

#7673: 9-Plex Spatial Co-Detection of Clinically Relevant Protein and RNA Targets in FFPE Tumors with HCR™ Gold and MoxiePlex

Wudy Yang¹, Randy Chen¹, Harry Choi¹, Aneesh Acharya¹, Minakshi Singh², Quyen Vu²
¹Molecular Instruments; ²Hamamatsu Photonics

BACKGROUND AND OBJECTIVES

High-plex spatial profiling of protein and RNA in FFPE tumors is increasingly important for understanding tumor-immune interactions. However, many existing approaches rely on predefined antibody panels, harsh stripping or elution steps, and iterative staining cycles that can compromise tissue integrity and make assay development and validation more burdensome. HCR Gold IF, enabled by the HiFi Encoder, supports enzyme-free fluorescent detection using unmodified user-supplied primary antibodies and operates on the same amplification platform as HCR Gold RNA-FISH. Combined with the Hamamatsu MoxiePlex multispectral imaging platform, this workflow provides a practical route to flexible, single-round 7- to 9-plex protein and RNA co-detection in FFPE tissue sections.

WORKFLOW AND METHODS

FFPE human colorectal tumor sections were stained with HCR™ Gold IF using encoded, clinically validated primary antibodies together with HCR™ Gold RNA-FISH.

- HCR™ Gold IF** enables:
- Parallel amplification of same-species primaries, no stripping required
 - Universal compatibility with unmodified primaries
 - Combined protein and RNA detection powered by the same HCR™ Gold amplification chemistry

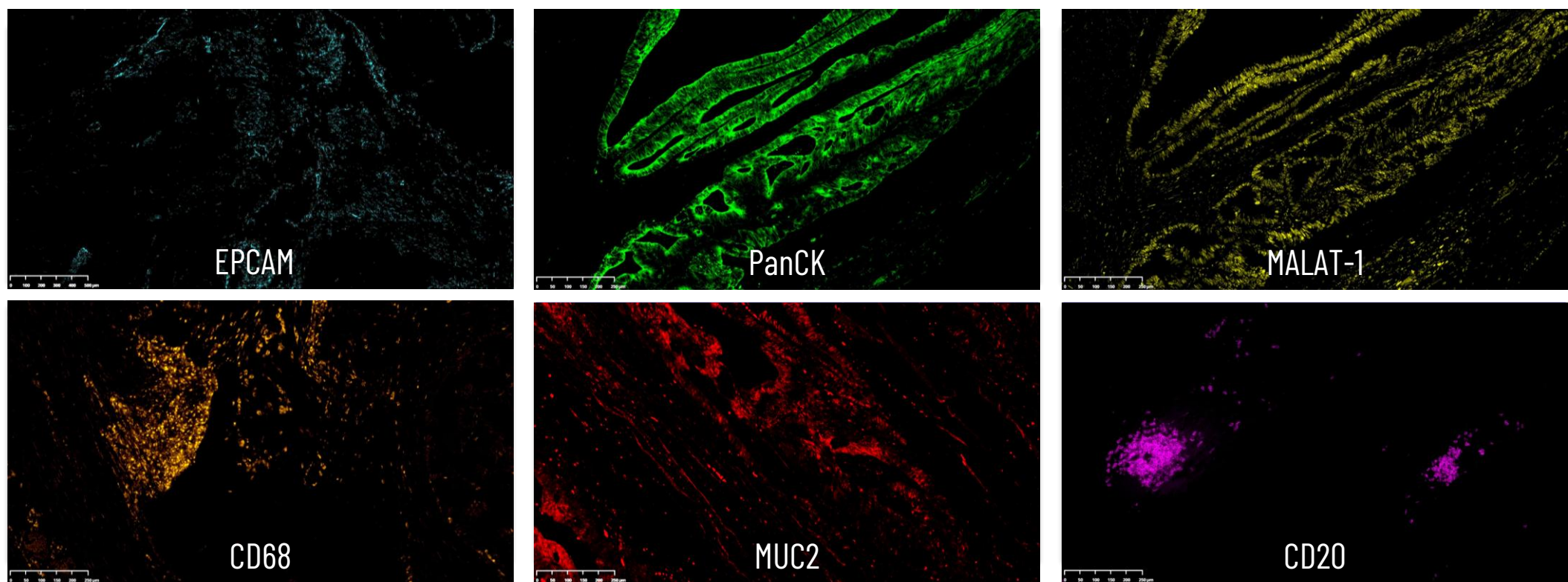
Imaging was performed on the Hamamatsu MoxiePlex spatial multiomics platform, which supports RNA and protein detection across a broad range of assay types.

- MoxiePlex** provides:
- Best-in-class image quality via a high-resolution 16-bit output CMOS camera, ultra-low readout noise, and high quantum efficiency across a 400 to 900 nm wavelength range
 - 9-color spectral unmixing and autofluorescence subtraction
 - Multi-band filters for fast scanning and single-band filters for no spectral unmixing of optimized 4-plex

For each fluorophore-target pairing, single-stain reference slides were generated to establish exposure settings, verify filter performance, and quantify spectral bleed-through for spectral unmixing. Multiplex slides were imaged in a single acquisition round and assessed for signal intensity, channel crosstalk, and concordance with single-stain references.

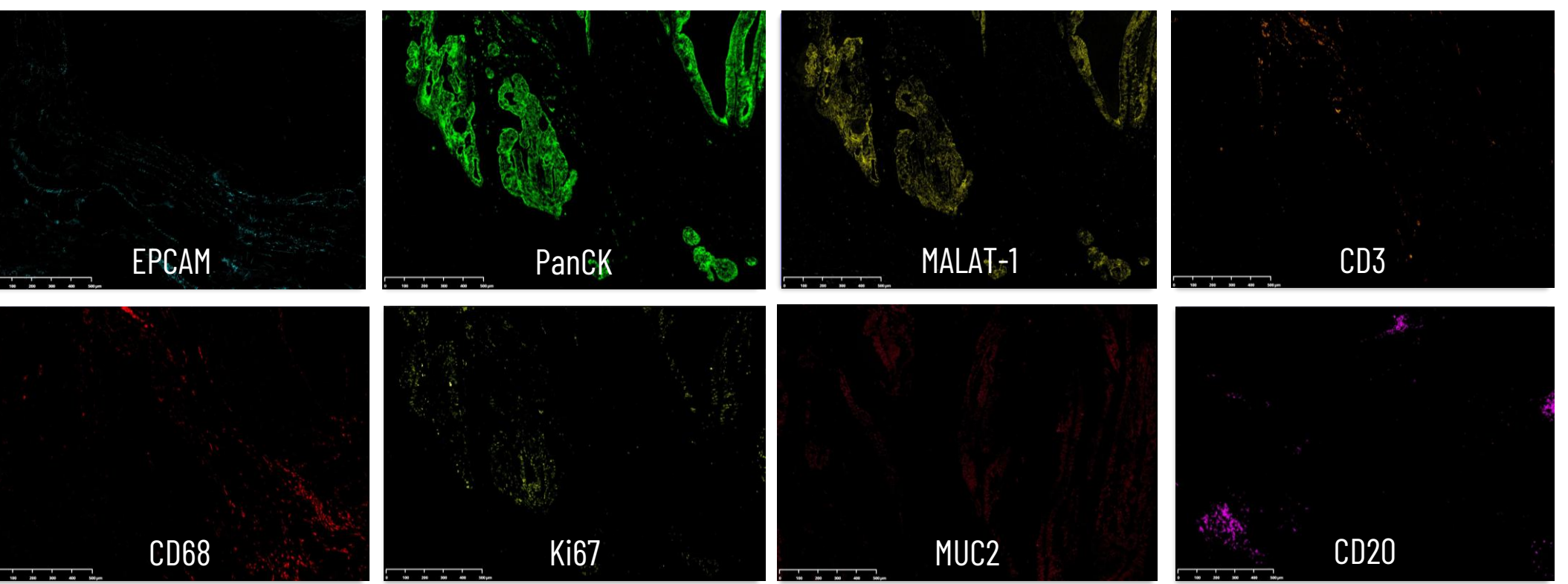
6-PLEX PANEL : 7 Fluorescent channels

System	Target	Biomarker
X6-425	EPCAM	RNA
X1-488	PanCK	Protein
X2-546	MALAT1	RNA
X8-594	CD68	Protein
X9-700	MUC2	RNA
X4-750	CD20	Protein
	DAPI	Nuclear stain

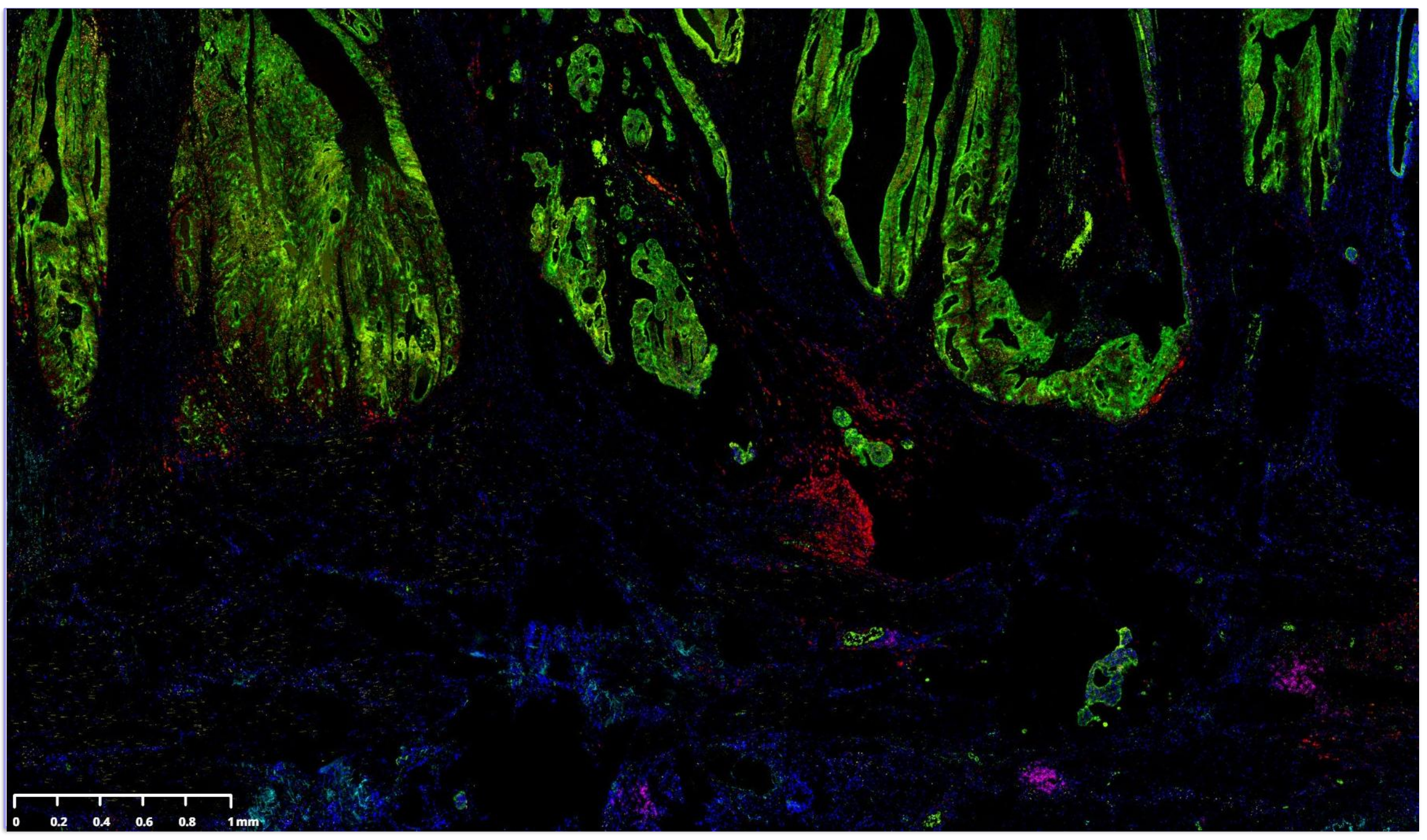
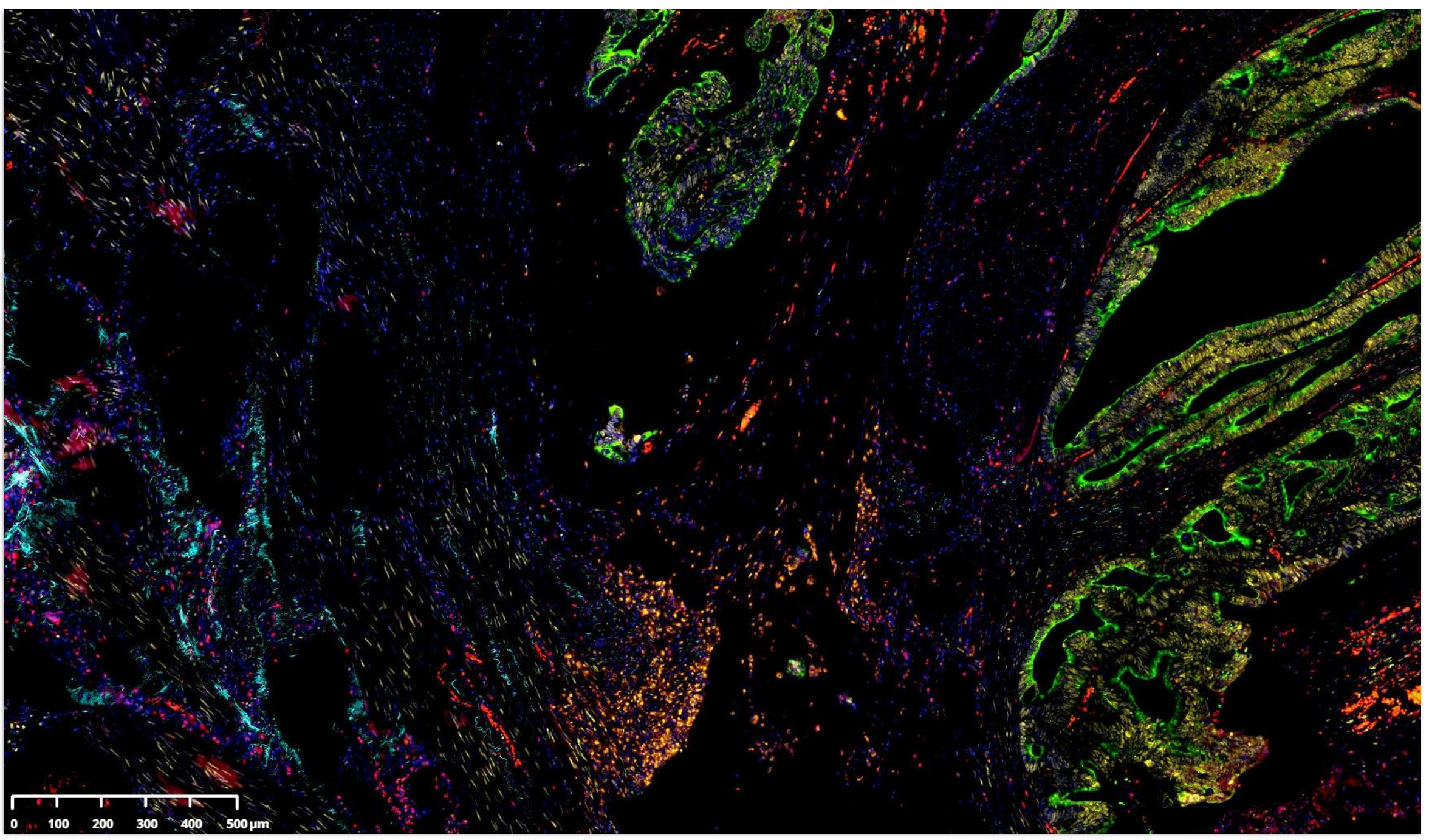


8-PLEX PANEL : 9 Fluorescent channels

System	Target	Biomarker
X6-425	EPCAM	RNA
X1-488	PanCK	Protein
X7-514	MALAT1	RNA
X2-546	CD3	Protein
X8-594	CD68	Protein
X3-647	Ki67	Protein
X9-700	MUC2	RNA
X4-750	CD20	Protein
	DAPI	Nuclear stain



RESULTS



CONCLUSIONS

HCR™ Gold IF and HCR™ Gold RNA-FISH can be combined in a single-round workflow on the MoxiePlex platform to achieve up to 9-plex imaging of clinically relevant protein and RNA targets in FFPE tissue. This approach avoids stripping and photobleaching, maintains compatibility with validated antibodies and standard filter sets, and provides a practical path to flexible spatial assays for translational and clinical research, while enabling high-quality whole-slide spatial profiling across mixed protein and RNA panels.