3873 High-multiplex automated staining and scanning workflow with Ultivue InSituPlex technology and the Olympus SLIDEVIEW VS200 slide scanner

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Introduction

Translational immuno-oncology research requires the visualization of multiple cell phenotypes in FFPE tissue sections. Ultivue's UltiMapper® I/O and U-VUE™ kits allow for pre-optimized staining of up to 8 targets in a single tissue section using exchange technology – sequential imaging of targets across two imaging rounds. Final high-plex images require careful image alignment between scans to identify cell phenotypes in downstream analysis without artifacts from inappropriately overlapped cells.

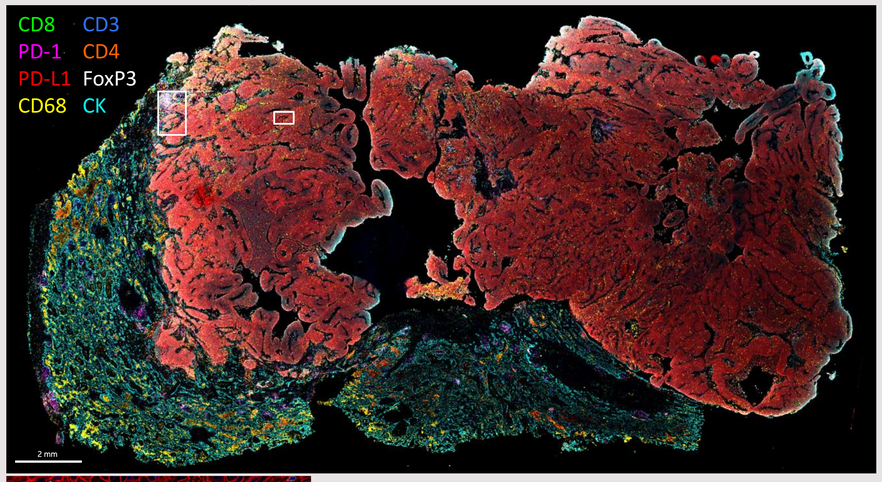
The newly released SLIDEVIEW™ VS200 slide scanner from Olympus™ provides rapid imaging of multiplexed IF slides with a 210 slide capacity for high-throughput, whole slide scanning. High quality hardware and software ensures minimal offsets between multiple images of the same slide.

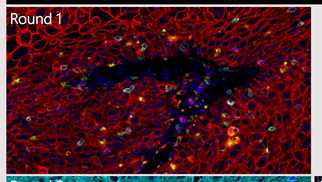
We present the newly established InSituPlex technology and DNA-Exchange workflow using the SLIDEVIEW VS200 scanner to produce high-quality, aligned images of eight targets in FFPE tumor sections.

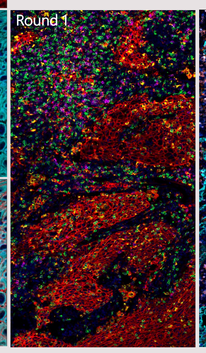
Methods

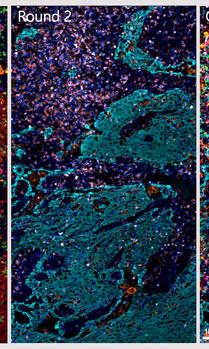
- De-identified FFPE tumor samples were stained with the UltiMapper I/O Immuno8 Kit labeling CD3, CD4, CD8, CD68, FoxP3, PD-1, PD-L1, and CK on an autostainer.
- The first four targets were imaged on the VS200, followed by signal removal and target re-probing (DNA-Exchange) on the autostainer.
- The last four targets were imaged on the VS200 using the overview from the initial image.
- The two images from each tissue section were co-registered with each other using Ultivue's UltiStacker™ software, which calculates a deformable mapping using the DAPI emission in each round of imaging.
- Final images were visualized in Indica Labs® HALO® software.
- Quality of overlap was assessed by calculating the offset between nuclei in multiple fields-of-view after alignment.

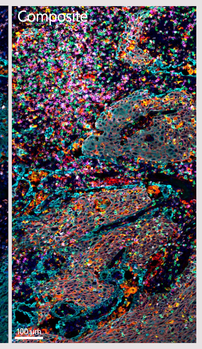
8-plex, whole slide images with the SLIDEVIEW VS200



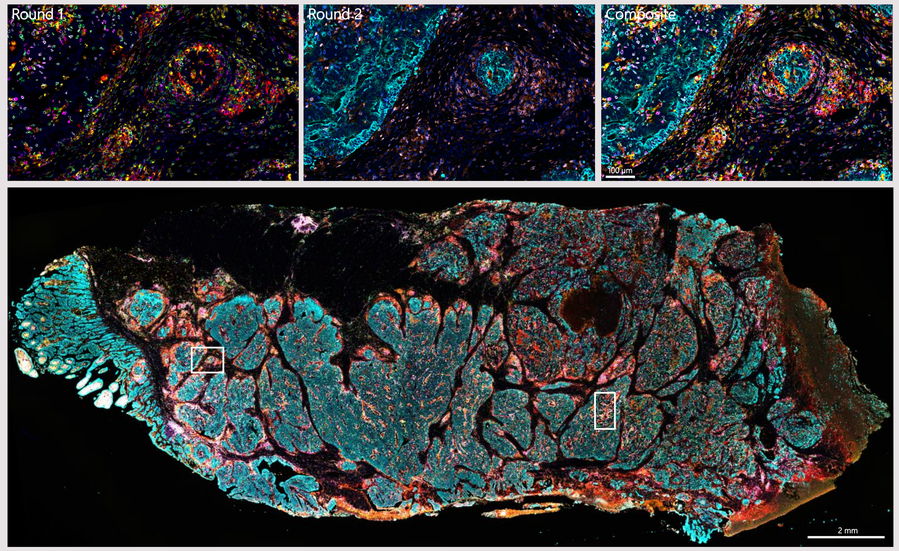


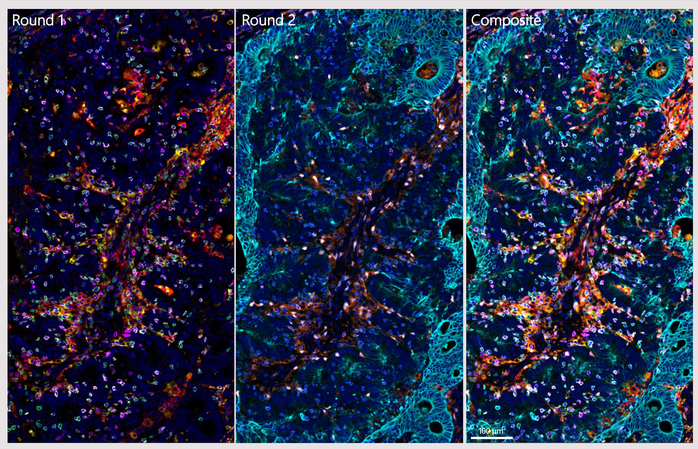






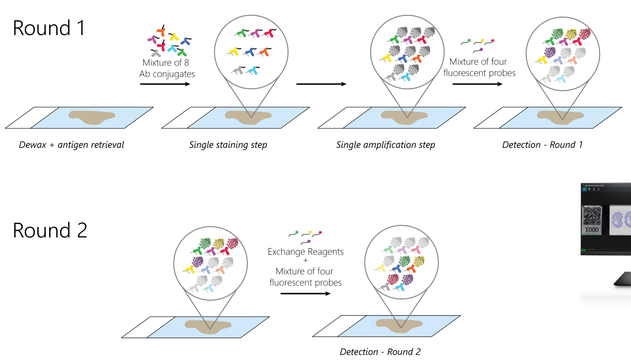
PD-L1+ non-small cell lung cancer (NSCLC) stained with the UltiMapper I/O Immuno8 Kit and scanned on the VS200 scanner. Tissue was scanned in DAPI, FITC, TRITC, Cy5, and Cy7 fluorescence channels. The 2.57 cm x 1.64 cm tissue area took 13:51 minutes to scan for Round 1 and 13:41 minutes for Round 2. The same slide overview was used for both scans.





Colorectal cancer (CRC) section stained with the UltiMapper I/O Immuno8 Kit and scanned on the VS200 scanner. The 2.33 cm x 1.16 cm tissue area took 8:50 minutes to scan for Round 1 and 8:47 minutes for Round 2. The same slide overview was used for both scans.

Workflow



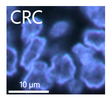
All eight targets were imaged on the VS200 scanner in two rounds of four targets. Images were exported into the UltiStacker software for co-registration.

SLIDEVIEW VS200

Quality of alignment

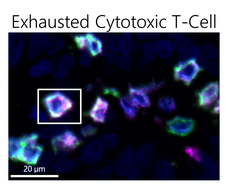
300 FOVs were randomly selected in the co-registered DAPI images. For each FOV, we calculated the cross-correlation of Round 1 and 2, and measured the (x, y) offset for each. Perfect co-registration would yield offsets of (0, 0). The mean and standard deviation offsets are a measure of alignment quality (accuracy and precision, resp.).

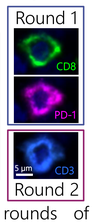
Metric	NSCLC Section	CRC Section
Mean Offset, x	+0.11 µm	+0.33 µm
Mean Offset, y	+0.30 µm	+0.05 µm
Standard Deviation, x	0.40 µm	0.14 µm
Standard Deviation, y	0.32 µm	0.15 µm



Round 1 DAPI signal in magenta, Round 2 in cyan. Areas of good overlap become light blue/white.

Exhausted Cytotoxic T-Cell





Minimal offsets between rounds of imaging enabled accurate colocalization and phenotyping of small cells such as T-cells.

Conclusion

- Ultivue kits leveraging DNA-exchange technology enable rapid, fully pre-optimized staining and imaging of eight protein targets in FFPE tissue sections.
- The Olympus SLIDEVIEW VS200 scanner provides a convenient, fast imaging tool for whole slide, multiplexed immunofluorescence imaging, compatible with Ultivue's UltiMapper (pre-set panels) and U-VUE™ kits (custom, up to eight targets).
- Images acquired from the VS200 scanner allow for highly accurate alignment between rounds of imaging on the same tissue section, enabling high-plex imaging without the need for spectral unmixing.
- Final high-plex images allow the user to identify increasingly complex cellular phenotypes in whole-tissue slides with accurate colocalization and a workflow inspiring confidence in staining and imaging accuracy.

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