

Expanding the boundaries of spatial proteomics with a universal antibody labeling strategy and EpicIF™ multiplexing chemistry



Thore Boettke¹, Jannik Boog¹, Charles Jackson¹, Matt Ingalls¹, Brian Lane¹, Arne Christians¹, Oliver Braubach¹

¹Bruker Spatial Biology, Research and Development, St. Louis, Missouri, USA

Introduction

Highly multiplexed immunofluorescence (mIF) has transformed cancer research by enabling the simultaneous detection of dozens of biomarkers and cell types within the spatial context of the tumor microenvironment (TME). However, mIF assays often require custom antibody content, which is difficult to attain with inflexible or proprietary chemistries. Alternative detection systems provide partial workarounds but frequently rely on harsh conditions that can compromise tissue integrity and reduce assay reproducibility.

To overcome these barriers, we developed VistaFlex™ Antibody Labeling Kit Powered by Proteintech Group, a universal antibody-labeling strategy compatible with the CellScope™ Precise Spatial Proteomics platform and EpicIF™ multiplexing chemistry. Our new workflow enables low-volume fluorophore labeling of virtually any IgG antibody, independent of antibody buffer formulations, using standard organic fluorophores in minutes and without specialized instrumentation. The resulting labeled antibodies integrate seamlessly into CellScope workflows.

Methods

To evaluate the compatibility of our new antibody labeling strategy with the CellScope™ Platform, we conducted a series of comparative staining experiments. We selected several well-characterized, routinely used antibodies with established IHC-P DAB performance and labeled them using the VistaFlex Antibody Labeling Kit Powered by Proteintech Group. Staining quality and target specificity were assessed by comparing the resulting immunofluorescence stain with their corresponding chromogenic reference stain.

Next, we expanded our commercially available VistaFlex™ Multiplexing Assay Kits by incorporating additional antibodies with a dedicated focus on metabolic markers. This extension, enabled by VistaFlex Kits, allowed a more detailed investigation into cellular metabolism in the TME.

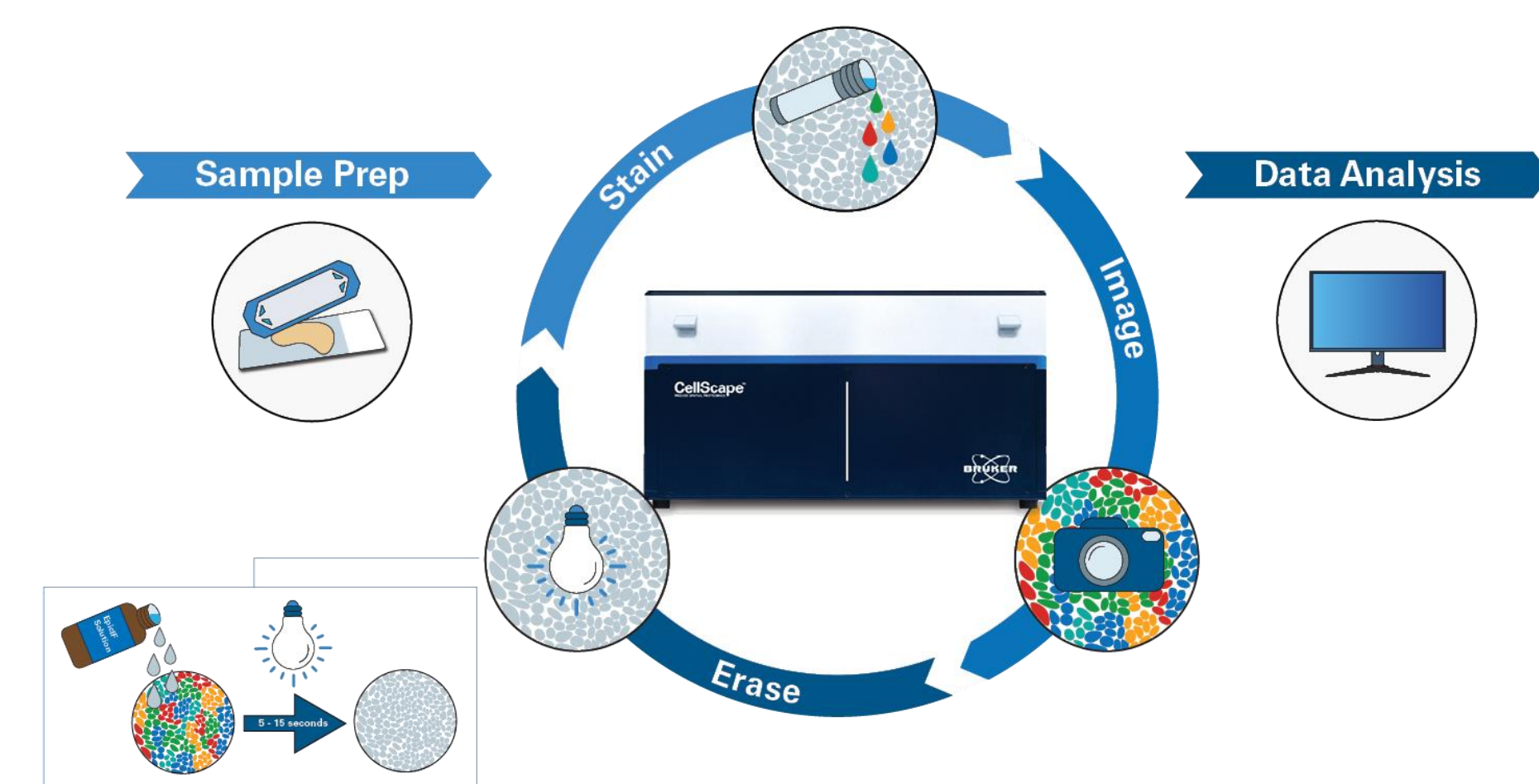


Figure 1. The EpicIF technology workflow on the CellScope platform. The instrument uses cycles of staining, imaging, and signal removal to detect biomarkers with spatial context at single-cell resolution.

Comparing VistaFlex™ Antibody Labeling to Chromogenic DAB Staining

To assess the labeling quality and selectivity of our universal labeling method we compared the staining of six routinely used markers on serial sections. On one section all six markers were stained using the VistaFlex Antibody Labeling Kit Powered by Proteintech Group, while another six serial sections were used to do single chromogenic DAB stains of each marker. The comparison shows that our universal labeling allows the seamless transfer of established primary antibodies to the CellScope platform. Staining patterns were comparable and specific. This experiment also demonstrates the utility of multiplexed immunofluorescence staining, as all six markers could be stained on a single slide, while the chromogenic DAB staining needed one slide for each marker (not shown).

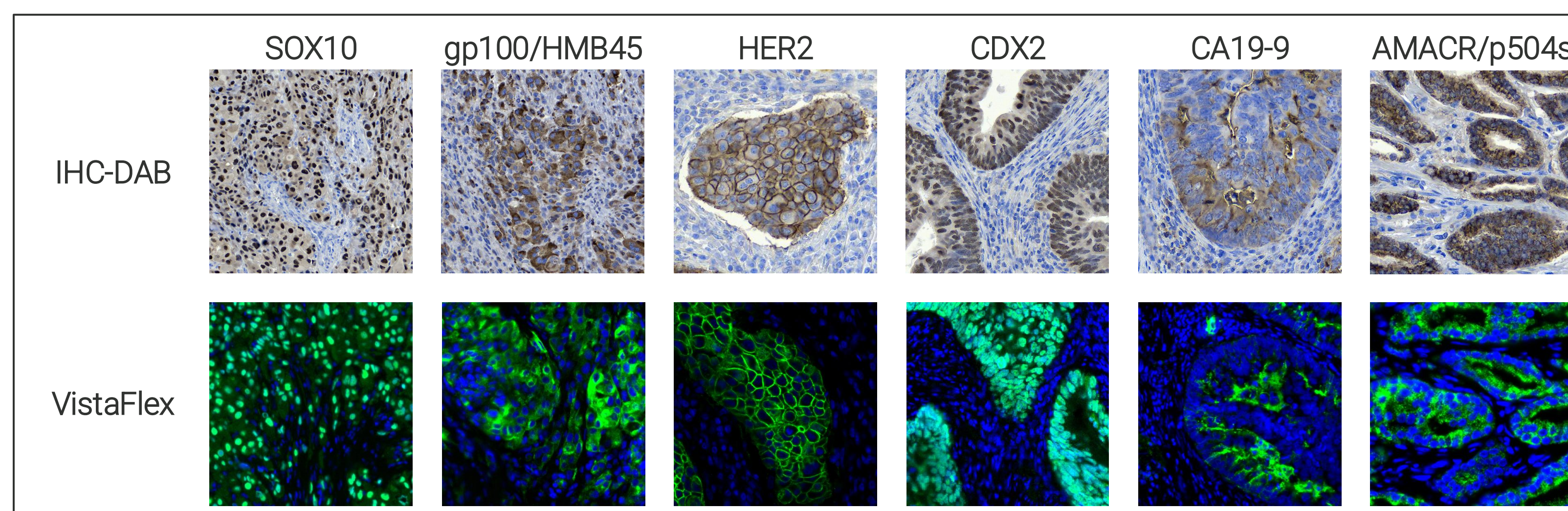


Fig. 2 Comparative DAB and VistaFlex labeled staining. Staining was done on a multi tumor array including melanoma (SOX10 and gp100/HMB45), breast cancer (HER2), colon cancer (CDX2 and CA19-9) and prostate cancer (AMACR/p504s). In the immunofluorescence stains, the antibody stain is shown in green and DNA in dark blue. For better comparison, the same antibody dilution was used for both staining methods.

VistaFlex™ Antibody Labeling Kits Powered by Proteintech Group Enabled Customization and Expansion of the VistaFlex™ Multiplexing Assay Kits

The universal labeling workflow enables flexible customization of immunofluorescence assays on the CellScope Platform. Using this approach, we expanded the standard VistaFlex Multiplexing Assay Kits - originally designed to profile cell boundaries, immune composition, and tissue architecture - by incorporating additional markers that capture cellular metabolic states (see Fig. 3). While the baseline VistaFlex™ Kits already support comprehensive characterization of tumors and the TME, the added customization enabled by VistaFlex Kits allowed us to further extend the panel and achieve more detailed tumor subtyping.

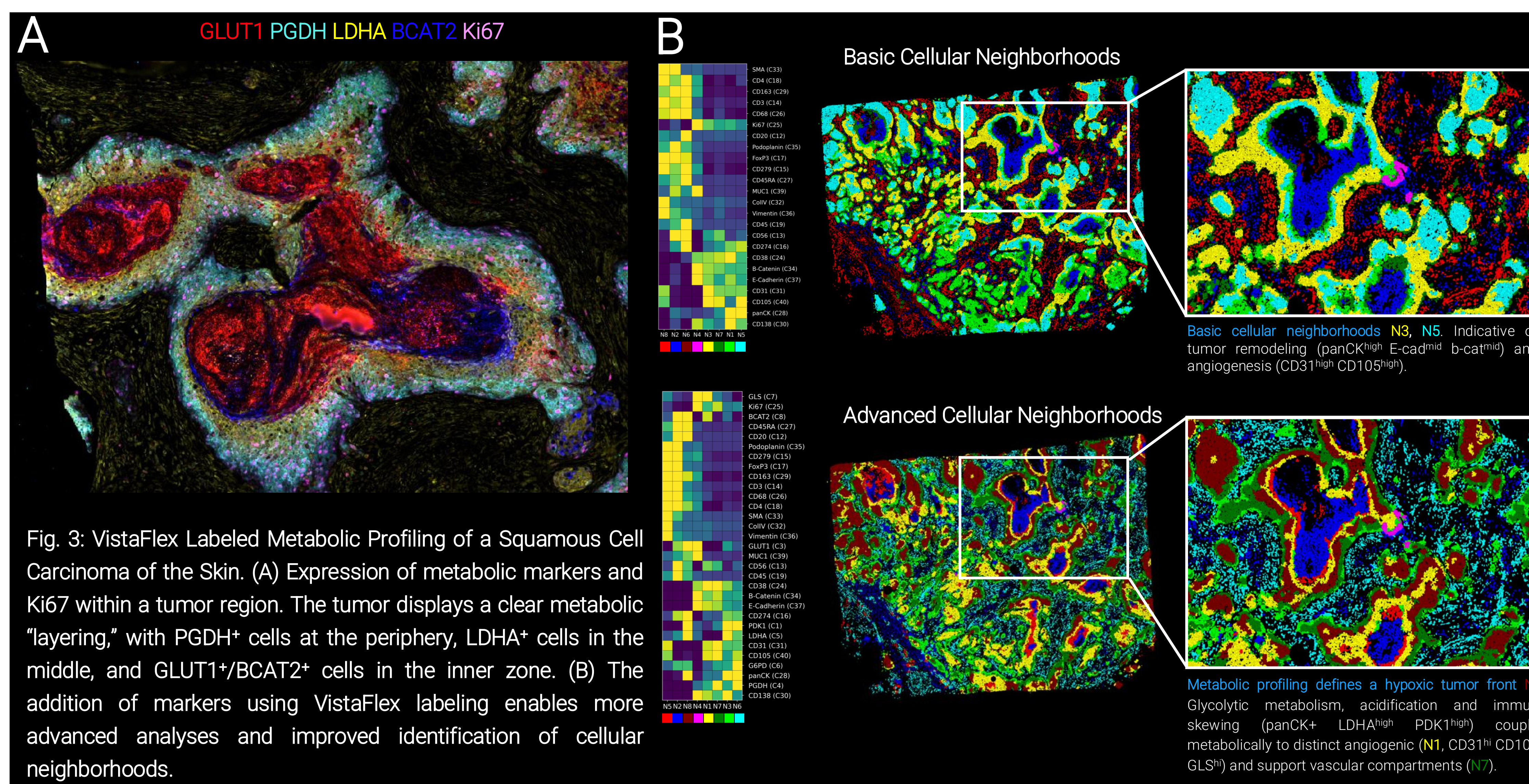


Fig. 3: VistaFlex Labeled Metabolic Profiling of a Squamous Cell Carcinoma of the Skin. (A) Expression of metabolic markers and Ki67 within a tumor region. The tumor displays a clear metabolic "layering," with PGDH⁺ cells at the periphery, LDHA⁺ cells in the middle, and GLUT1⁺/BCAT2⁺ cells in the inner zone. (B) The addition of markers using VistaFlex labeling enables more advanced analyses and improved identification of cellular neighborhoods.

Summary

VistaFlex™ Antibody Labeling Kits powered by Proteintech Group provide a universal, low-effort antibody-labeling strategy that removes long-standing barriers in custom multiplex immunofluorescence. By enabling rapid fluorophore labeling of virtually any IgG antibody and seamless integration into the CellScope™ Platform, VistaFlex™ Kits expand the flexibility and applicability of high-plex spatial proteomics. Our validation experiments demonstrate that VistaFlex Kit labeled antibodies retain the staining quality and specificity of their chromogenic reference stains while allowing all markers to be assessed on a single section. Furthermore, the ability to customize and extend VistaFlex™ Kits - illustrated here through the addition of metabolic markers - enables deeper biological insights, including refined tumor subtyping and more granular analysis of cellular neighborhoods. Together, these results highlight VistaFlex™ Kit antibody labeling as a powerful tool for researchers seeking adaptable, high-quality, and scalable mIF workflows for advanced tumor and TME characterization.

Conclusion

- VistaFlex™ Kits powered by Proteintech Group enables rapid, universal labeling of virtually any IgG antibody, removing common barriers to custom mIF assay development.
- VistaFlex Kit-labeled antibodies perform comparably to established chromogenic stains, allowing direct transfer of validated antibodies onto the CellScope™ Platform.
- Customized expansion of VistaFlex™ Kits is easily achieved, supporting the addition of new biological themes such as metabolic profiling. Enhanced panel flexibility enables deeper tumor characterization, including refined subtyping and improved identification of cellular neighborhoods.
- Together, VistaFlex Kits and the CellScope platform provide a scalable, high-quality workflow for advanced spatial proteomics in tumor and TME research.

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