuno-Oncology (I/O) Core Panel assay on the Lunaphore COMET platform across two global CRO laboratories, assessing the reproducibility and transferabilityof a hyperplex spatial proteomics assay within a global laboratory ecosystem.

METHODS

- **Platform deployment:** Lunaphore COMET instruments were installed at CellCarta laboratories in Antwerp (Belgium) and Lake Forest (USA).
- **Assay implementation:** The 13-plex SPYRE Immuno-Oncology Core Panel was developed and optimized.
- **Assay optimization:** Marker performance was benchmarked against internally validated single-plex and low-plex assays.
- **Background reduction and image processing:** Isotype control antibodies were incorporated to minimize non-specific staining. Whole-slide images were processed in Lunaphore Horizon viewer software to remove autofluorescence and background signal.
- **The SPARTA framework (CellCarta's proprietary) for image and data analysis:** Whole-slide digital image analysis performed using HALO AI allowed the generation of summary and object data. Cell phenotypes can be assigned via a configurable JSON schema using object data and a phenotyping module. Spatial distances and spatial colocalization may be computed using a combination of proprietary functions and scimap.

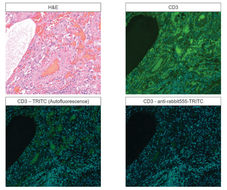


Figure 1. Background subtraction in the Horizon viewer software using isotype control antibodies substantially reduces residual background signal compared to autofluorescence-channel subtraction alone.

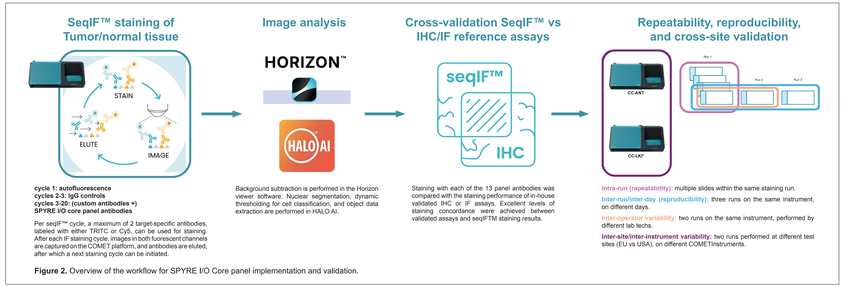


Figure 2. Overview of the workflow for SPYRE I/O Core panel implementation and validation.

RESULTS

High analytical accuracy and strong correlation with reference methods

The SPYRE I/O Core Panel produced marker-specific staining patterns consistent with biological expectations across normal tonsil and solid tumor tissues and showed close agreement with validated single-plex and multiplex IHC/IF reference assays. All targets met the predefined validation criteria, demonstrating reliable detection across a broad range of expression levels. Representative images illustrate preservation of expected cellular localization and tissue distribution, while semi-quantitative comparisons support concordance between the sequential IF method and reference assay readouts.

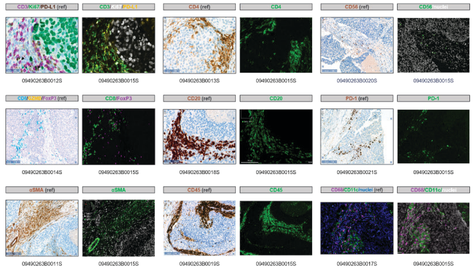


Figure 3. Representative images of the SPYRE I/O Core Panel staining compared with validated single-plex and multiplex IHC/IF reference assays.

Table 1. Correlation between the percentage of positive cells or density of positive cells obtained with the SPYRE I/O Core Panel and validated single-plex or multiplex IHC/IF reference assays, assessed using marker-specific correlation coefficients.

Marker	Correlation coefficient	Interpretation
CD20*	0.99	Very strong
FoxP3*	0.99	Very strong
CD56	0.98	Very strong
CD4	0.96	Very strong
Ki67	0.96	Very strong
PD-L1	0.89	Strong
PD-1	0.88	Strong
CD3	0.87	Strong
CD8*	0.83	Strong
aSMA**	0.81	Strong
CD11c	0.80	Strong
CD68	0.78	Moderate–strong
CD45	0.78	Moderate–strong

*Densities of positive cells.
**Quantified as relative marker area in the singleplex IHC reference assay.

Excellent inter-laboratory reproducibility

Assay transferability was assessed by comparing serial sections stained at the Antwerp and Lake Forest laboratories on independent COMET instruments. Side-by-side review showed consistent marker specificity, intensity, localization and overall staining quality across breast cancer, colorectal cancer and NSCLC tissues. Semi-quantitative comparison of marker positivity, supported by ICC analysis and Bland–Altman analysis (not shown in this poster), demonstrated strong agreement between sites, confirming reproducible performance across laboratories and instruments.

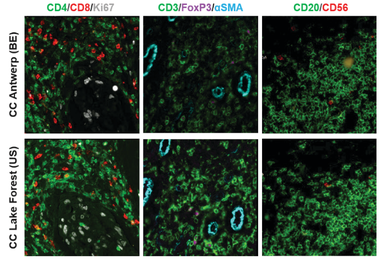


Figure 4. Representative images of a subset of targets included in the SPYRE I/O Core Panel stained and imaged on independent COMET instruments.

Table 2. Concordance between the percentage of positive cells measured in serial sections stained on independent COMET instruments, assessed using a two-way absolute agreement intraclass correlation coefficient (ICC).

Marker	ICC	Interpretation
PD-L1	0.97	Excellent
CD68*	0.97	Excellent
FoxP3	0.96	Excellent
CD8	0.96	Excellent
CD20	0.96	Excellent
CD56	0.96	Excellent
CD3	0.95	Excellent
PD1	0.95	Excellent
aSMA	0.94	Excellent
Ki67	0.94	Excellent
CD11c	0.94	Excellent
CD4	0.77	Acceptable
CD45	0.78	Moderate–strong

*Quantified as relative marker area.

Assay robustness evaluation

Robustness testing evaluated the effect of introducing additional upstream staining and elution cycles before execution of the SPYRE I/O Core Panel. Qualitative assessment and analysis of marker positivity showed that the expected staining patterns and signal levels were maintained, supporting flexible placement of supplementary markers ahead of the core panel. These findings indicate that the workflow has the required flexibility to allow for addition of additional markers before the SPYRE I/O core panel without compromising panel performance.

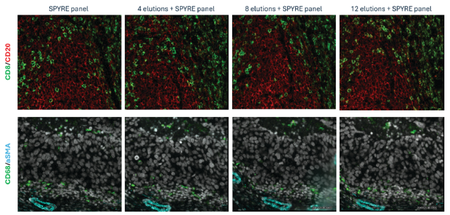


Figure 5. Representative images of a subset of targets included in the SPYRE I/O Core Panel using the default protocol and following additional upstream staining and elution cycles.

Table 3. Concordance between the percentage of positive cells obtained with the default protocol and after 4, 8, and 11 additional upstream staining and elution cycles, assessed using a two-way absolute agreement intraclass correlation coefficient (ICC).

	ICC (4 EC)	ICC (8 EC)	ICC (11 EC)
CD3	0.99	0.97	0.98
FoxP3	0.99	0.81	0.99
CD4	0.97	0.94	0.97
CD8	0.96	0.99	0.99
CD20	0.80	0.72	0.76
CD56*	0.50	0.47	0.52
CD11c	0.92	0.96	0.97
CD68	0.81	0.81	0.87
aSMA	0.84	0.77	0.83
PD-L1	0.99	0.99	0.99
PD-1	0.90	0.94	0.87
Ki67	0.93	0.99	0.92

*The analyzed samples displayed very low levels of CD56 positivity, which might have affected the concordance analysis.

The SPARTA framework (CellCarta's proprietary) for image and data analysis

The combination of Comet™ hyperplex technology and CellCarta's proprietary SPARTA framework enables comprehensive characterization of the tumor microenvironment, including immune cell diversity, spatial organization, and tumor-immune proximity metrics. The resulting analytes support comparative analyses across preclinical and clinical cohorts, including treatment groups and response categories. A real-world application of the SPARTA framework is presented in Poster #476.

CONCLUSIONS

High-plex spatial immunophenotyping using the COMET platform can be implemented in a robust and reproducible manner without compromising data quality. By combining reproducibility, accuracy, and enhanced signal quality with automation and flexibility, the SPYRE I/O Core Panel translates the promise of multiplex spatial proteomics into a practical and scalable solution within CellCarta CRO.

Send inquiries to isales@cellcarta.com with SITC 2026 COMET Hyperplex Assay Within a Global CRO in email subject.

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