Antibody Development and Validation in the Era of Spatial Proteomics

Ashley Oliver, Kristine Trueman, Jodi Hagen, Alex Kalyuzhny, and Nidhi Nath

R&D Systems, a Bio-Techne brand, Antibody Development, Minneapolis, MN

bio·techne®
R&D SYSTEMS

Learn more about multiplex IF (mIF) antibody validation.

01 // Overview

- Spatial proteomics can create high resolution map of large number of proteins at subcellular resolution within tissues, enabling understanding of complex cellular processes.
- Visualizing large array of proteins within complex tissue environment requires highly sensitive and specific antibodies.
- In collaboration with Lunaphore, we have deployed processes to meet the exacting standards for antibodies on the spatial biology COMET™ platform.
- Antibodies designed for purpose will allow broad adoption of spatial proteomics and multiomics in research, drug discovery and diagnostic fields.

02 // COMET™ an Automated Sequential Immuno-fluorescence (seqIF™) Platform for Spatial Biology
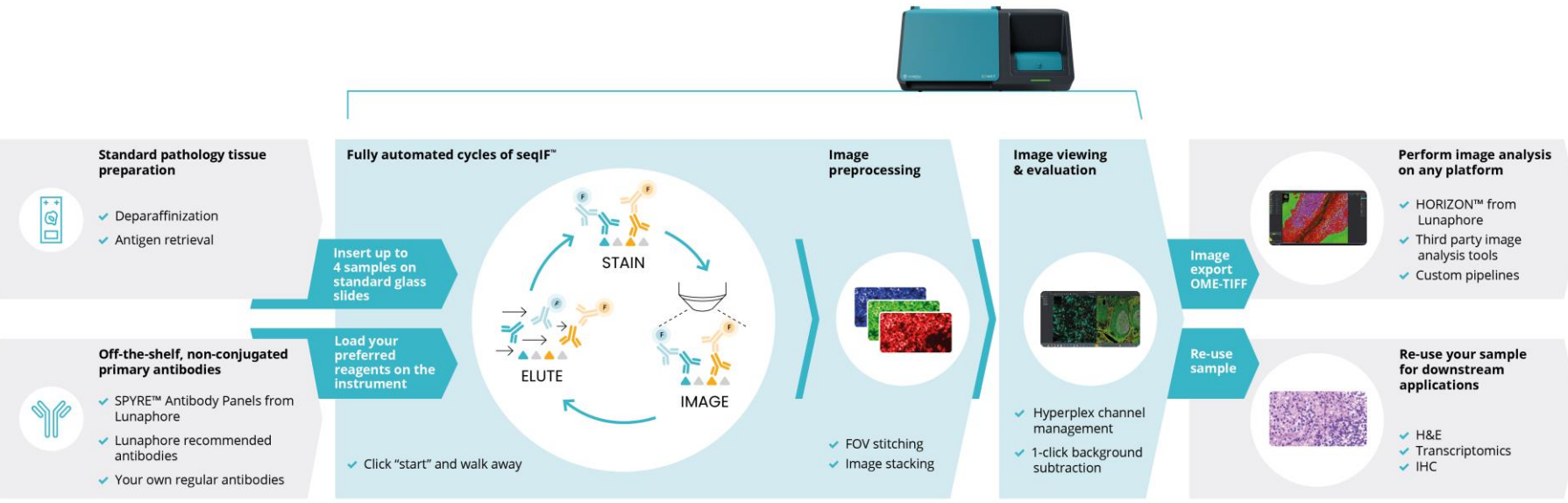

Figure 1. COMET™ - automated staining & imaging platform for multiplex spatial biology

- Unmatched multiplex throughput with walk-away automation
- Rapid and flexible panel development using label-free antibodies
- True reproducibility and tissue preservation

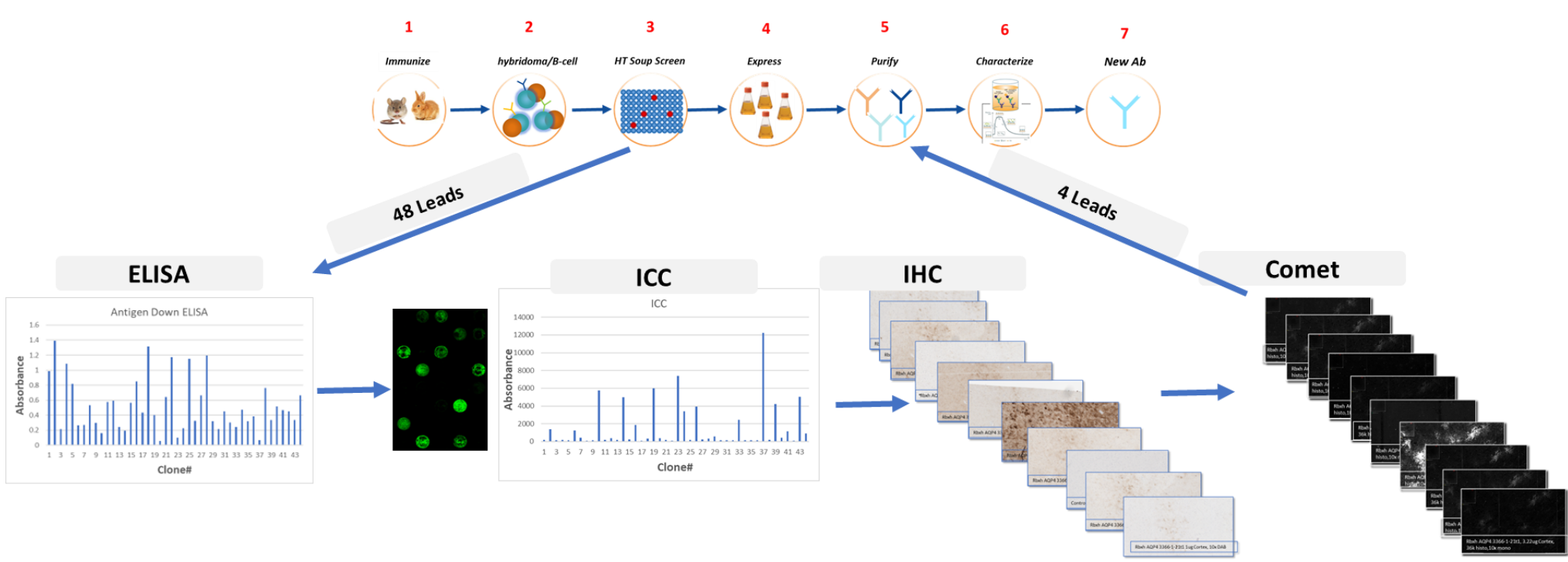## 03 // Expanded COMET™ Antibody Discovery Workflow

Figure 2. Antibody discovery and validation workflow to select sensitive and specific antibodies for COMET™

- Improved methodology for immunization
- Expanded primary screening to identify lead candidates
- Enhanced testing for platform specific performance

04 // COMET™ Specific Optimization

For best performance on COMET™, antibodies are optimized for, concentration, elution condition, elution cycles, sensitivity, and specificity.
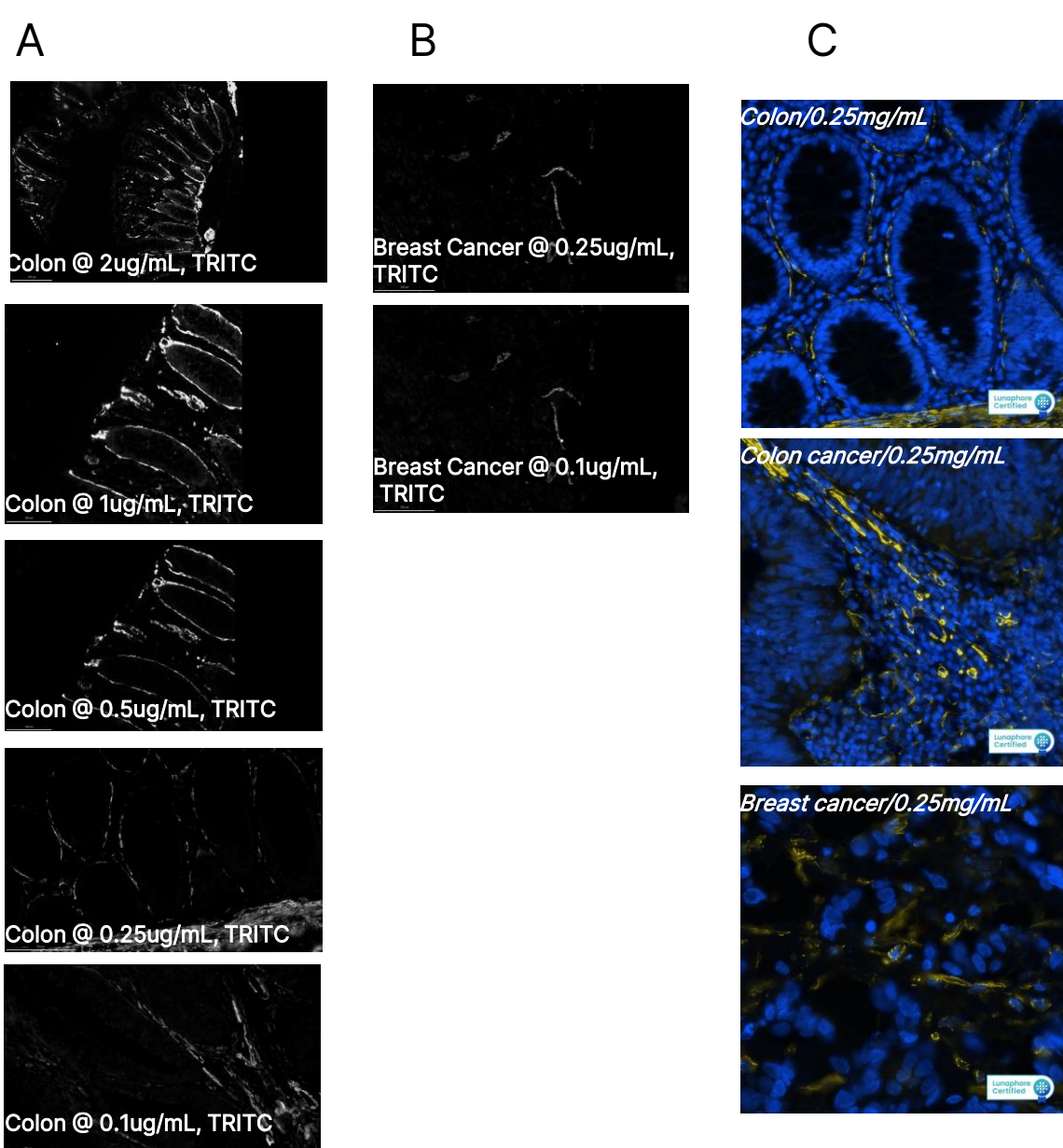
Smooth Muscle Actin (α-SMA) Antibody Optimization

Figure 3. Representative images showing impact of SMA antibody concentration on tissue staining in colon **(A)** and breast cancer **(B)**. **(C)** Final images using optimized antibody concentration of 0.25 µg/mL in colon, colon cancer, and breast cancer tissues

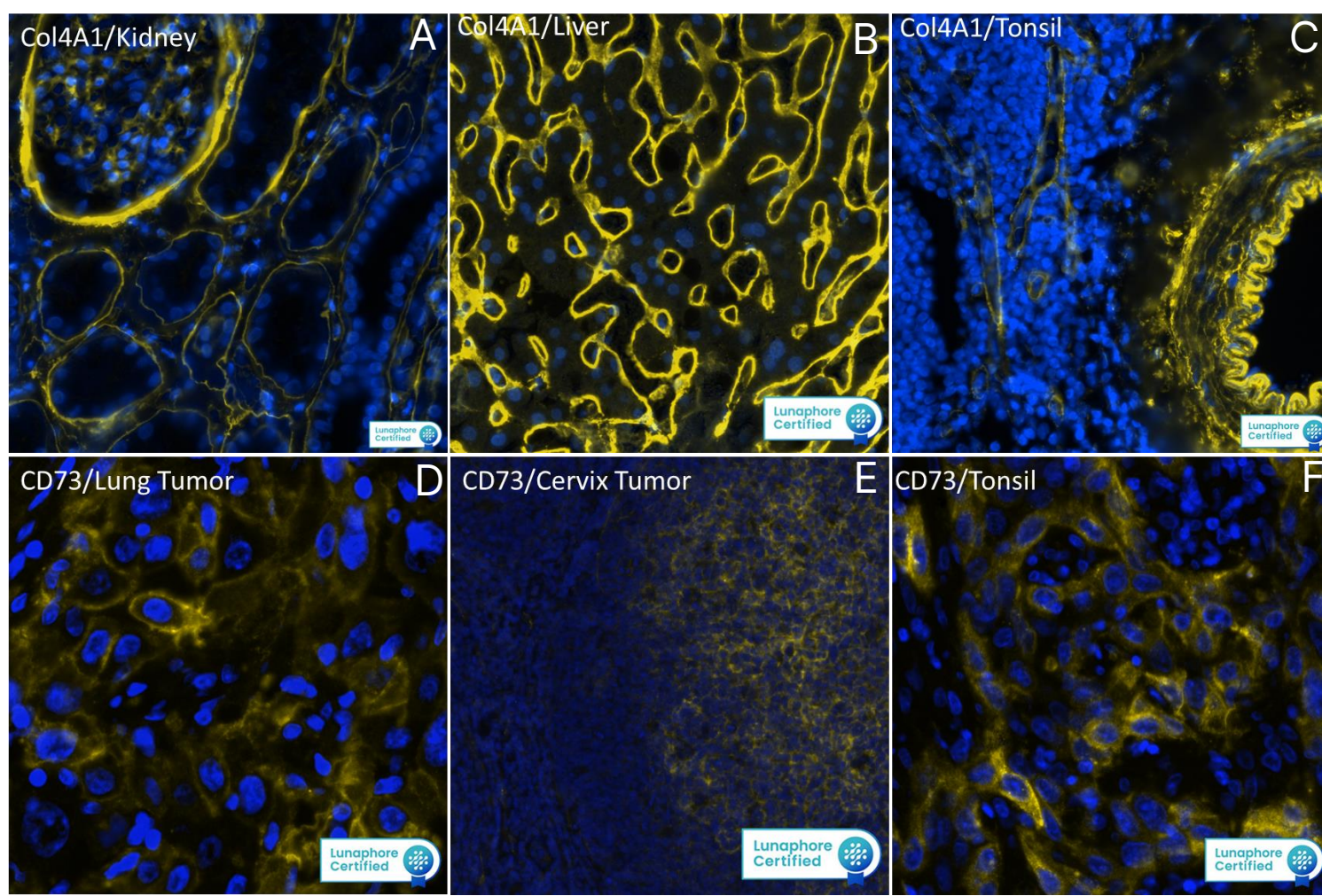## 05 // COMET™ Antibodies Optimized for Specificity

Figure 4. COMET™ optimized antibodies are tested for specificity using multiple tissues. Collagen type IV alpha 1 chain (COL4A1t) localization in basement membrane in kidney **(A)**; liver **(B)** and tonsil **(C)**. CD73 localization in lung tumor **(D)**; cervix tumor **(E)**; and tonsil **(F)**.

06 // Multiplexed Tissue Image using COMET™ Optimized Antibodies
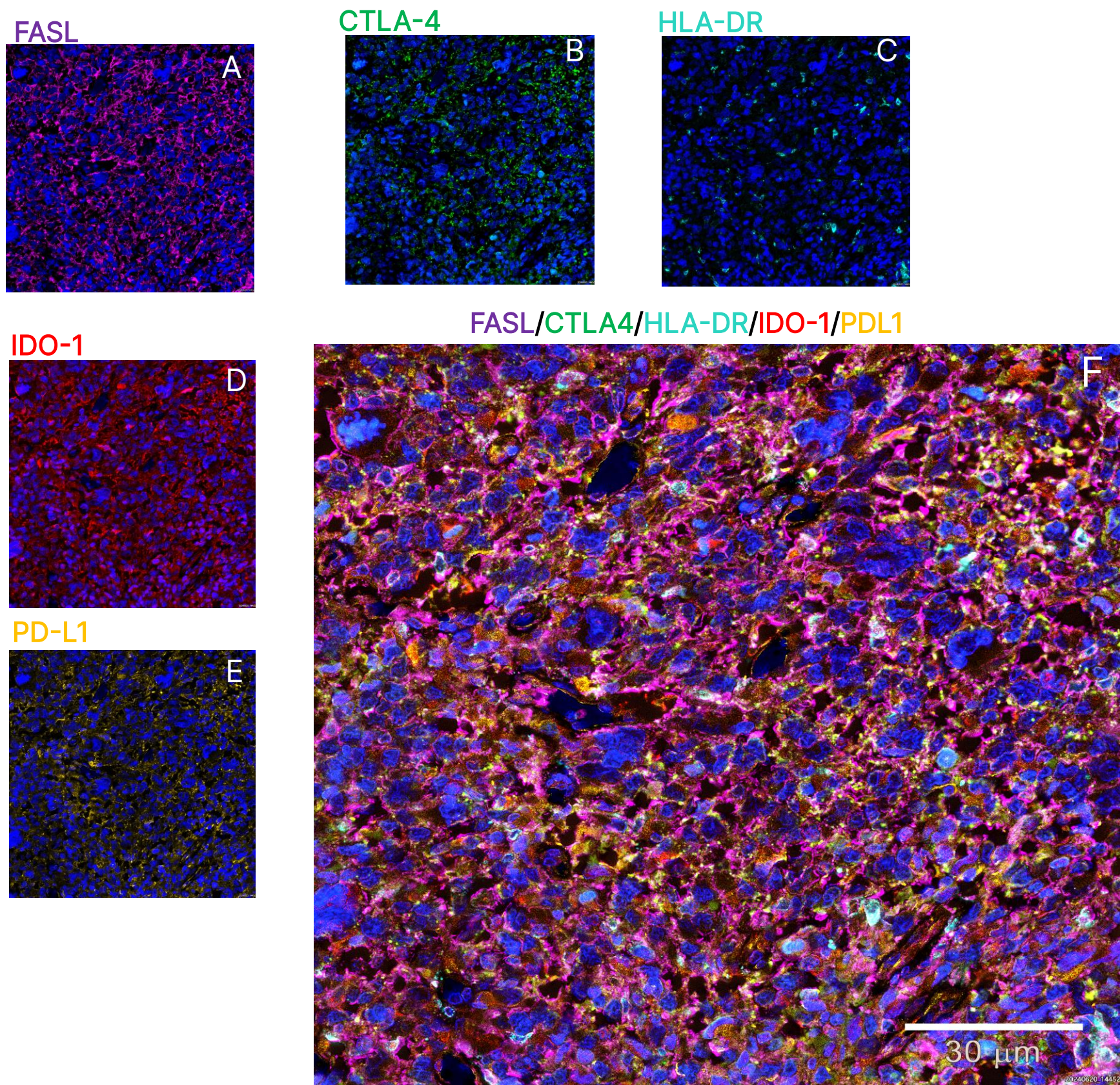

Figure 5. Multiplex fluorescence imaging in glioblastoma tissues for five different protein markers-FASL **(A)**, CTLA-4 **(B)**, HLA-DR **(C)**, IDO-1 **(D)** and PD-L1 **(E)**. **(F)** Composite image highlighting the spatial distribution of five markers on the tissue.
Antibodies used were optimized for COMET™

07 // Results and Conclusions

- We demonstrate that enhanced development and testing strategies can meet the needs for high quality spatial biology antibodies.
- COMET™ platform stands out for multiplexed imaging by eliminating the need for antibody conjugation while enabling automation and high-throughput analysis.
- We are actively expanding and qualifying our antibody catalog to provide customers with an increasingly diverse and robust portfolio of COMET™ tested antibodies for their research needs.