Ultivue

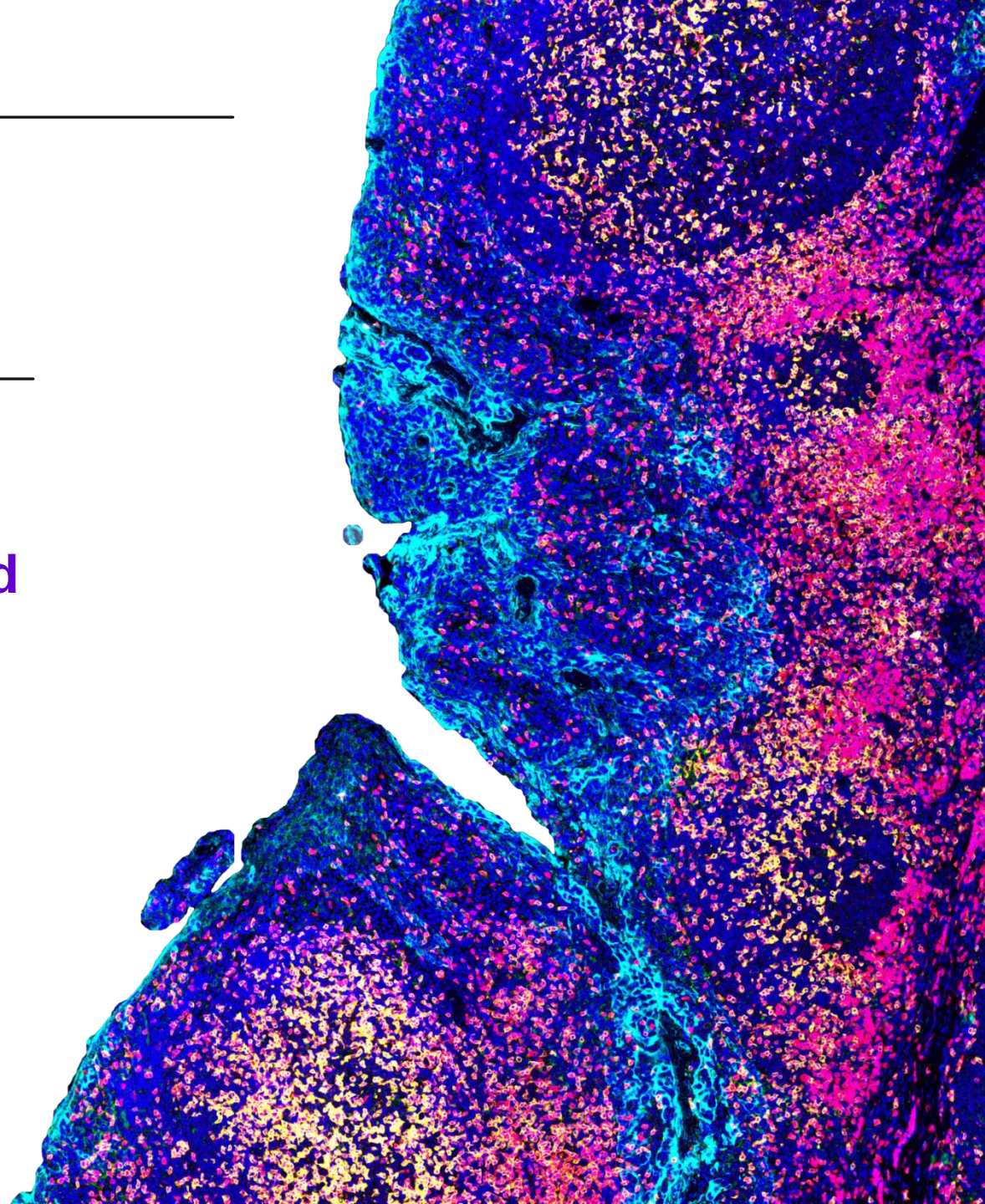
Profiling Cancer Biology

Advancing Precision Oncology through AI-Enhanced Spatial Image Analysis and Multiplex Assays

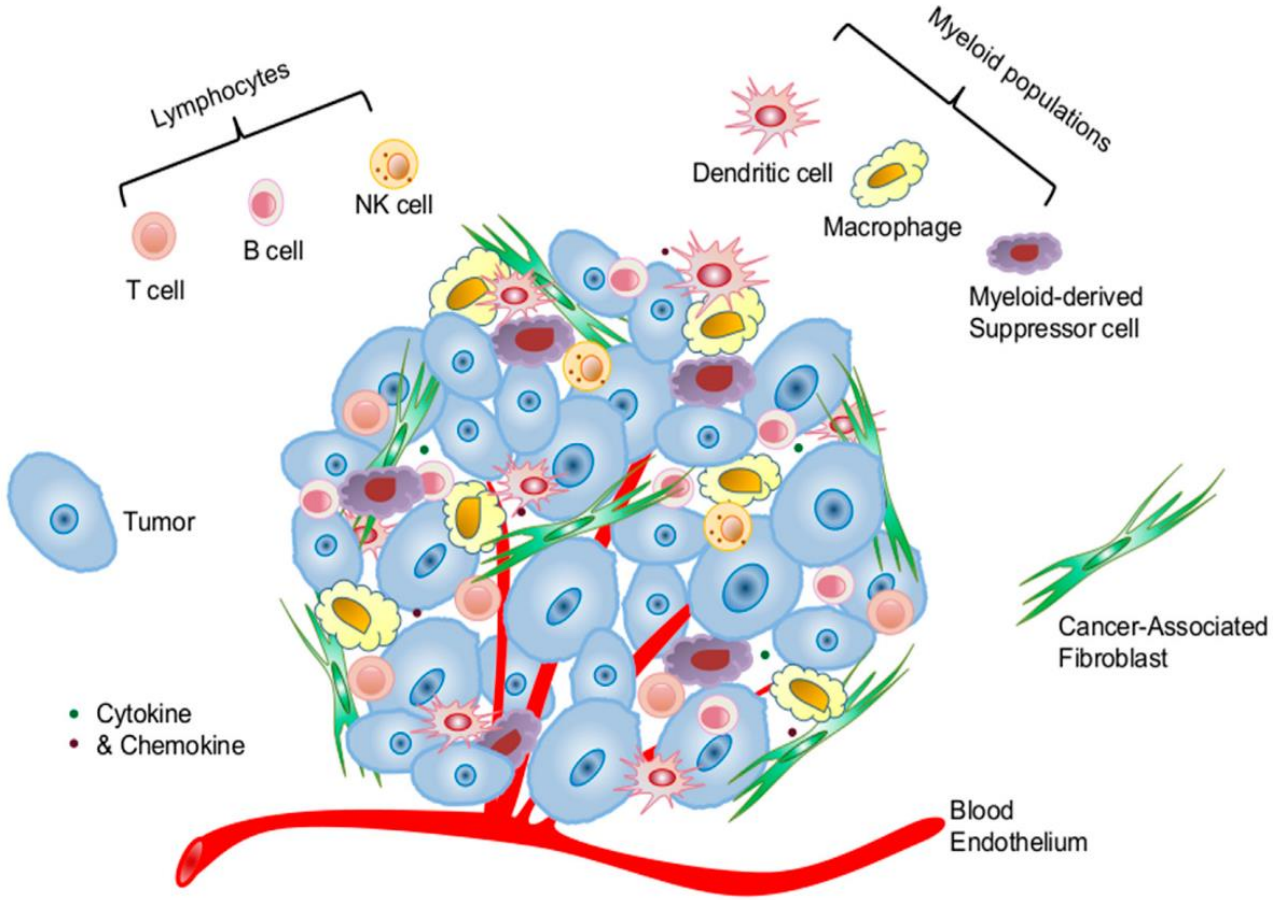
Lorenz Rognoni, PhD

Director Image Data Science, Ultivue

11th July 2024



The Tumor Microenvironment



Cui et al., Int. J. Mol. Sci. (2016) 17, 1942

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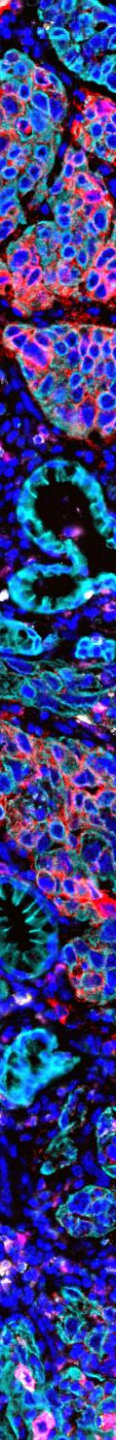
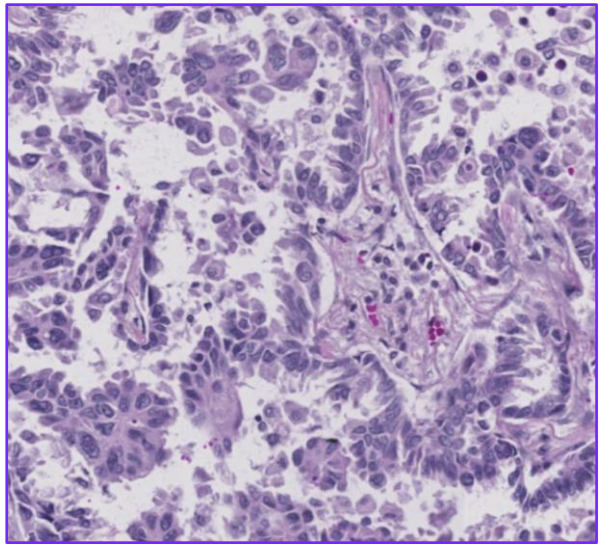


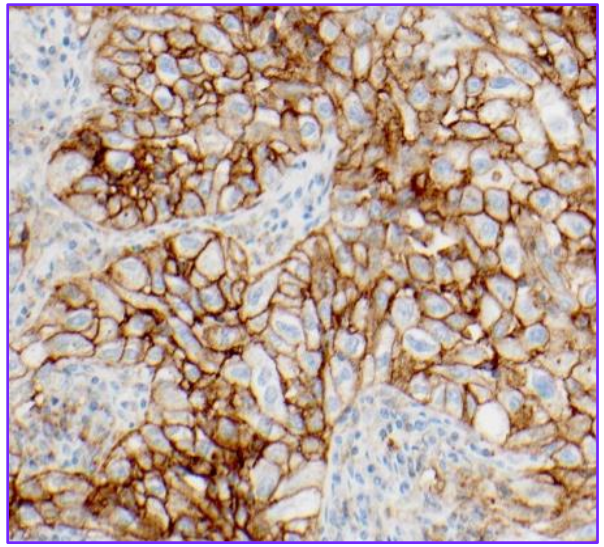
Complexity of the tumor microenvironment is driving the need for multiplex marker detection in tissue samples

Hematoxylin & Eosin (H&E)



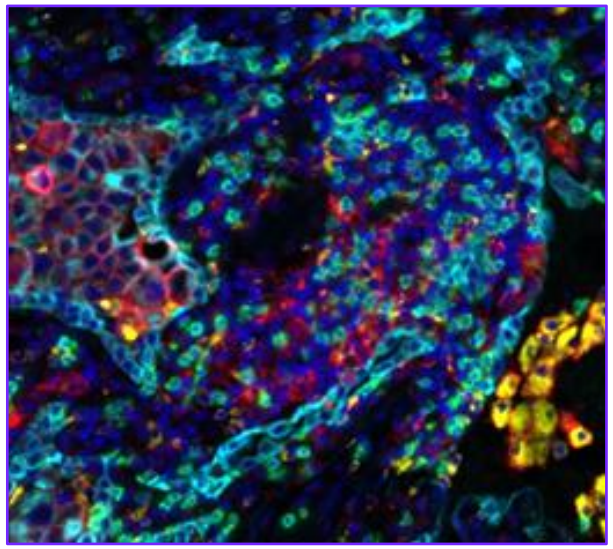
- Morphology determines diagnosis

Immunohistochemistry (IHC)



- Single protein detection (DAB)
- Cell identity (cytokeratin), state (Ki67), diagnostic algorithms, predictive biomarkers (Her2, PD-L1)

Multiplex Immunofluorescence (mIF)



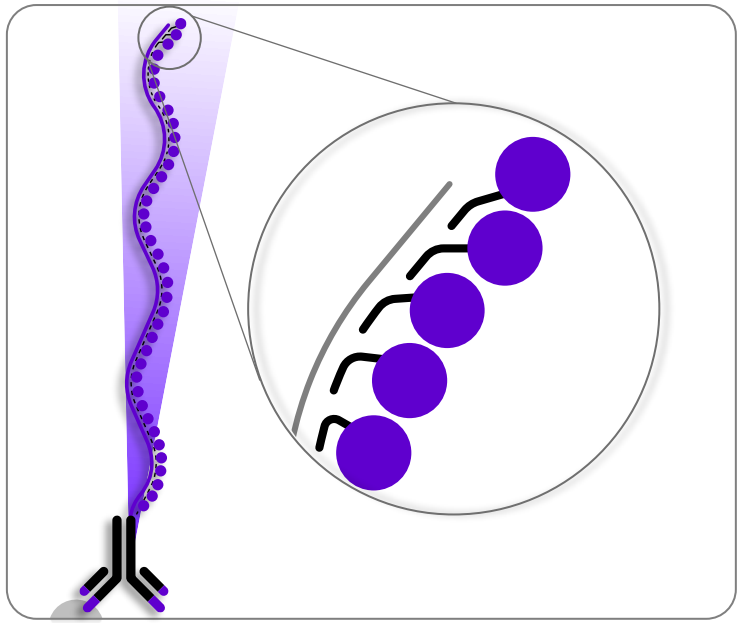
- Multiple protein detection
- Deeper cell phenotyping
- Wider expression range
- Intercellular interactions and networks

Ultivue

At the Heart of Ultivue are Two Core Technologies

Combine to form an integrated, high-throughput analytical platform

InSituPlex®
Multiplex Assay

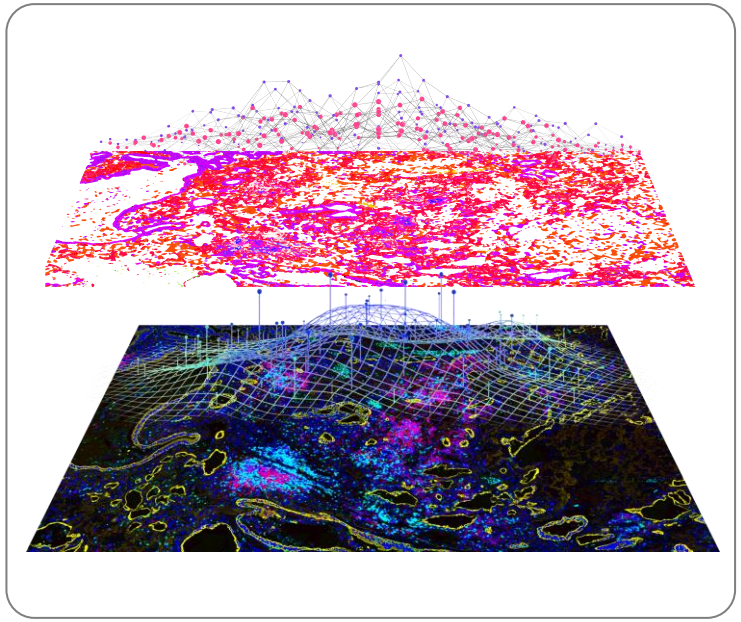


Massively parallel single-molecule amplification

- Highly configurable multiplexed biomarker detection
- Clinical-grade assay performance
- Fast and gentle high-throughput workflow

+

STARVUE™
Image Data Science



Deep learning enabling faster image-to-insight

- Biomarker morphology and signal intensity-driven cell classification
- Highly scalable and adaptable image processing
- Enables precise accurate results faster at a lower cost

Ultivue

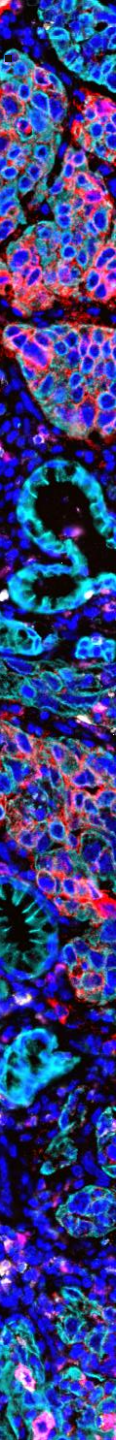
Next Generation Integrated “Samples-to-Insights”

mIF Image

- Configurable panels of <12 targets
- Simple high-throughput workflow
- High specificity & dynamic range

Sample

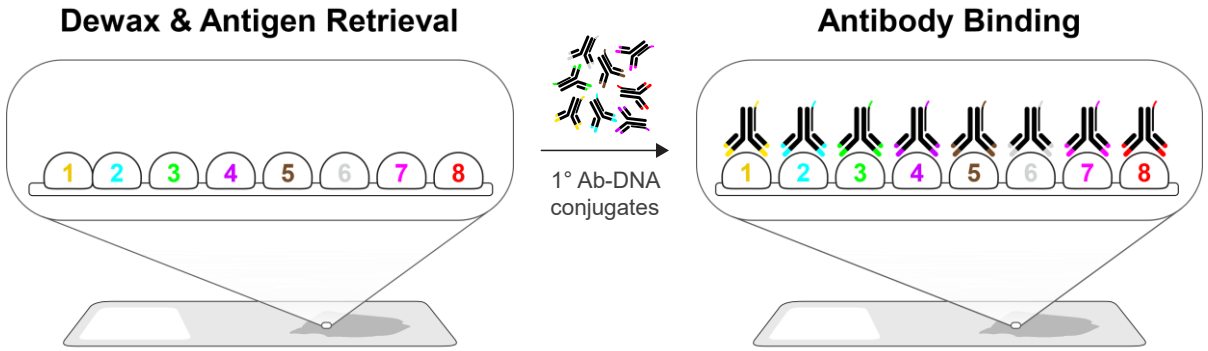
InSituPlex®
Multiplex Assay



InSituPlex® (ISP) Overview: Fast and Simple Workflow

The TME is Complex: Your Assay Shouldn‘t Be!

InSituPlex®
Multiplex Assay



Direct detection of target sites
1º Ab-DNA conjugates: high specificity

Ultivue

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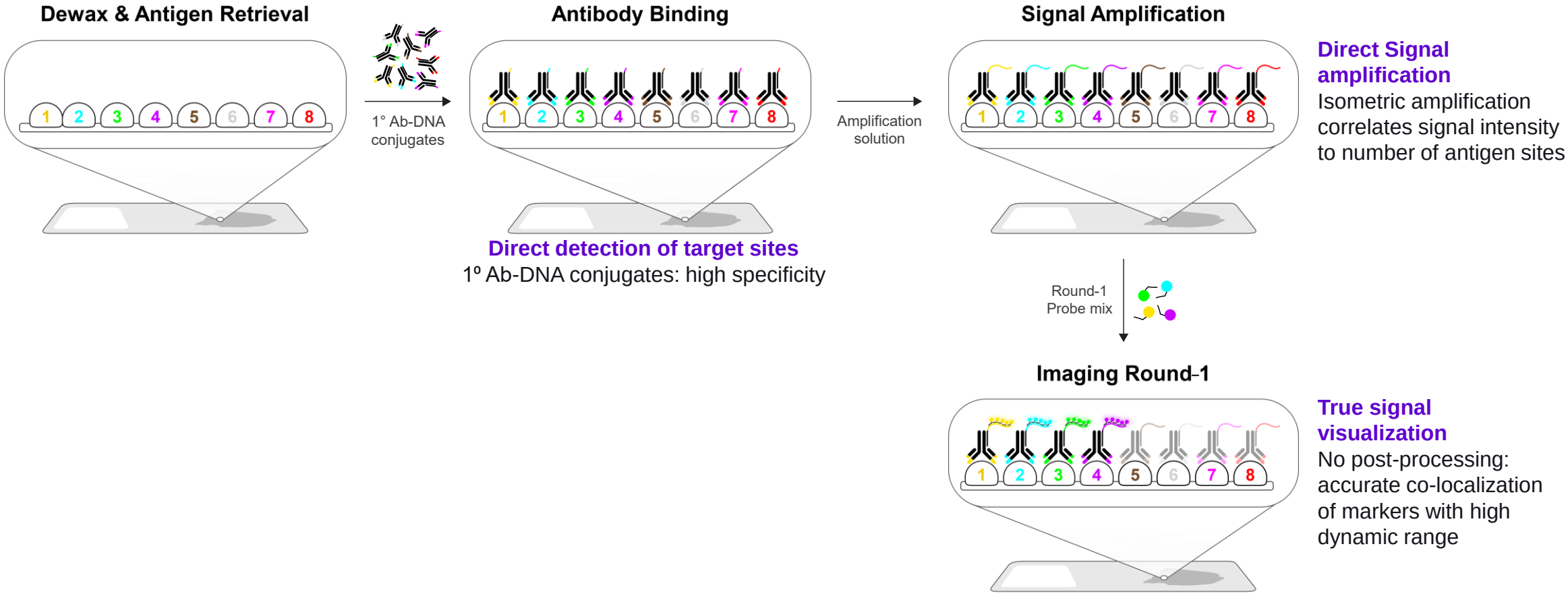

Direct detection of target sites
1º Ab-DNA conjugates: high specificity

Direct Signal amplification
Isometric amplification correlates signal intensity to number of antigen sites

True signal visualization
No post-processing: accurate co-localization of markers with high dynamic range

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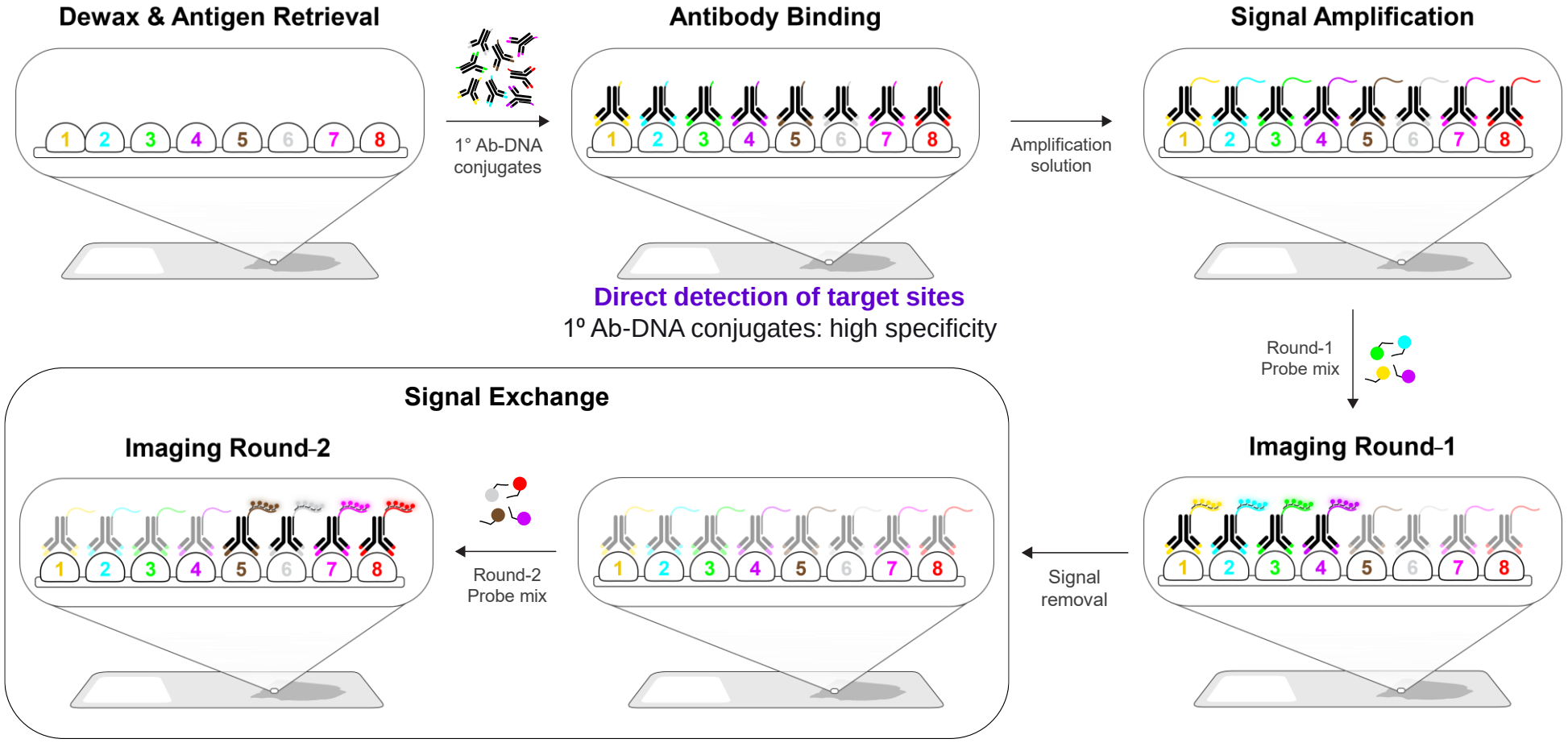


Direct detection of target sites
1º Ab-DNA conjugates: high specificity

Direct Signal amplification
Isometric amplification correlates signal intensity to number of antigen sites

True signal visualization
No post-processing: accurate co-localization of markers with high dynamic range

Fast and gentle workflow
Preserves tissue morphology

Ultivue

Next Generation Integrated “Samples-to-Insights”

mIF Image

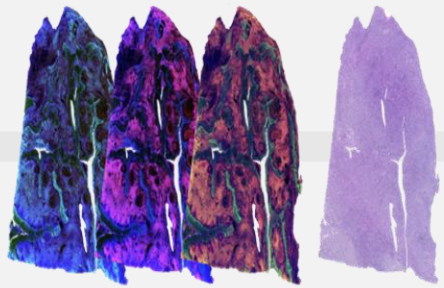
- Configurable panels of <12 targets
- Simple high-throughput workflow
- High specificity & dynamic range

Image Stacking

- Robust co-registration, micron-level accuracy
- Extremely high throughput (100’s of samples/hr)
- Nearly perfect co-registration even with large tissue defects

Sample

InSituPlex®
Multiplex Assay



UltiStacker.AI™
Image Co-registration

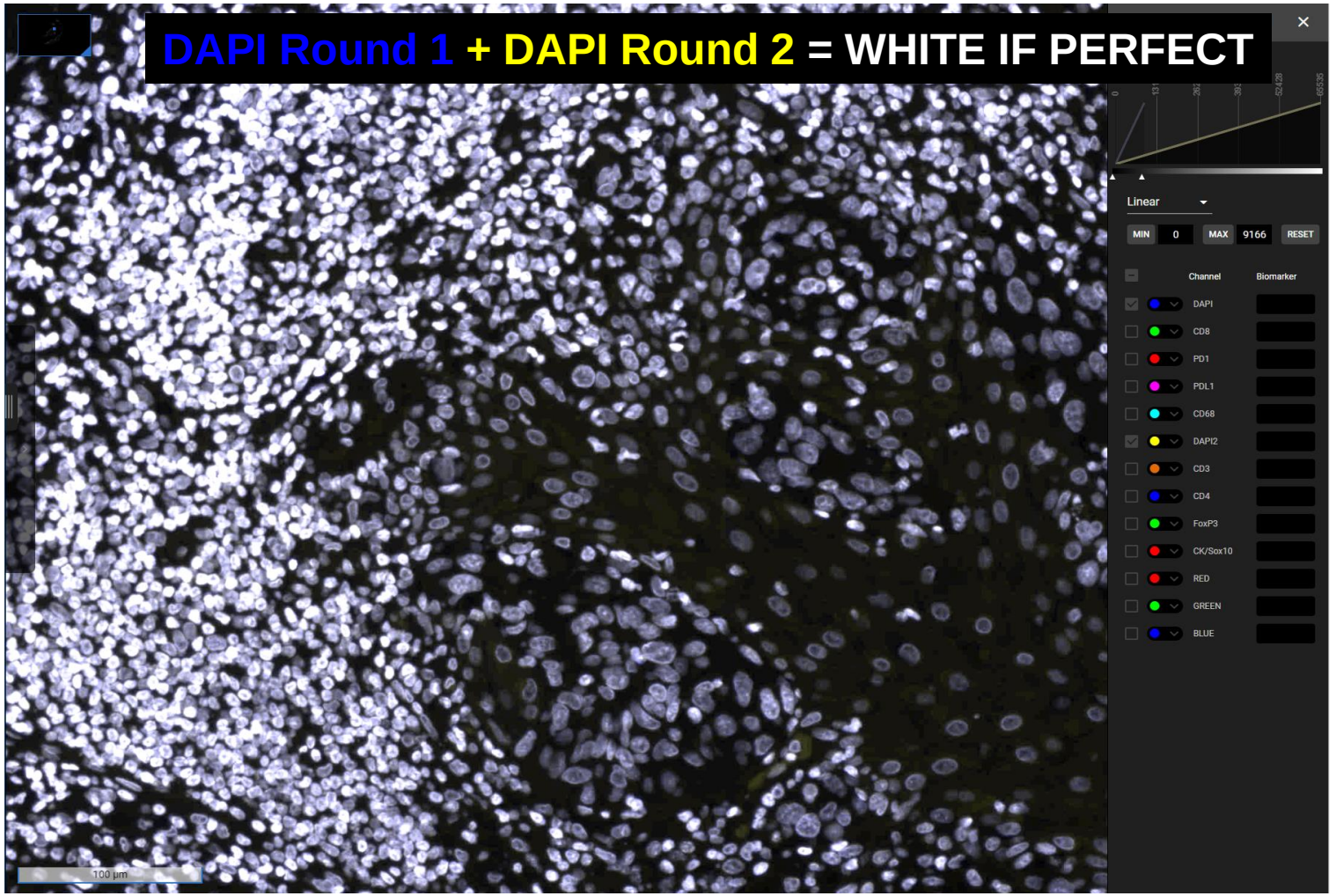
Essentially perfect IF co-registration even with image defects

UltiStacker.AI™
Image Co-registration

Detail view

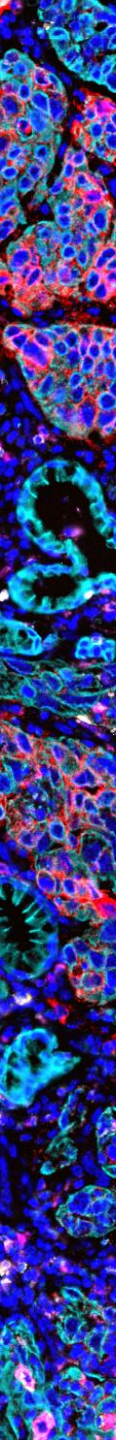
This is NOT just a single DAPI image

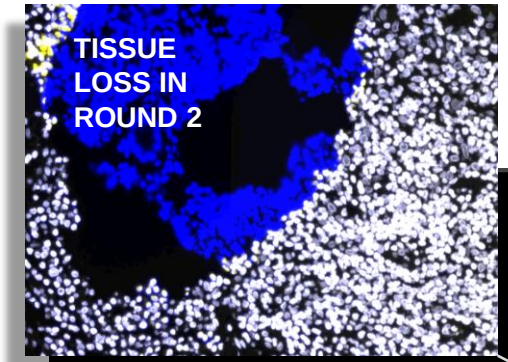
It is a <u>composite</u> of the two DAPI scans of the same slide.

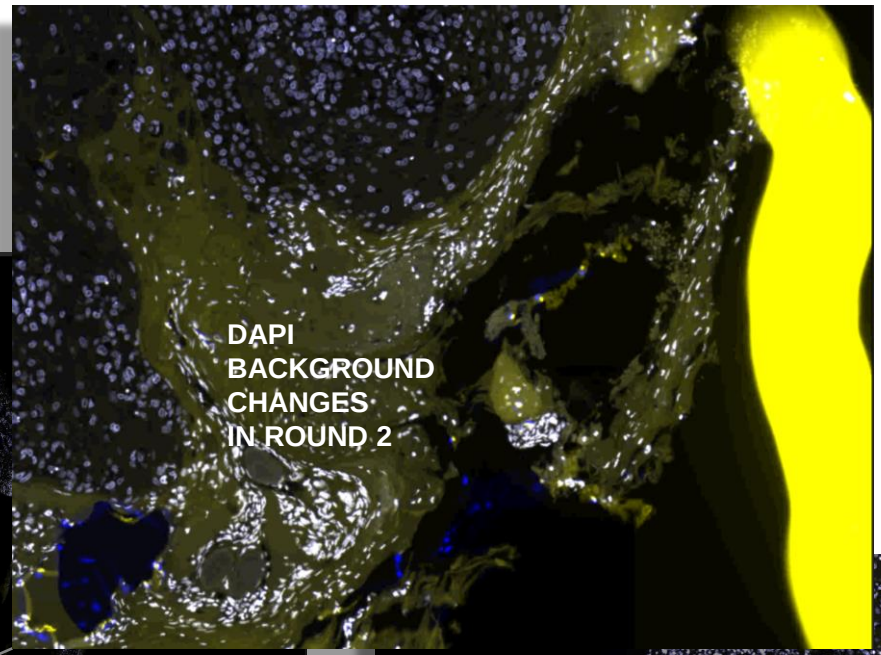


Essentially perfect IF co-registration even with image defects

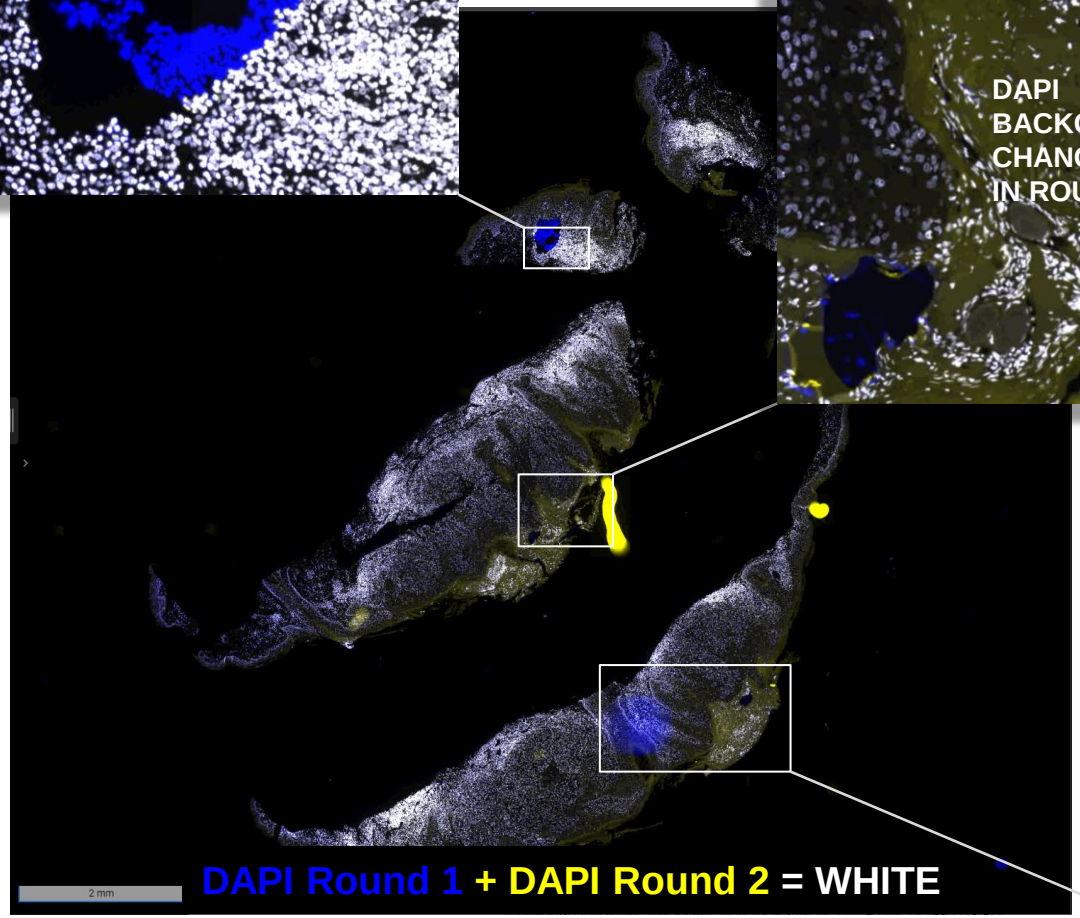
UltiStacker.AI™
Image Co-registration

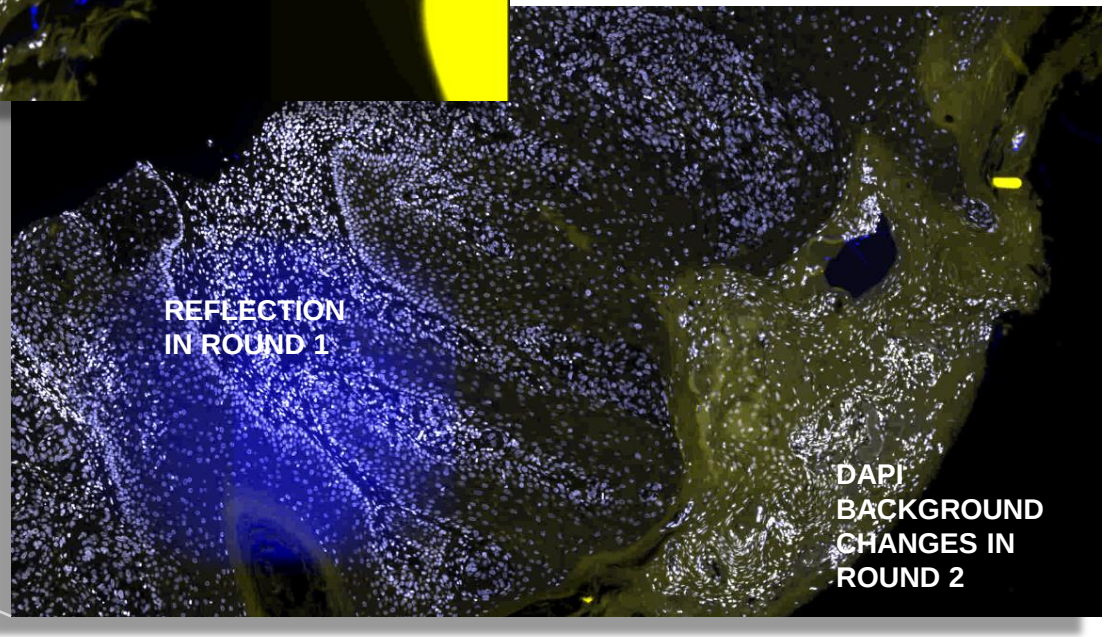






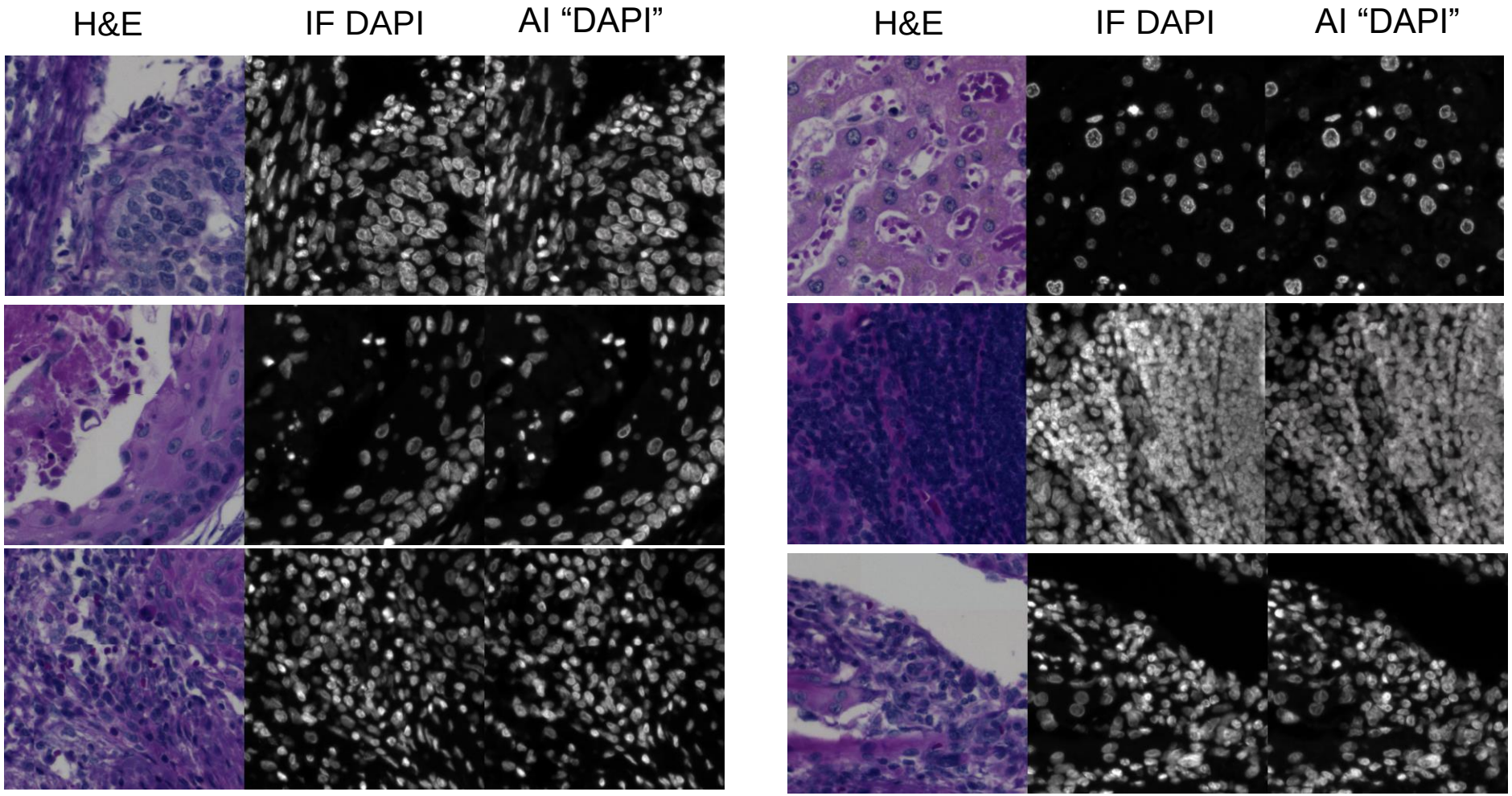
DUST
IN
ROUND 2





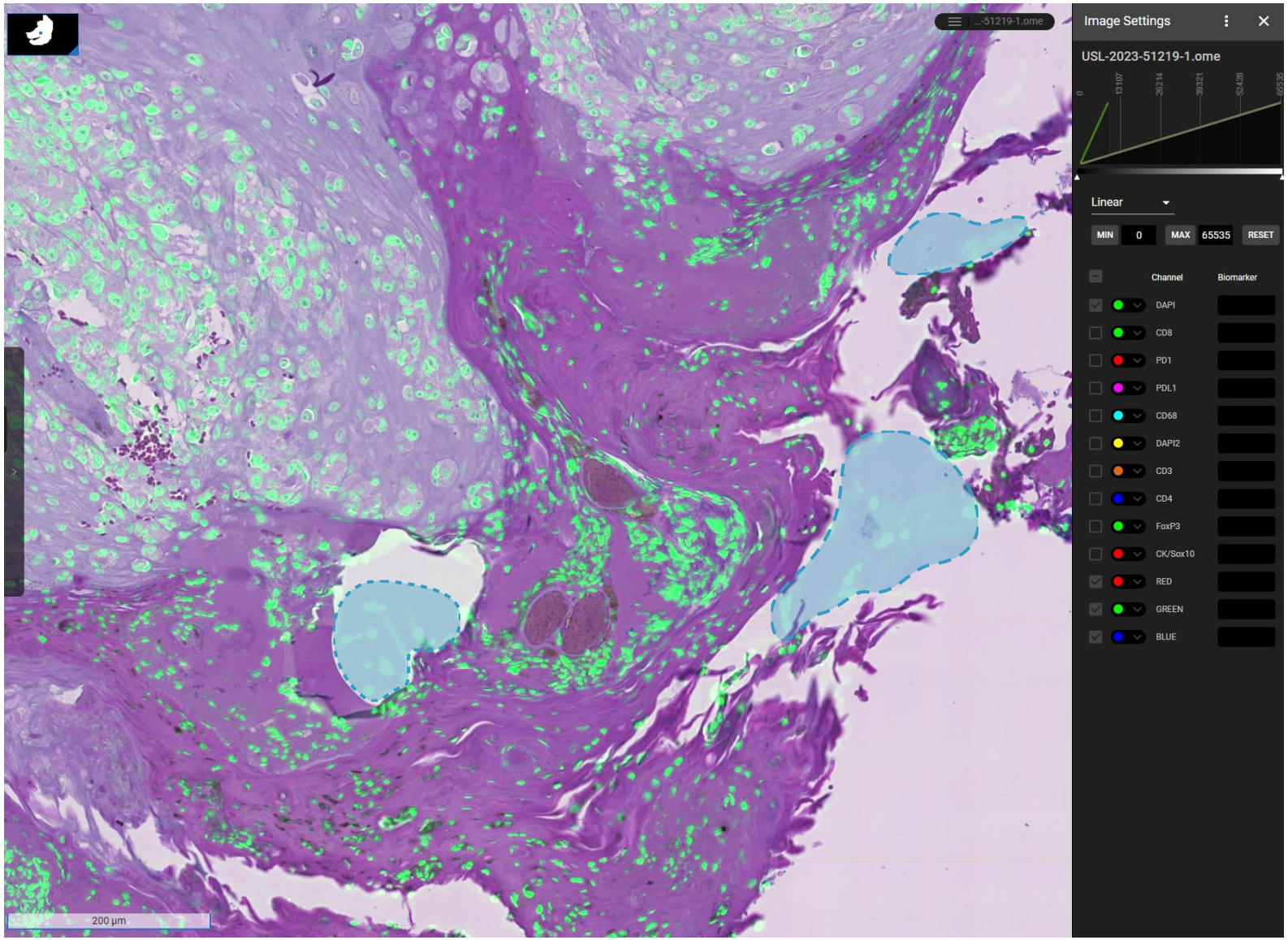
AI: H&E matching using a Generative Adversarial Network (GAN)

We have trained AI models which accurately predict the appearance of the IF DAPI from an H&E brightfield scan. We then use this AI "DAPI" to co-register with the IF DAPI for **subcellular accuracy multimodal stacking**.



- Models were created using Ultivue's extensive catalog of images produced by our lab
- H&E matching is fully automatic: does not require any input from the user

Co-registration of mIF and brightfield (H&E) images with high precision



Note: Good results even in the vicinity of tissue loss or poor focus

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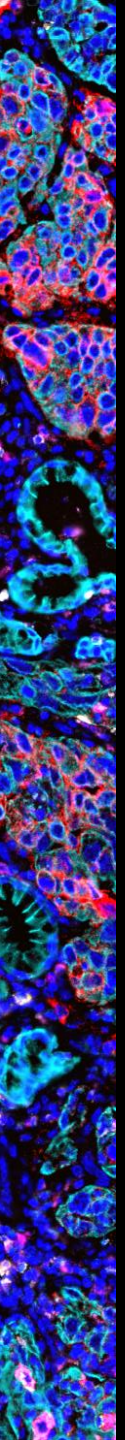
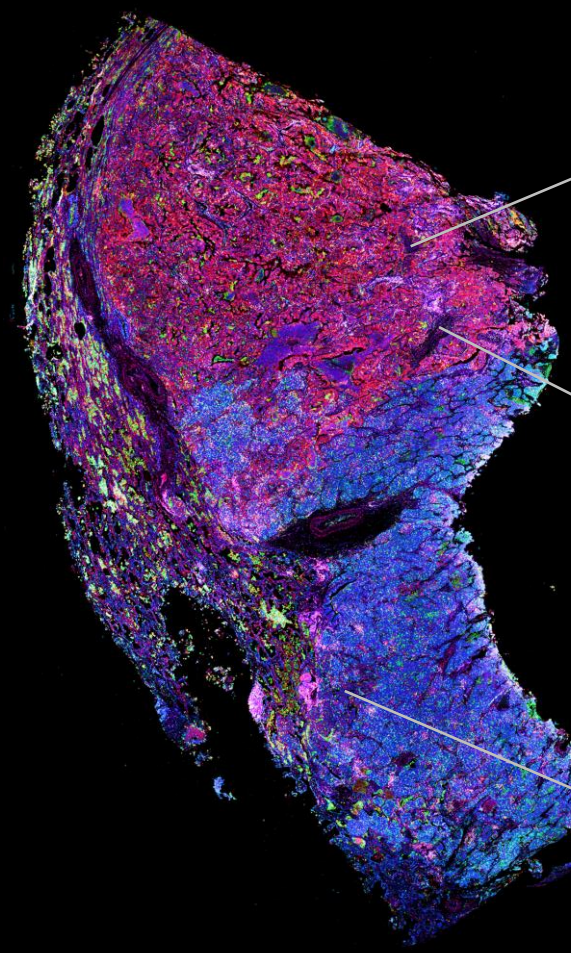
ISP facilitates high resolution cellular phenotyping through co-detection of multiple biomarkers in a single cell



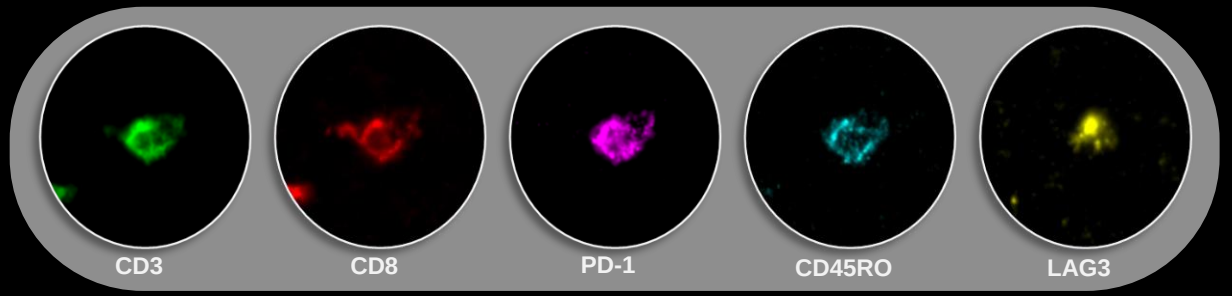


Overlay

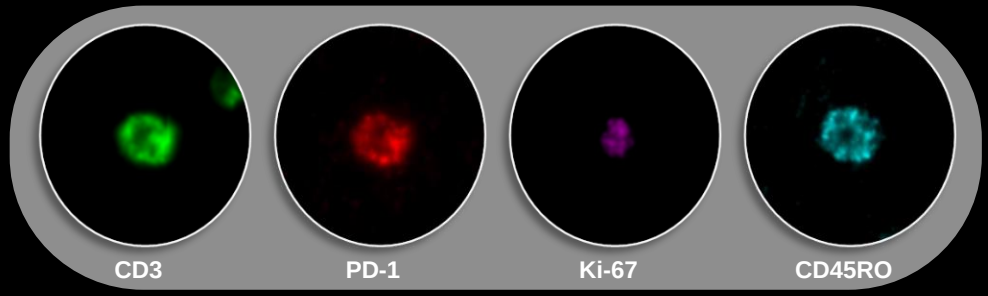
Overlay

Overlay

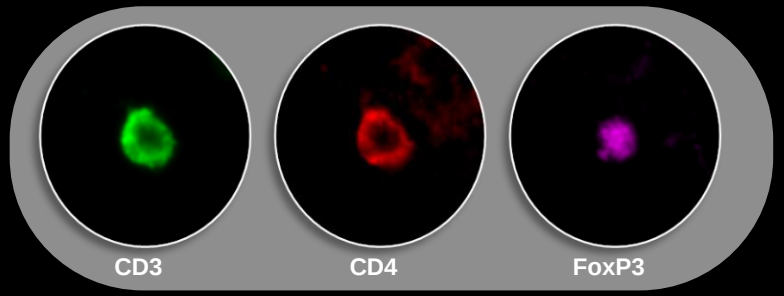
Exhausted memory cytotoxic T cells



Proliferating exhausted memory T cells



Naïve regulatory T cells



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- Simple high-throughput workflow
- High specificity & dynamic range

Image Stacking

- Robust co-registration, micron-level accuracy
- Extremely high throughput (100’s of samples/hr)
- Nearly perfect co-registration even with large tissue defects

Image Analysis

- Deep-learning enabled whole-slide analysis
- Highly scalable cloud-based infrastructure
- Custom phenotyping through robust co-detection of multiple biomarkers

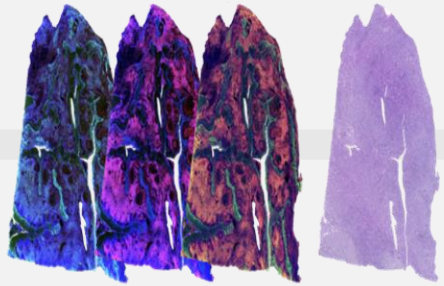
Biological Insights

- Intercellular dynamics: ROIs vs. whole section
- Advanced spatial phenomics: Clinical data integration, Endpoint analysis, Cohort-level data stratification, and more.

Sample

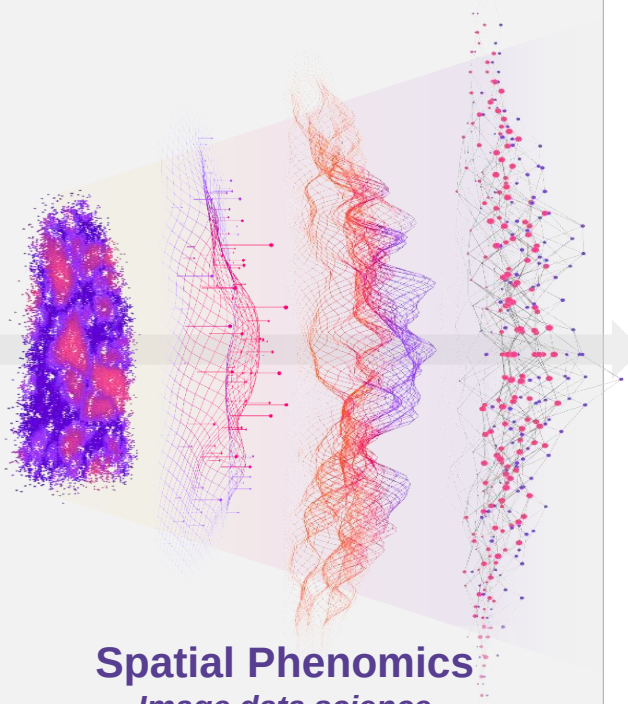
InSituPlex®
Multiplex Assay

STARVUE™
Image Data Science



UltiStacker.AI™
Image Co-registration

UltiAnalyzer.AI™
Image Analysis

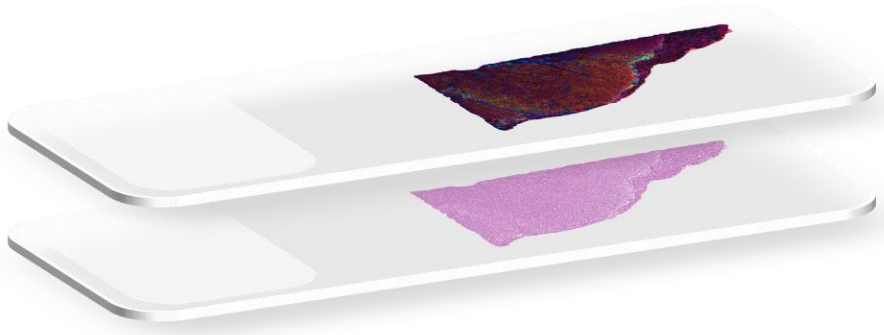


Spatial Phenomics
Image data science

Spatial Tissue Analytics and Reporting

Image Analysis: Input

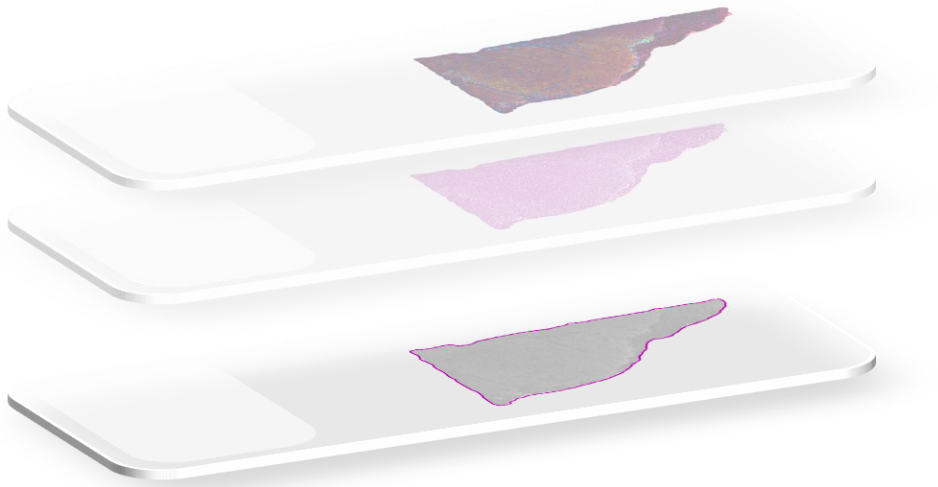
UltiStacker.AI™
Image Co-registration



Stacked mIF Image with optional same-section H&E

Image Analysis: Semantic Segmentation

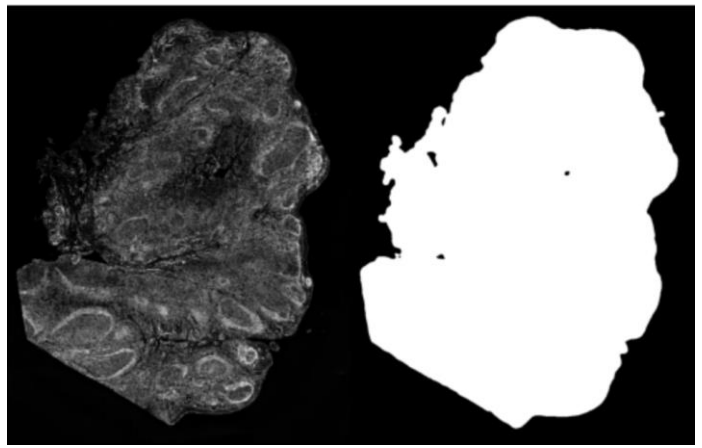
UltiStacker.AI™
Image Co-registration



Stacked mIF Image with optional same-section H&E

Tissue Segmentation

UltiAnalyzer.AI™
Image Analysis

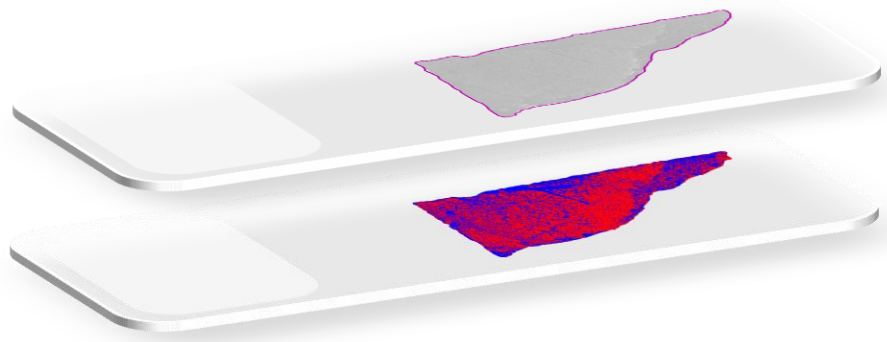


DAPI

Ultivue

State of the art AI applied to mIF image data

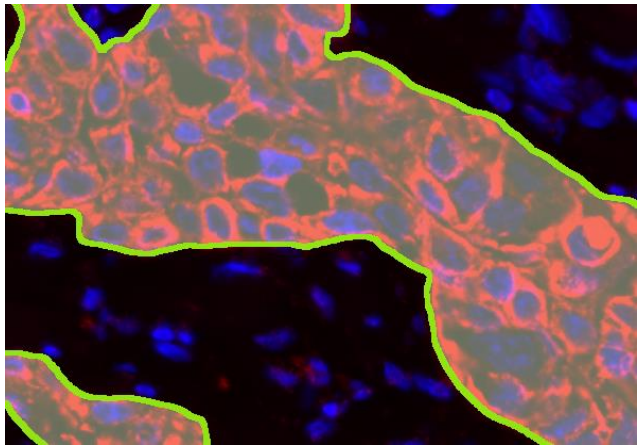
Image Analysis: Semantic Segmentation



Tissue Segmentation

Region Segmentation: Tumor vs Stroma

UltiAnalyzer.AI™
Image Analysis

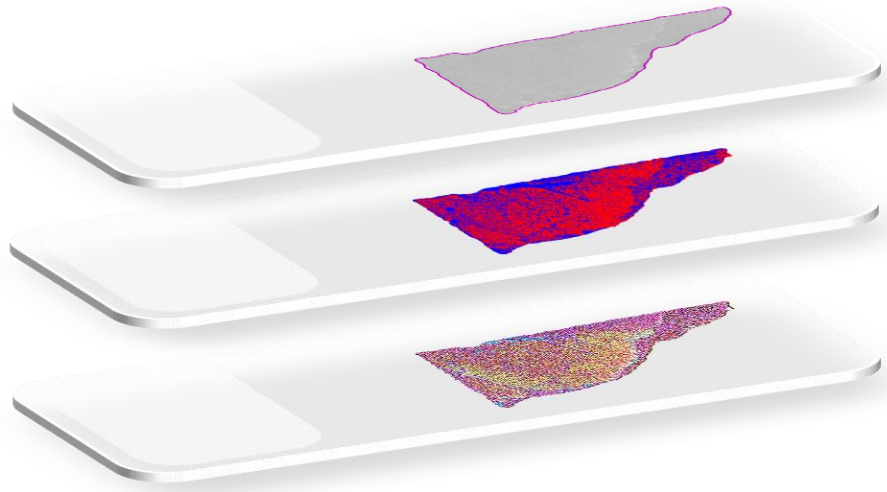


DAPI
CK

Ultivue

State of the art AI applied to mIF image data

Image Analysis: Instance Segmentation

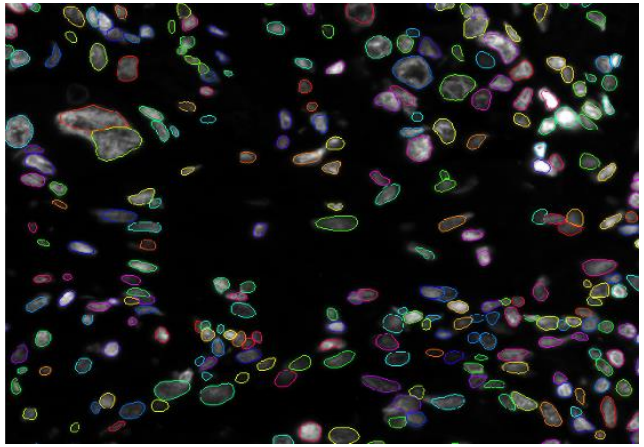


Tissue Segmentation

Region Segmentation: Tumor vs Stroma

Cell Segmentation

UltiAnalyzer.AI™
Image Analysis

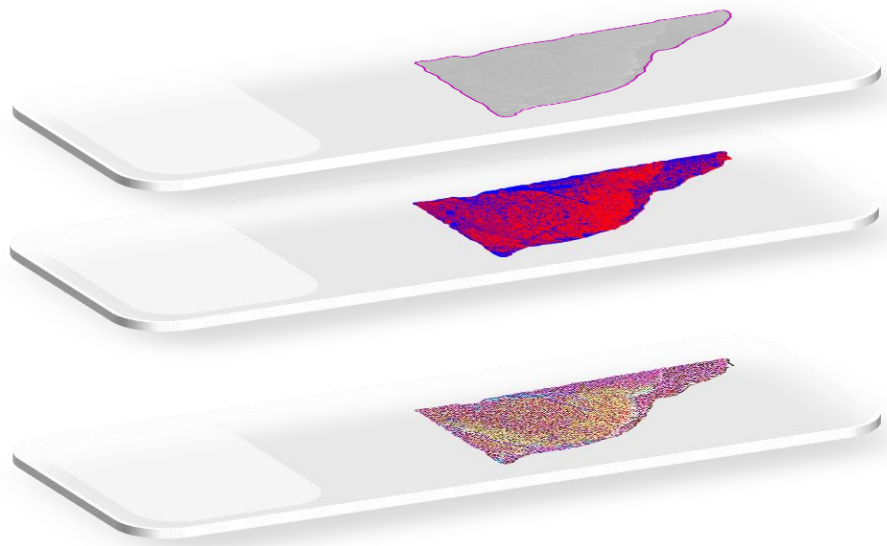


DAPI

Ultivue

State of the art AI applied to mIF image data

Image Analysis: Object Detection



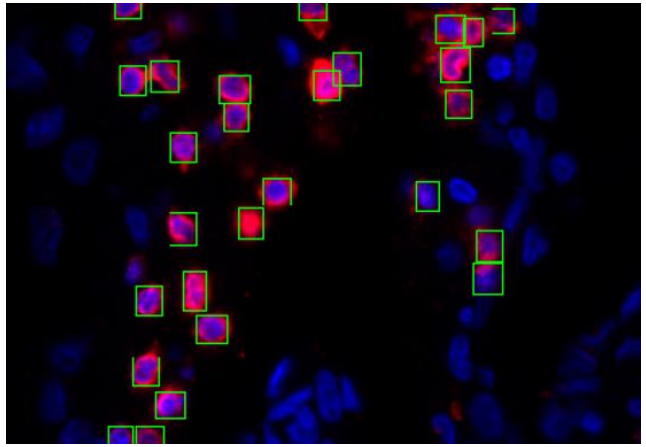
Tissue Segmentation

Region Segmentation: Tumor vs Stroma

Cell Segmentation

Marker Positive Classification

UltiAnalyzer.AI™
Image Analysis



DAPI
CD3

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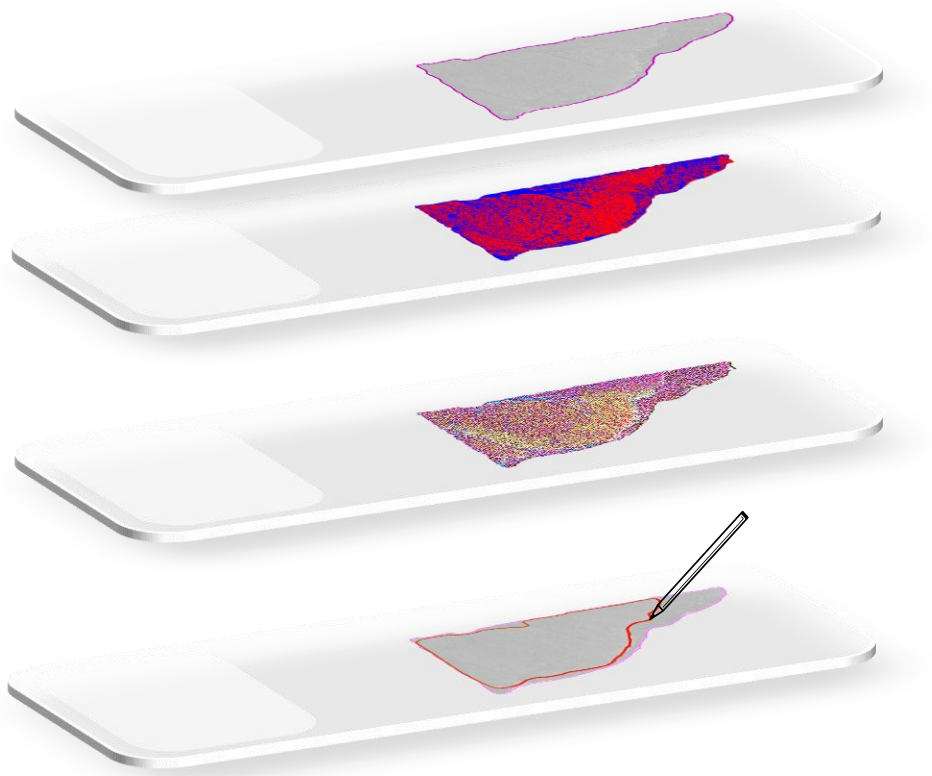
State of the art AI applied to mIF image data

Image Analysis: Expert Annotations

UltiAnalyzer.AI™
Image Analysis



Tissue Segmentation

Region Segmentation: Tumor vs Stroma

Cell Segmentation

Marker Positive Classification without thresholds

Expert Annotations: ROIs or Exclusions

post IA

Flexible phenotyping based on marker positive information

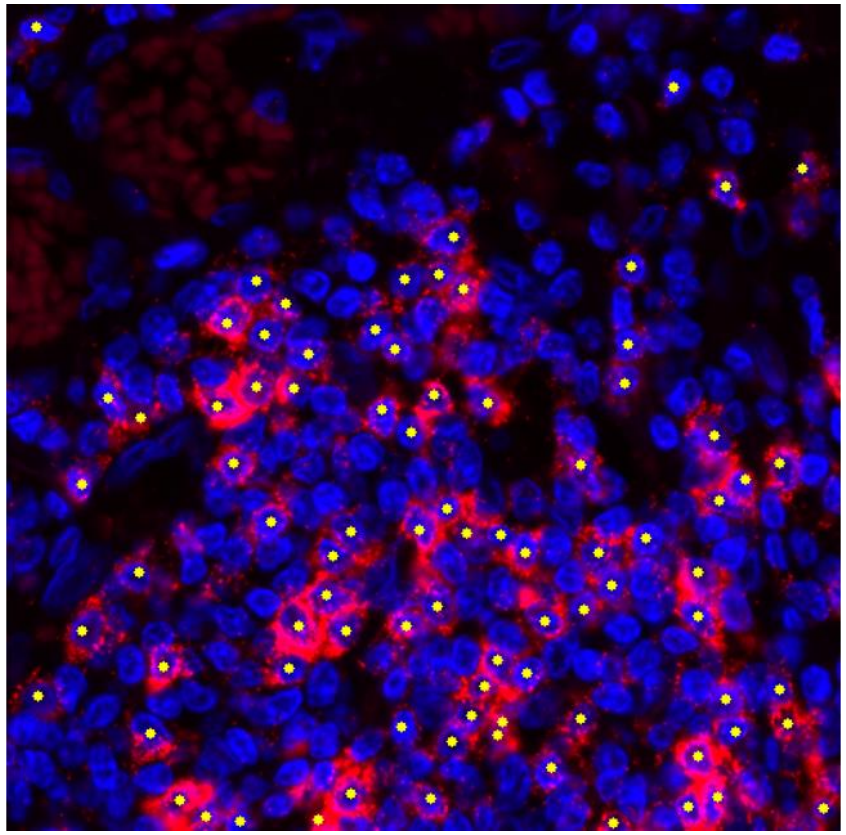
Ultivue

IMAGE ANALYSIS

Superior Sensitivity and Robustness with Deep Learning

Deep Learning for all analysis tasks including cell classification

UltiAnalyzer.AI™
Image Analysis



DAPI **PD1** PD1+ Cell

Unprecedented cell detection in difficult regions where threshold-based methods fail.

With Deep Learning:

- Improved accuracy
- Reduced need for manual feature engineering
- Ability to handle complex data
- Deep Learning algorithms improve with growing training data
- Pre-trained on proprietary InSituPlex® data

Ultivue

IMAGE ANALYSIS

Superior Sensitivity and Robustness with Deep Learning

Deep Learning for all analysis tasks including cell classification

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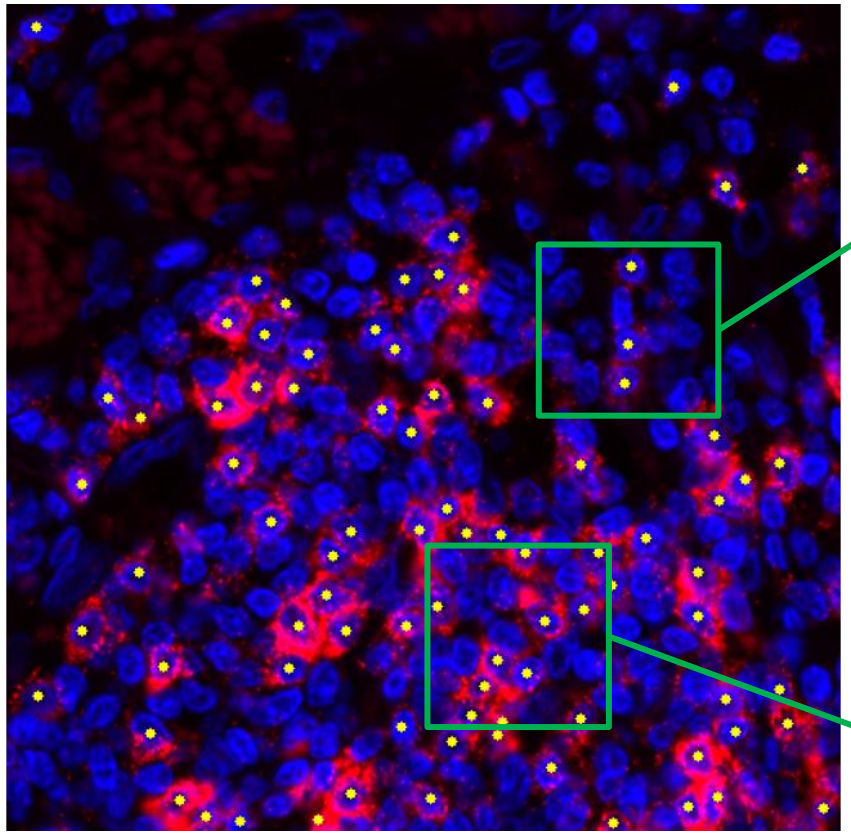
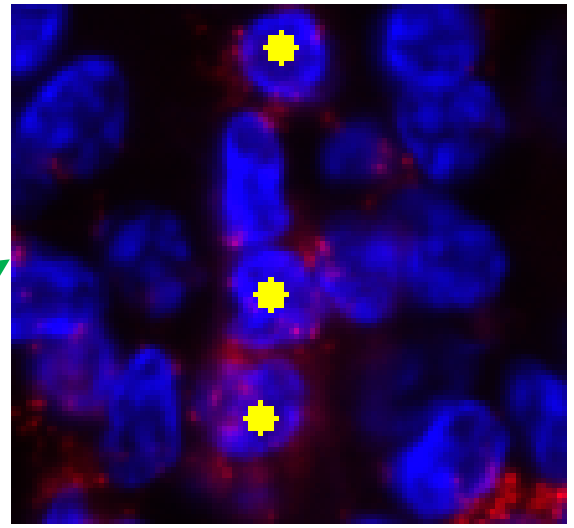
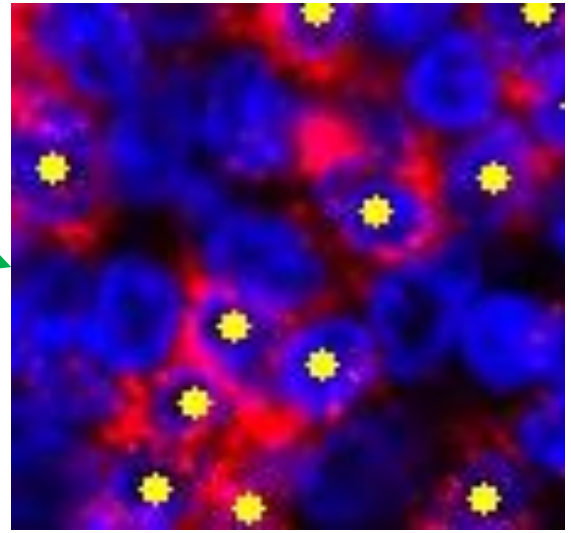
Image Analysis





Low expressers



High expressers

DAPI **PD1** PD1+ Cell

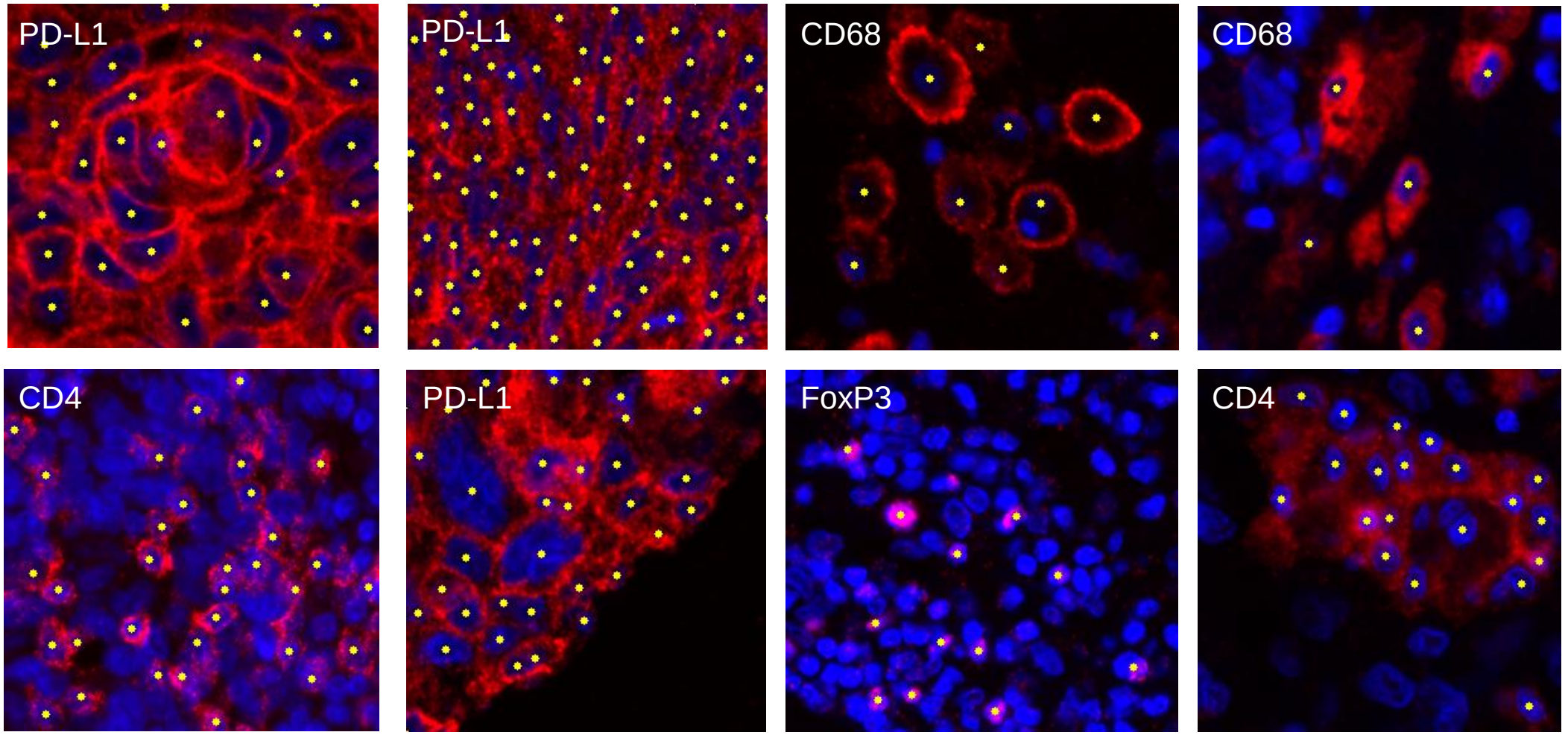
Ultivue

IMAGE ANALYSIS

Superior Sensitivity and Robustness with Deep Learning

Diversity of shapes

UltiAnalyzer.AI™
Image Analysis

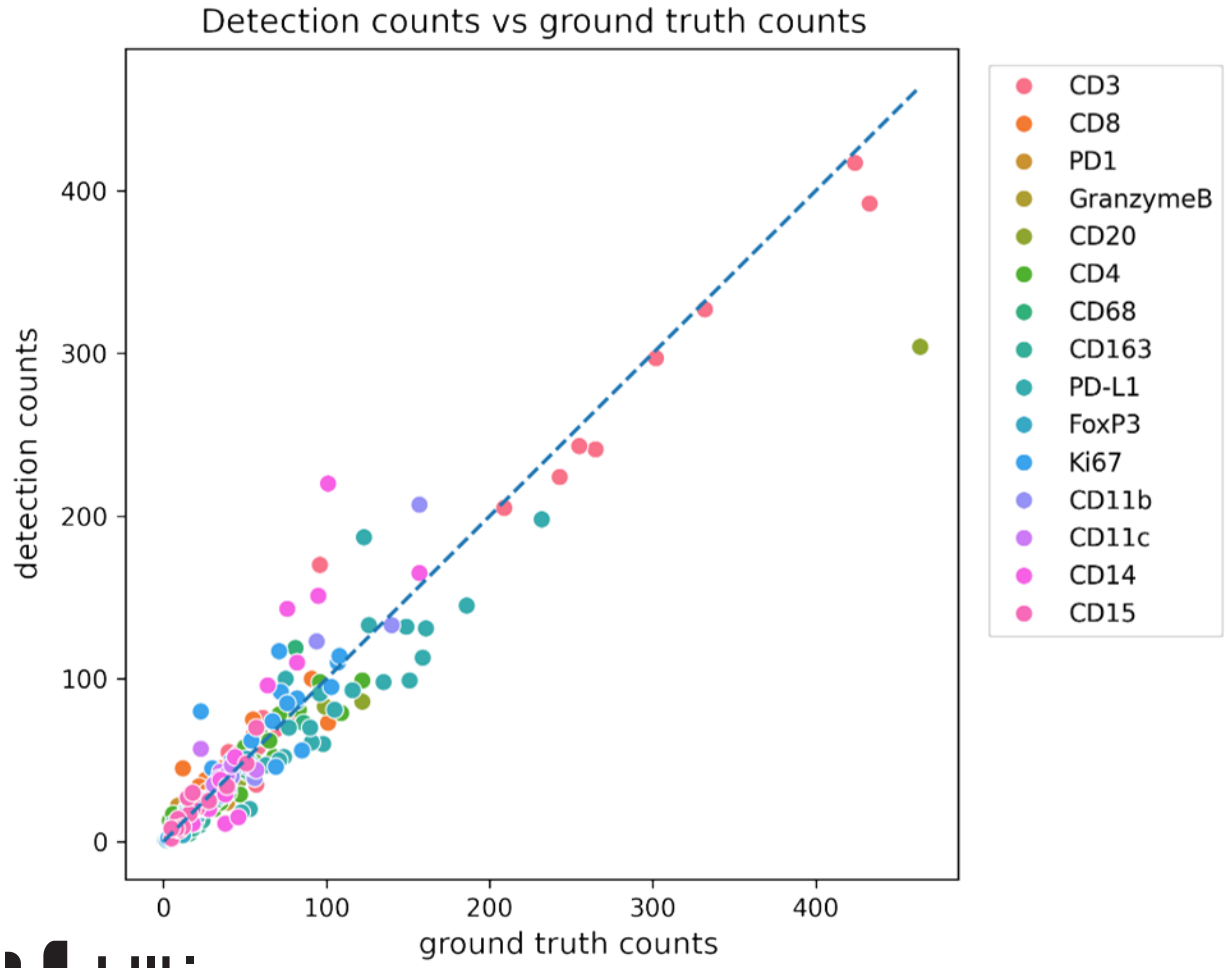


Ultivue

Model performance on manual annotated validation data

UltiAnalyzer.AI™
Image Analysis

All detection models > 0.8 Lin's Correlation
Global Lin's Corr: 0.954



Marker	Lin's Corr	F1-score
CD3	0.990	0.916
CD8	0.861	0.861
PD1	0.870	0.806
Granzyme B	0.901	0.833
CD20	0.913	0.747
CD4	0.942	0.802
CD68	0.955	0.703
CD163	0.828	0.685
PD-L1	0.922	0.746
FoxP3	0.940	0.875
Ki67	0.834	0.803
CD11b	0.989	0.686
CD14	0.945	0.617
CD15	0.925	0.731

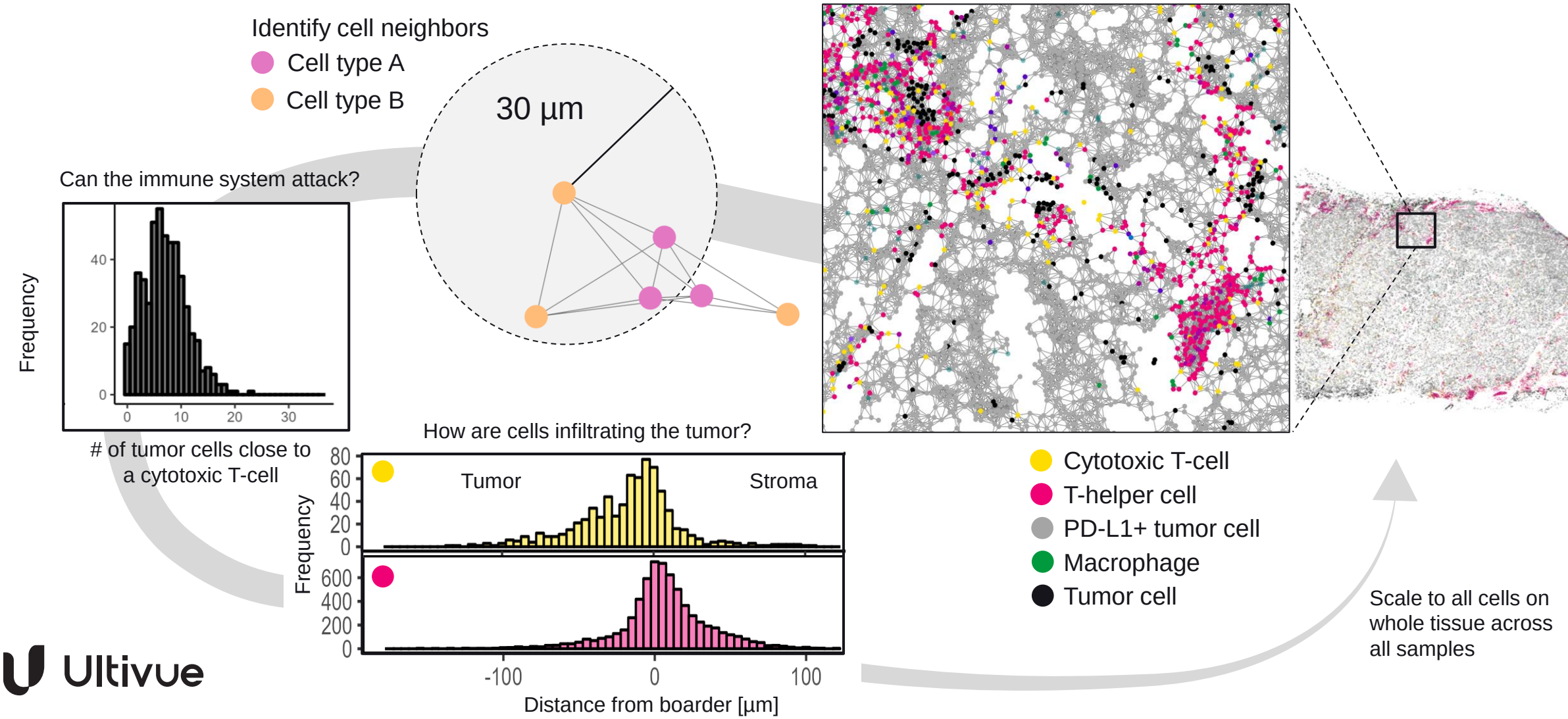
Ultivue

IMAGE ANALYSIS

Spatial Image Analysis

UltiAnalyzer.AI™
Image Analysis

Additional slide- and cohort-level spatial analysis based on a graph representation of all cells including phenotypes



Ultivue

Next Generation Integrated “Samples-to-Insights”

mIF Image

- Configurable panels of <12 targets
- Simple high-throughput workflow
- High specificity & dynamic range

Image Stacking

- Robust co-registration, micron-level accuracy
- Extremely high throughput (100’s of samples/hr)
- Nearly perfect co-registration even with large tissue defects

Image Analysis

- Deep-learning enabled whole-slide analysis
- Highly scalable cloud-based infrastructure
- Custom phenotyping through robust co-detection of multiple biomarkers

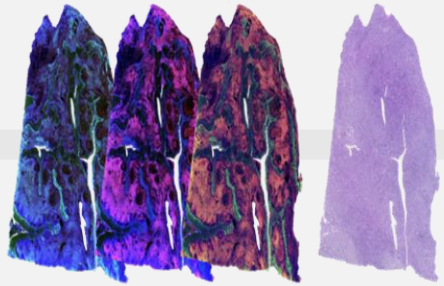
Biological Insights

- Intercellular dynamics: ROIs vs. whole section
- Advanced spatial phenomics: Clinical data integration, Endpoint analysis, Cohort-level data stratification, and more.

Sample

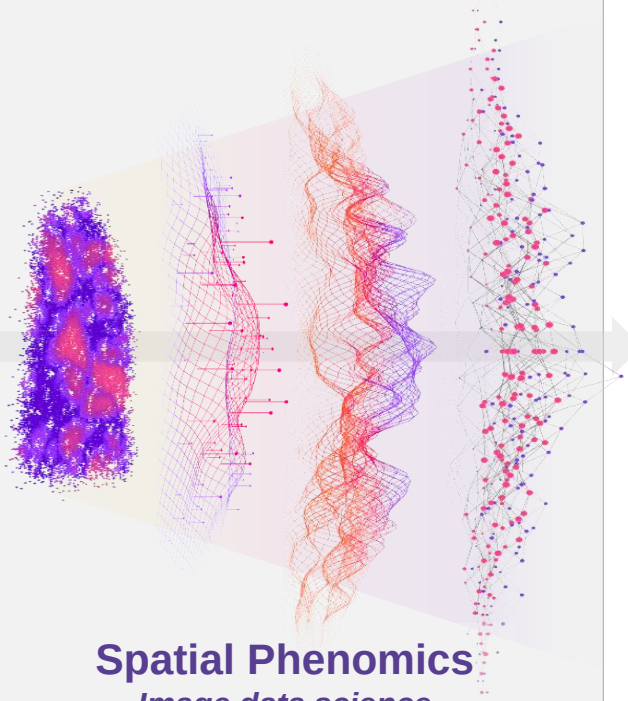
InSituPlex®
Multiplex Assay

STARVUE™
Image Data Science



UltiStacker.AI™
Image Co-registration

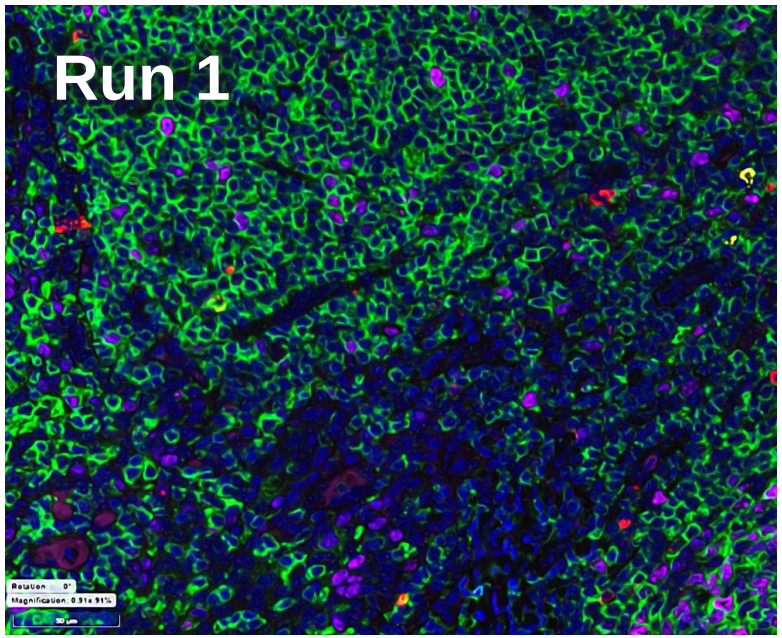
UltiAnalyzer.AI™
Image Analysis

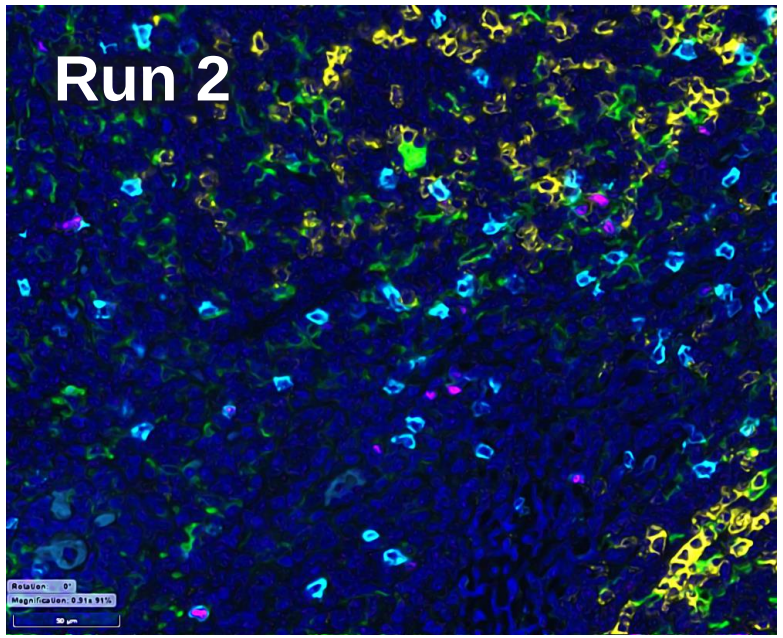


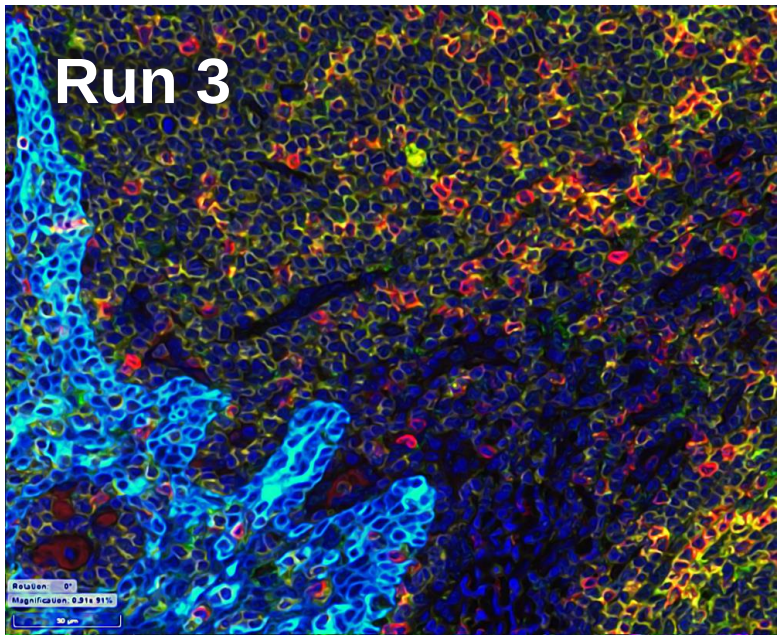
Spatial Phenomics
Image data science

Assay verification with 12-plex OmniVUE™ panel

Round	Counter	FITC	TRITC	Cy5	Cy7
1	DAPI	Ki67	Granzyme B	Lag3	HLA-DR
2	DAPI	CD8	PD-1	FoxP3	CD11c
3	DAPI	CD3	CD4	CD20	CK





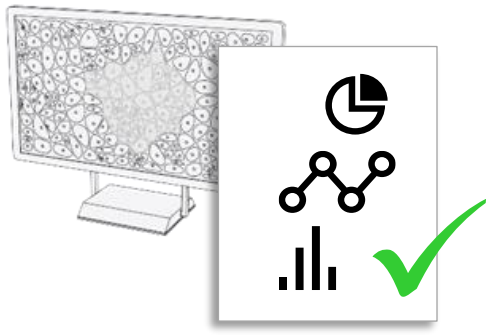


Ultivue

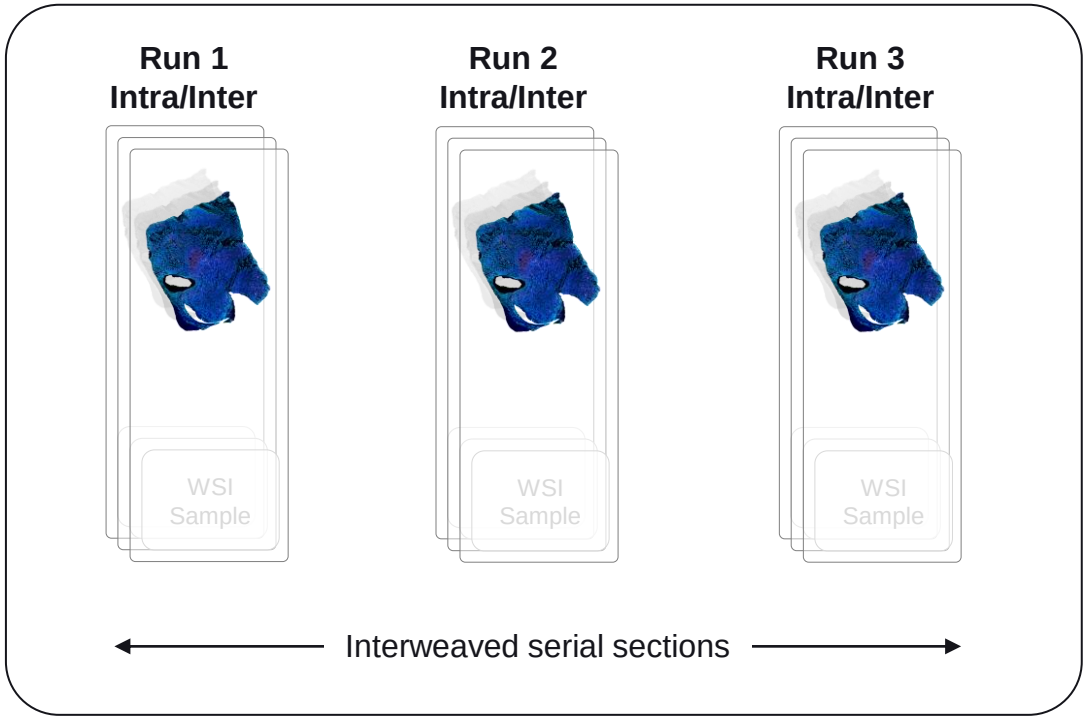
Ultivue

Intra- and Inter-Day Reproducibilty

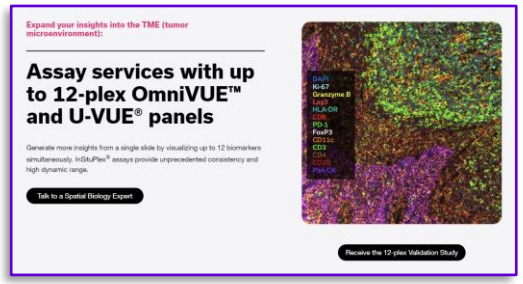
Image Analysis



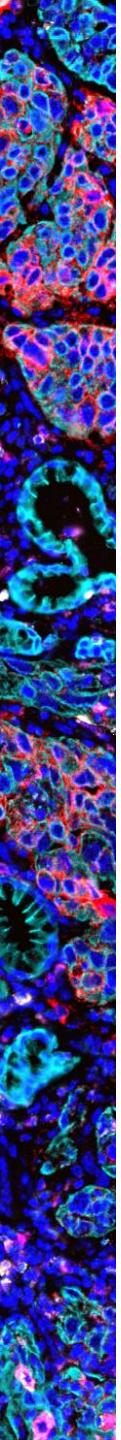
Mean positive signal intensities
Positive cell densities
Intra vs. Inter-day analysis



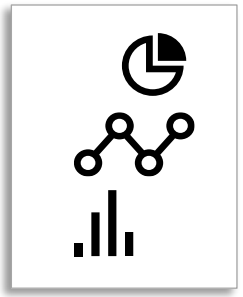
LINK



Ultivue



CV Calculation and Assessment Criteria



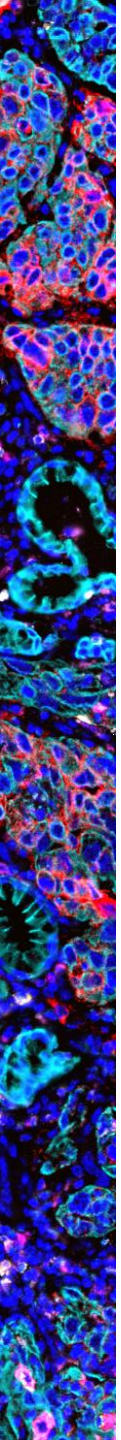
Coefficient of Variation

Mean signal intensity/density over all detected cell objects was calculated for each image. The Coefficient of Variation (CV) is calculated for each marker across all images of the same replicate (see previous slide). Finally, to obtain one CV per marker, the mean of all per-replicate CVs is calculated.

Assessment Criteria

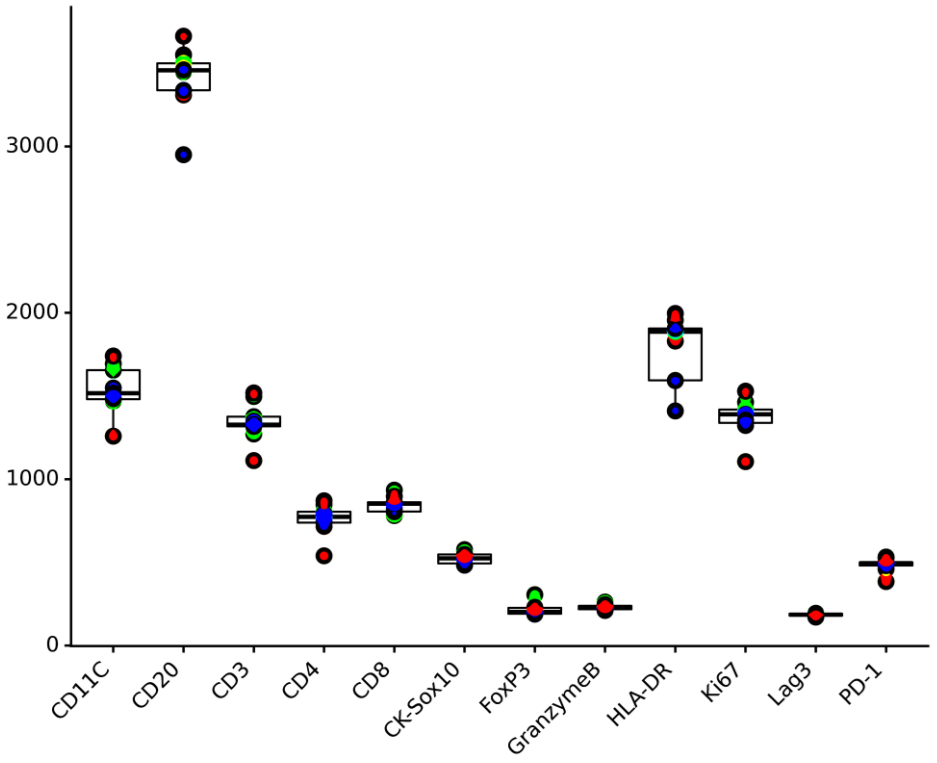
Staining is within the acceptable range of variability given an aggregate CV of **20% or less.**

Ultivue

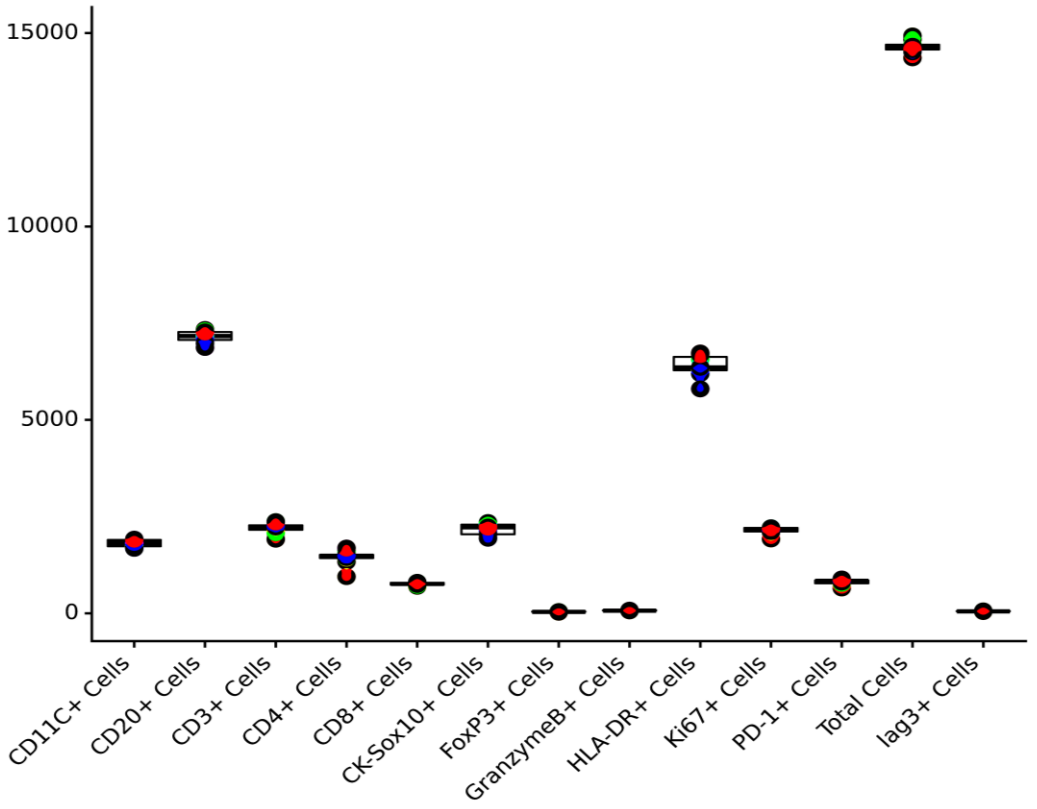


Highly consistent biomarker detection in 12-plex panels

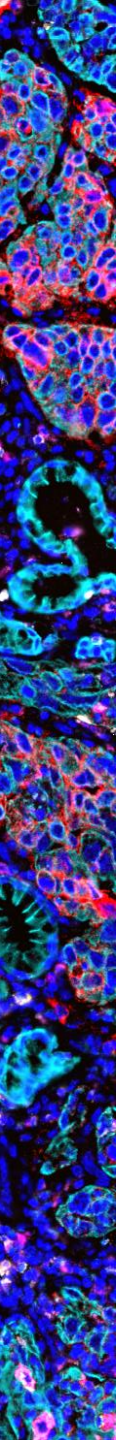
Mean positive signal Intensities



Positive cell densities



Ultivue



Highly consistent biomarker detection in 12-plex panels

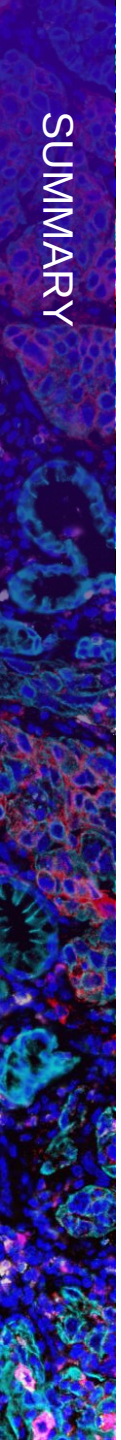
Mean positive signal Intensities

Marker	Intra-day CV	Inter-day CV
CD11C	9.88	7.46
CD20	4.77	5.53
CD3	9.18	7.6
CD4	10.99	11.28
CD8	6.61	5.61
CK-Sox10	6.64	6.38
FoxP3	13.32	15.45 Max. CV
GranzymeB	6.48	7.02
HLA-DR	10.18	12.53
Ki67	7.55	7.59
Lag3	3.26	4.51
PD-1	8.62	6.7

Positive cell densities

Marker	Intra-day CV	Inter-day CV
CD11C	5.11	5.1
CD20	2.22	1.73
CD3	6.8	4.02
CD4	14.11	10.82
CD8	5.24	3.09
CK-Sox10	6.99	6.36
FoxP3	13.06	12.72
GranzymeB	6.91	5.86
HLA-DR	4.35	4.84
Ki67	3.79	3.68
Lag3	8.69	9.88
PD-1	9.3	5.45

Ultivue



Summary

- **Flexible multiplexed immunofluorescence (mIF)** solutions are essential for tailoring multiplex assays to precisely fit the unique demands of tumor microenvironment research within clinical trials.
- **mIF allows** the study of cell sub populations and their spatial interaction.
- **InSituPlex® assay** is highly sensitive, customizable, and reproducible.
- **Specialized computational tools** are required to analyze mIF images due to their complexity and size challenges.
- **Deep Learning** robust and highly automated image analysis without manual thresholding, improving consistency and throughput.
- **End-to-end workflow** from assay to quantitative cellular data shows excellent reproducibility.