Case Study

A multiplex immunofluorescence assay to assess immune checkpoint inhibitor-targeted CD8 activation and tumor co-localization in FFPE tissues

Background:

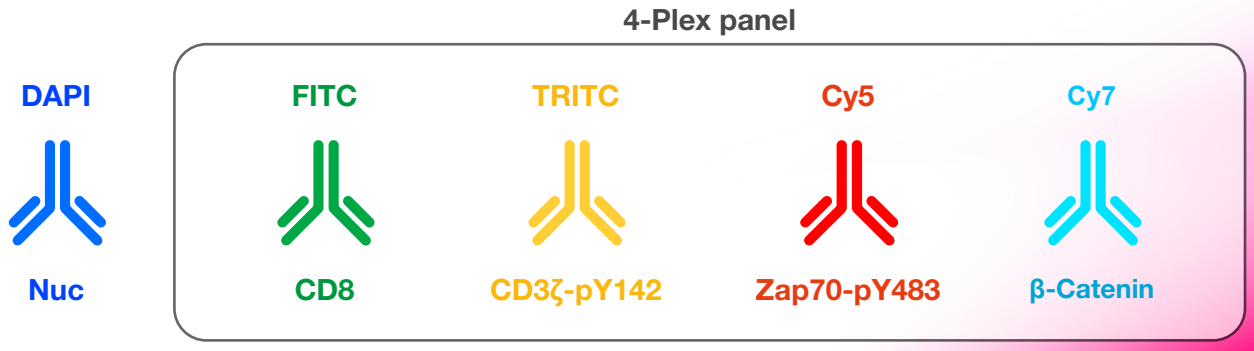
Immune checkpoint inhibitors promote anti-tumor immune responses by enhancing T-cell activity. Measuring the pharmacodynamic effects of these drug types presents a challenge as both the immune and cancer cell populations must be separated and assessed individually.

Researchers at the Pharmacodynamics (PD) Biomarkers group at the Frederick National Laboratory for Cancer Research wanted to develop a multiplex immunofluorescence assay for the in depth characterization of activated T cells in tumor tissue samples.

The conceptualized panel included the need to detect the tumor marker, β-Catenin, in the Cy7 channel on the fluorescence spectrum and ability to identify unique phosphorylation specific sites of marker expression.

Solution:

The PD Biomarkers group contracted the Ultivue Services Lab to utilize the InSituPlex® technology to develop a 4-plex quantitative multiplex immunofluorescence assay consisting of the following panel:

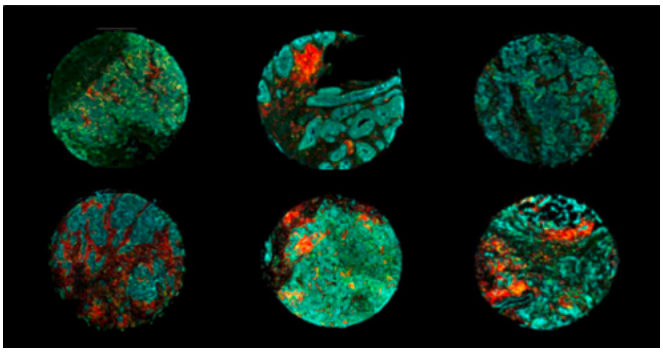


Outcome:

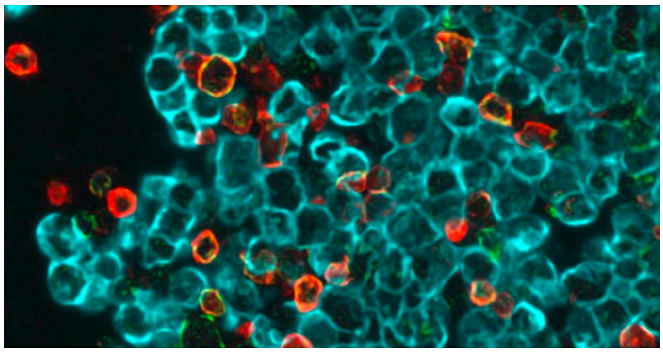
The custom assay was successfully developed and allowed for:

- Robust identification of β-Catenin in Cy7
- Seamless profiling of co-localized cell phenotypes
- Ability to apply the same optimized assay across multiple tissue types

This assay is now in the process of being internalized in order for the PD Biomarkers group at NCI to offer this panel in support of future clinical trials hosted at the National Cancer Institute.



Multiplex immunofluorescence staining across tumor tissue samples with CD8 (yellow), CD3ζ-pY142 (green), Zap70-pY483 (red), β-Catenin (cyan), and nuclear counterstain (blue).



Multiplex immunofluorescence staining in bead-activated CD3 cells + IFNγ-induced ACHN cell lines. Markers shown: CD3ζ-pY142 (yellow), CDS (red), β-Catenin (cyan).

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