#3962: Same-Species Multiplex IF with HCR™ Gold IF: Cross-Clone Benchmarking Using Abcam's Oncology-Validated Primaries

Wudy Yang[1], Randy Chen[1], Harry Choi[1], Aneesh Acharya[1], Will Howat[2]
[1]Molecular Instruments; [2]Abcam

progress happens together abcam | MOLECULAR INSTRUMENTS

BACKGROUND AND OBJECTIVES

Spatially resolved, multiplexed immunofluorescence (mIF) is essential for oncology research to profile tumor-immune architecture, track pathway activation, and stratify biomarkers in precious FFPE specimens. However, conventional mIF workflows are often limited by species constraints, secondary antibody cross-reactivity, custom conjugation requirements, and the need to validate multiple antibody clones, all of which can slow panel development. Here, we present a plug-and-play workflow that pairs HCR™ Gold IF and HiFi Encoder reagents with off-the-shelf Abcam primaries to enable robust same-species multiplexing with minimal optimization and rapid turnaround. Our main objective here is to establish a practical order-to-image workflow that can generate imaging-ready multiplex data in about one work week.

WORKFLOW AND METHODS

To first confirm that encoding preserved expected antibody performance, we performed 1-plex validations comparing HCR™ Pro IHC with Leica's BOND Polymer Refine Detection kit. After establishing comparable staining patterns with encoded antibodies, we benchmarked different Abcam clones for the same targets in human FFPE colorectal cancer tissue to assess whether the workflow could support flexible clone selection while preserving expected localization patterns. We then advanced into a 4-plex HCR™ Gold IF assay to demonstrate same-species multiplexing enabled by the HCR™ HiFi Encoder.

From Off-the-Shelf Antibodies to Multiplex IF in 3-5 Days

Order to image in just 3-5 days

DAY 0-2
Order & receive validated Abcam antibodies
Abcam qualifying US delivery in ~48 hours gets the workflow moving quickly.

DAY 2-3
Encode & stain
No custom conjugation and no iterative stripping workflow are required with the HCR™ HiFi Encoder.

DAY 4-5
Image & analyze
Reach imaging-ready multiplex IF data in less than one work week.

CROSS-CLONE BENCHMARKING IN 4-PLEX HCR™ GOLD IF

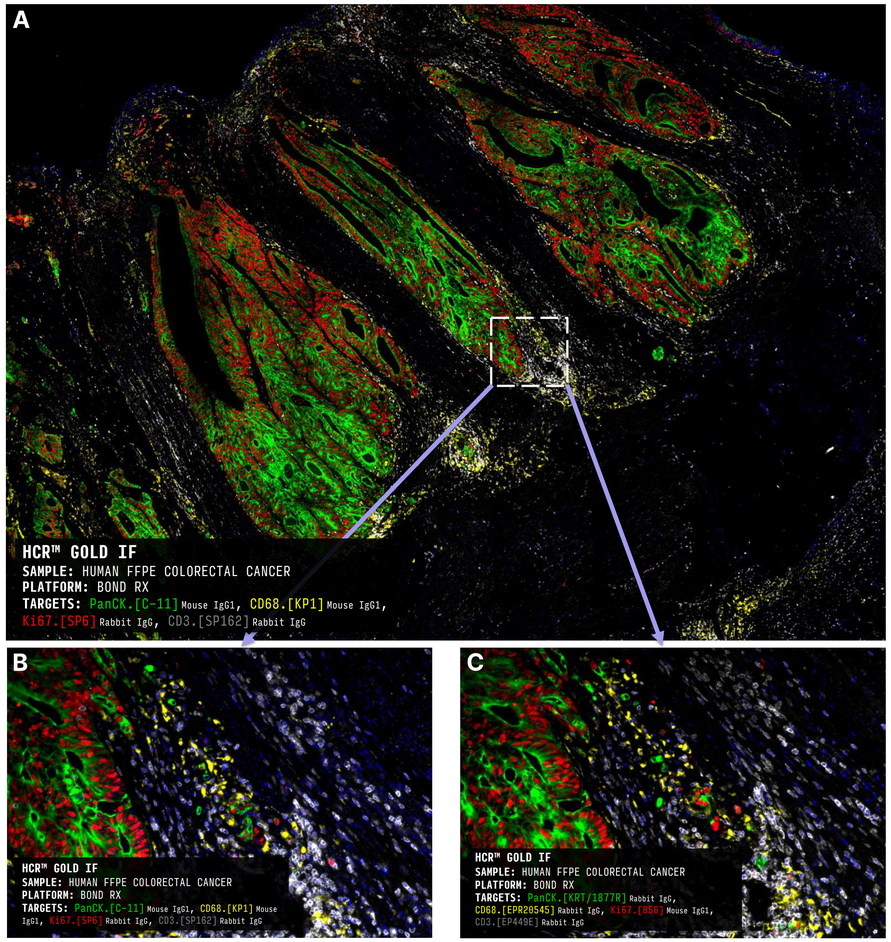
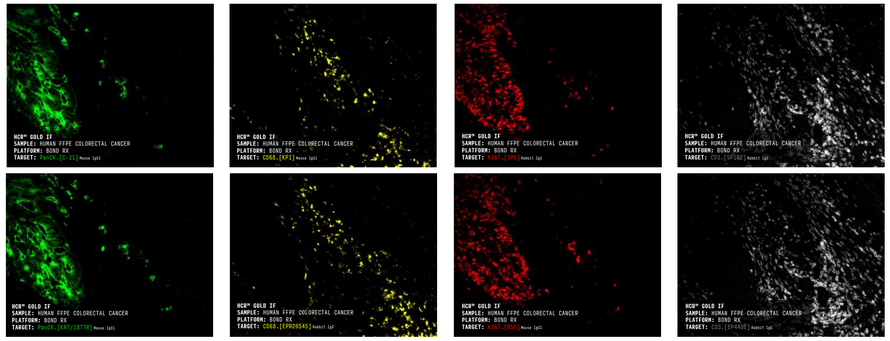
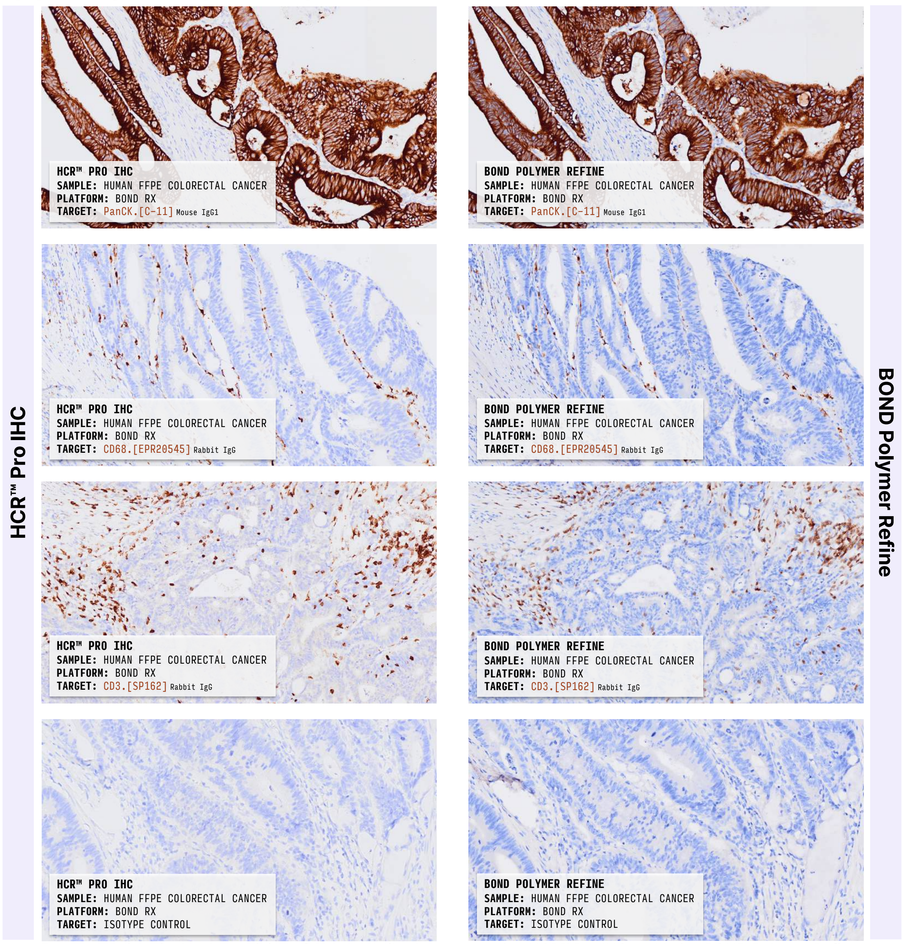


Figure A. Whole-slide scan of a 4-plex HCR™ Gold IF assay in human FFPE colorectal cancer tissue. **Figure B.** Higher-magnification view highlighting multiplex signal and tissue architecture. **Figure C.** Higher-magnification view using alternate antibody clones for all targets, demonstrating robust staining patterns across clone combinations.



ENCODING PRESERVES IHC PERFORMANCE



Representative single-plex staining of Abcam antibody targets using HCR™ Pro IHC and Leica BOND Polymer Refine Detection on serial tissue sections demonstrates comparable signal pattern and tissue localization. Clean isotype controls were observed in both workflows, supporting staining specificity and minimal nonspecific background.

CONCLUSIONS

This study demonstrates an automated, flexible mIF workflow that combines HCR™-based staining on the Leica Biosystems BOND RX Research Staining System with quantitative analysis in Indica Labs' HALO platform. In single-plex format, HCR™ Pro IHC produced staining patterns comparable to Leica BOND Polymer Refine Detection, supporting the use of HiFi Encoder-based detection with clinically relevant Abcam antibody clones. Building on that validation, HCR™ Gold IF enabled same-species mIF without iterative staining, simplifying panel development while preserving tissue morphology. Together, these results support a practical end-to-end approach for scalable tumor microenvironment profiling in FFPE tissues.

CONTACT: info@molecularinstruments.com