2783 Fast and accurate spatial phenotyping and immuno-profiling using Ultivue UltiMapper kits and the ZEISS Axioscan 7 slide scanner

Catherine Benson*, Keith Wharton*, Gourab Chatterjee*, Markus Neumann†, Volker Döring†, Maël Manesse*

*Ultivue, Inc. 763D Concord Avenue, Cambridge, MA 02138 • +1-617-945-2662 • www.ultivue.com • contact@ultivue.com • Twitter: @Ultivue

†Carl Zeiss Microscopy GmbH, Carl-Zeiss-Promenade 10, 07745 Jena • +49-3641-64-1901 • www.zeiss.com/microscopy • microscopy@zeiss.com • Twitter: @zeiss_micro

Introduction

Understanding the complexities of the tumor micro-environment in detail can vastly improve the accuracy of immuno-oncology research and accelerate the discovery of potential immunotherapy targets. Multiplexed immunofluorescence (mIF) assays have emerged as a critical approach for identifying complex cellular phenotypes in the tumor micro-environment, powered by the value of the spatial correlation of such phenotypes in a tissue specimen.

The Ultivue UltiMapper® I/O and U-VUE® kits enable rapid, pre-optimized staining of multiple targets simultaneously in a single FFPE tissue section. This technology is ready-to-use with conventional automated staining workflows and commercially available automated imaging systems.

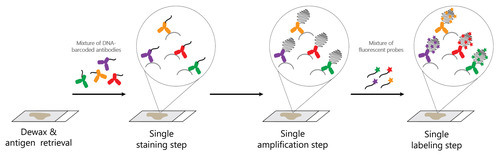
Here, we demonstrate the potential of accurate cellular phenotyping using the InSituPlex technology with fast whole-slide scanning using ZEISS Axioscan 7, an automated slide scanning system for fluorescence and transmitted light applications with modern cameras, a sophisticated focusing method and a powerful imaging software. The compounded effect of integrating these technologies can significantly improve the throughput of immuno-oncology research.

Methods

- Two de-identified FFPE non small cell lung cancer (NSCLC) tissue samples were stained with the UltiMapper I/O PD-L1 Kit labeling CD8, CD68, PD-L1, and CK/Sox10.
- Slides were fully stained in one run with a cocktail of primary antibodies using a Leica Biosystems BOND RX autostainer.
- Serial sections for each sample were stained with H&E to provide additional morphological information.
- Post-staining, the slides were coverslipped and imaged on ZEISS Axioscan 7. The resulting images were analyzed using IndicaLabs HALO analysis software.

Workflow

Staining- UltiMapper I/O PD-L1



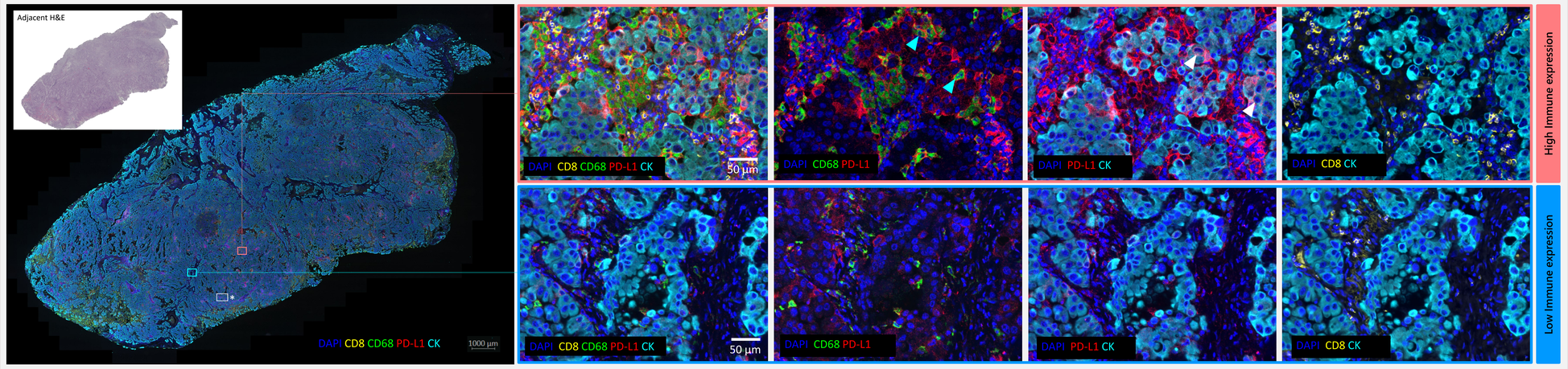
- Pre-optimized assay panel, rapid and automated protocol
- Same-day results

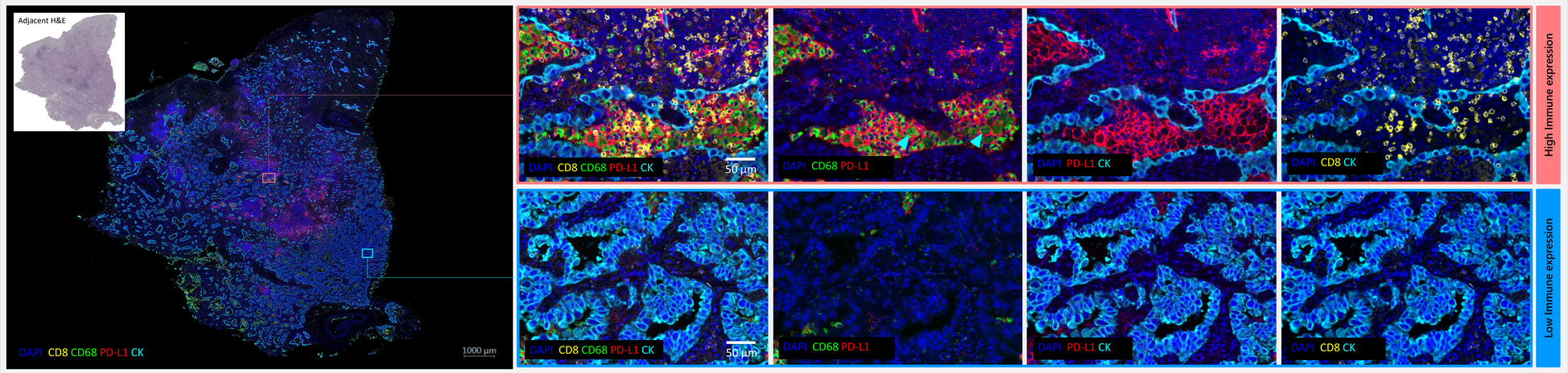
Brightfield and fluorescence scanning

- ZEISS Axioscan 7 – next generation slide scanner
- Fast brightfield based focusing method – Transfer Intensity Equation – enables increased scan speeds and fluorescence preservation

- New cameras:
  - Axiocam 712 mono – 12-megapixel camera for highest resolution and sensitivity in fluorescence
  - Axiocam 705 color – for brilliant color representation
- Dedicated filter sets offer crosstalk-free multiplexed acquisition

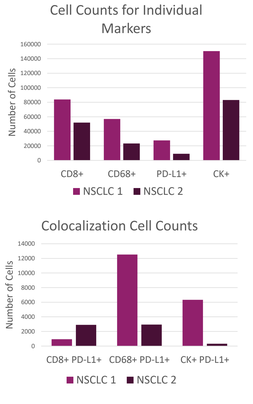
Results: H&E and 4-plex whole slide images with ZEISS Axioscan 7



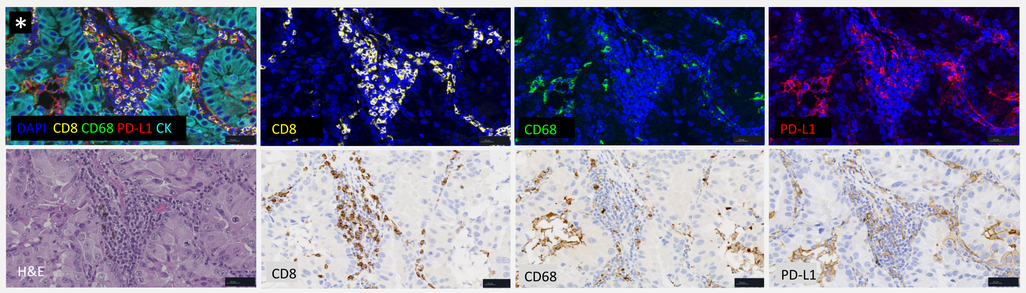


Two NSCLCs (top and bottom) exhibiting distinct histological growth patterns and heterogeneous inflammatory infiltrates. Right panels show magnified regions of interest for each tumor. Red and blue boxes, respectively, illustrate high and low immune expression regions with markers indicated in each subpanel. Note PD-L1 positivity in both macrophage (CD68+) (blue arrows), and tumor populations (CK+) (white arrows), with distributed CD8 positive T-effector cells.

Cell phenotyping quantification and IHC comparison



Whole slide analysis of both tissue samples yielded total cell counts and colocalization cell counts, identifying relevant cell and phenotype populations.



To confirm the distribution of each marker and cell type, serial sections of the NSCLC samples were stained with singleplex DAB for CD8, CD68, and PD-L1. The images were used as a confirmation of the specificity of the multiplex IF assays. Note high concordance of staining pattern between IF (top row, magnified view of white box in top panel) and IHC stains (bottom row).

Conclusion

We describe an efficient and streamlined workflow for mIF staining, imaging, and analysis of tumor samples. With this workflow, a 4-plex (4 biomarkers plus nuclear counterstain) whole slide immunofluorescence image of FFPE tumor sections is acquired in less than 6 hours. Using the automated slide scanning capability of the ZEISS Axioscan 7, up to 100 slides can be imaged at a time with minimal user input.

The combination of optimized, fast-staining UltiMapper reagents with the ZEISS Axioscan 7 automated whole-slide fluorescence scanner enables rapid, deep immunoprofiling within the spatial context of the tumor, empowering translational and immuno-oncology research.

For Research Use Only. Not for use in diagnostic procedures.