CASE STUDY

# Actionable, Qualified Spatial Profiling of Pharmacodynamic-Relevant Tumor–Immune Biology in Non-Small Cell Lung Cancer

Aliri BIOANALYSIS

A qualified multiplex spatial approach designed to generate reproducible tumor–immune metrics associated with pharmacodynamic-relevant biology in immuno-oncology studies.

## Moving Beyond Descriptive Spatial Biology

Spatial biology has transformed our ability to visualize the tumor microenvironment. However, many translational workflows remain largely descriptive, generating complex images without necessarily producing controlled and reproducible biological outputs.

In non-small cell lung cancer (NSCLC) immuno-oncology studies, the challenge is no longer simply identifying immune cells within tissue, but understanding whether their spatial organization reflects biologically meaningful mechanisms associated with therapeutic activity, immune exclusion, suppression, or cytotoxic engagement.

At Aliri, we believe the next evolution of spatial biology is the transition from exploratory imaging toward controlled generation of quantitative spatial metrics associated with pharmacodynamic-relevant tumor biology.

## Case Study Overview

This case study illustrates how a qualified multiplex spatial panel can be deployed on FFPE NSCLC tissue to generate reproducible and biologically interpretable spatial metrics associated with pharmacodynamic (PD)-relevant tumor–immune biology.

The objective is not yet large-scale clinical outcome validation, but rather the implementation of a controlled multiplex framework capable of moving beyond descriptive multiplex imaging toward more quantitative spatial characterization of immune biology.

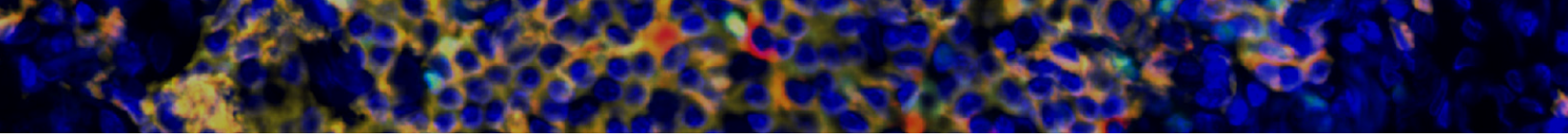
The panel was developed as a fit-for-purpose assay for translational PD assessment in immuno-oncology studies. Qualification activities were implemented early during development to ensure:

- controlled multiplex performance
- reproducible image acquisition
- analytical consistency
- specificity
- intra- and inter-run reproducibility

This qualification strategy supports the intended context of use: generation of reproducible and interpretable spatial metrics suitable for translational pharmacodynamic assessment.

## Clinical Context

Despite routine PD-L1 testing, response to immune checkpoint inhibitors remains highly heterogeneous in NSCLC. Tumors with similar PD-L1 profiles may exhibit very different biological behaviors due to differences in immune infiltration, cytotoxic engagement, suppressive niches, and tumor accessibility.



Many of these mechanisms cannot be adequately resolved using conventional single-marker assays or qualitative tissue assessment alone.

This creates a need for multiplex spatial approaches capable of generating quantitative and reproducible characterization of tumor–immune states within FFPE tissue.

## Biologically Relevant Multiplex Panel

The Tumor–Immune Spatial Fingerprint panel was intentionally designed around markers associated with biologically relevant immunotherapy mechanisms rather than maximizing plexity alone.

## Panel Composition

CD8, Granzyme B, FoxP3, PD-1, CD68, Ki-67, CD20, PanCK

Together, these markers enable simultaneous assessment of:

- immune infiltration
- cytotoxic activation
- suppressive immune regulation
- checkpoint engagement
- tumor proliferation
- lymphoid organization
- tumor accessibility

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This creates a mechanistically focused view of tumor–immune biology within a single FFPE tissue section.

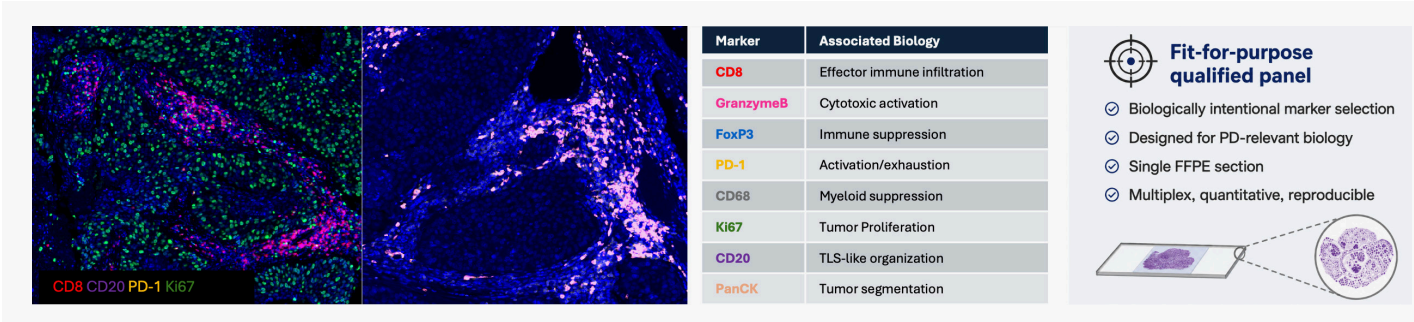
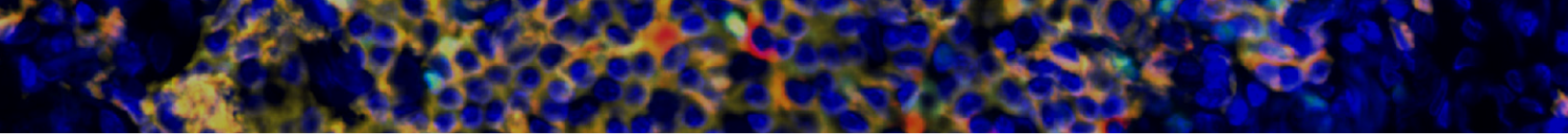



**FIGURE 1.** Biologically Relevant Multiplex Spatial Profiling



## Qualification and Analytical Control

A major differentiator of this approach is the implementation of a qualified and controlled multiplex workflow designed to improve reproducibility and confidence in generated spatial metrics.

Qualification activities included:

- optimization on human FFPE tissues
- multiplex signal balancing
- controlled staining conditions
- predefined acquisition parameters
- segmentation QC
- intra-run reproducibility assessment
- inter-run reproducibility assessment
- isotype specificity controls
- predefined acceptance criteria

Importantly, qualification focused not only on image quality, but on reproducible generation of biologically interpretable spatial metrics.

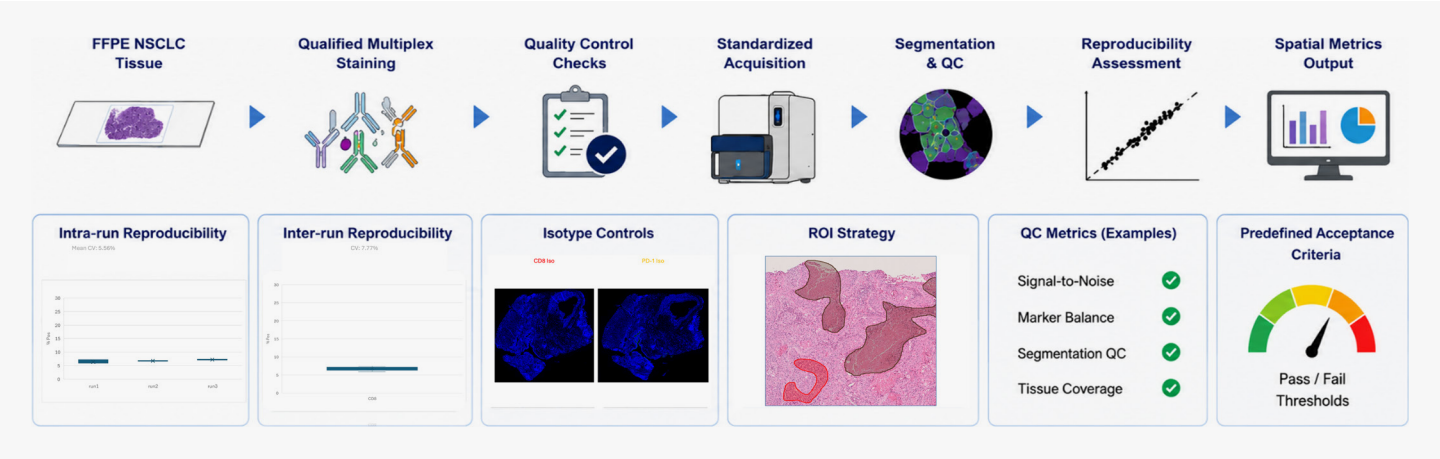



**FIGURE 2.** Qualification and Reproducibility Framework

## Quantitative Spatial Metrics

The assay generates quantitative outputs associated with pharmacodynamic-relevant tumor–immune biology, including:

- CD8-positive T-cell density
- cytotoxic Granzyme B-positive fractions
- CD8-to-tumor proximity
- CD8/FoxP3 immune balance
- suppressive macrophage localization
- TLS-like immune organization
- tumor–immune interface characterization

These metrics provide a more structured and quantitative framework for interrogation of tumor–immune states within FFPE tissue.

While not yet intended as clinically validated efficacy biomarkers, these outputs establish a controlled foundation for future translational and pharmacodynamic integration.

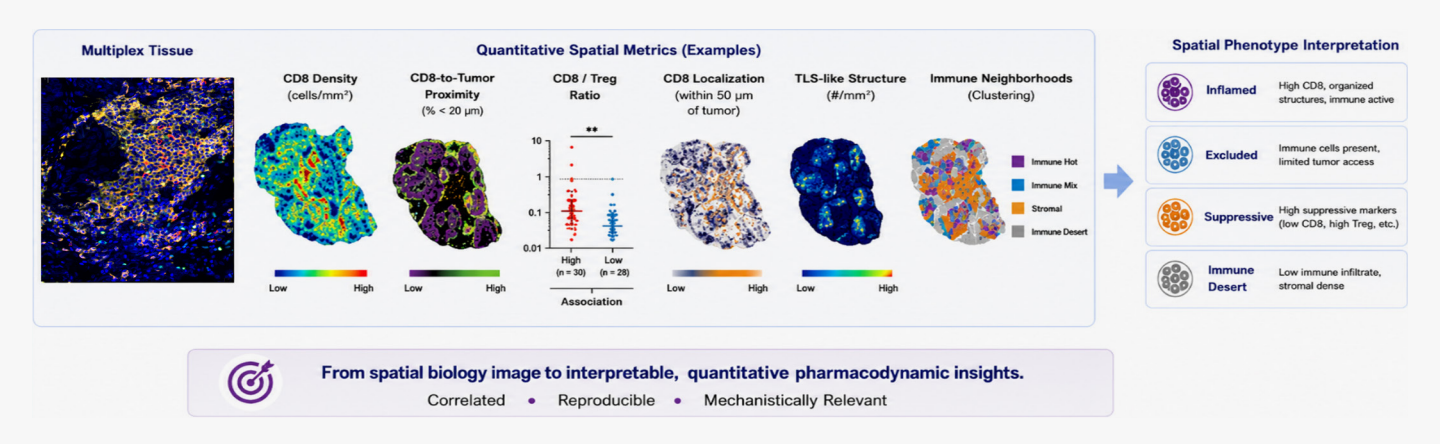



**FIGURE 3.** Quantitative Spatial Metrics Associated with Tumor–Immune Biology

## Conclusion

This case study represents an early but important step toward more clinically deployable spatial pharmacodynamic assessment in immuno-oncology.

Rather than focusing solely on descriptive multiplex imaging, the approach combines biologically relevant marker selection, qualified workflows, reproducibility assessment, and quantitative spatial analysis to generate controlled tumor–immune metrics associated with pharmacodynamic-relevant biology.

While not yet intended as a clinically validated predictive framework, this work establishes a reproducible and mechanistically interpretable foundation for future translational studies integrating spatial biology with therapeutic response assessment.



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