Ultivue technology

ULTIVUE

InSituPlex® (ISP) Overview

ANTIGEN RETRIEVAL

Mixture of DNA-barcoded antibodies

STAINING

AMPLIFICATION

Mixture of fluorescent probes

DETECTION

✓ **Single Antigen Retrieval Step**

Dewax and retrieve samples

✓ **Single Staining Step**

Incubate sample with a mixture of DNA barcoded antibodies

✓ **Single Amplification Step**

Increases ratio of barcodes per antibody to allow for more complimentary probe strands to bind

✓ **Single Detection Step**

Complementary fluorescent DNA probes hybridize to targets
Samples are ready to be imaged

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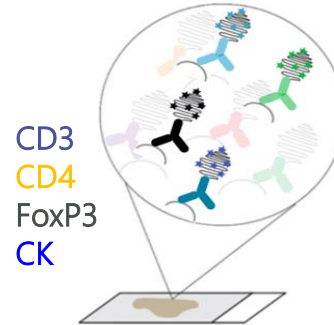
Ultivue Technology affording High-Plex, Single Slide Assays

Round 1

CD8
PD-1
PD-L1
CD68
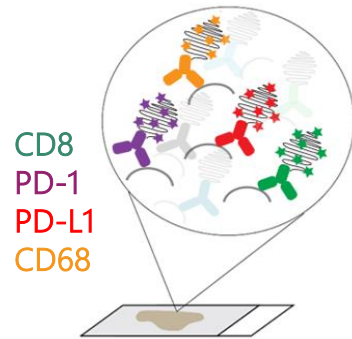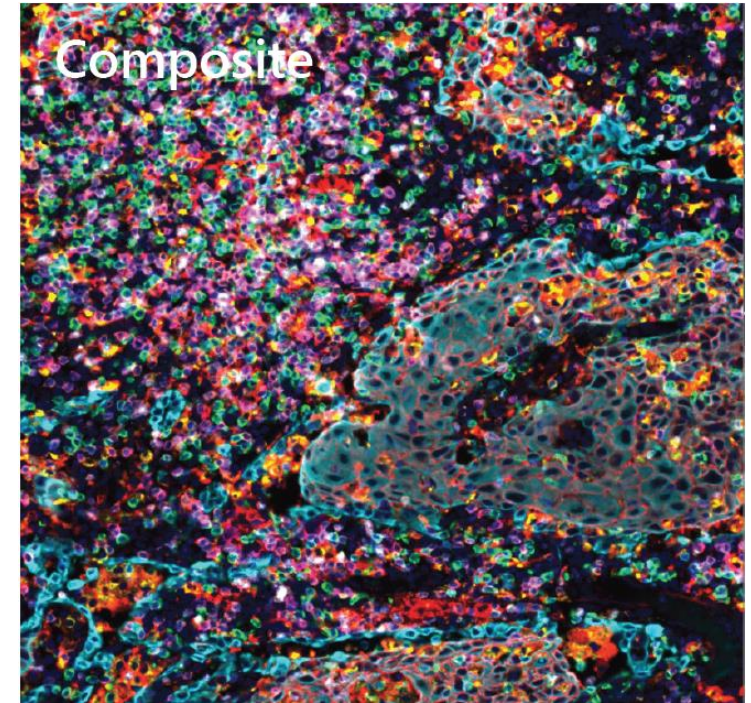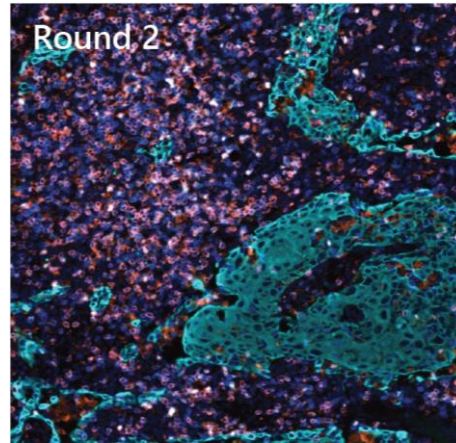
- Add 1st set of fluorescent probes to bind targets
- Image sample

Remove 1st set of probes

Round 2

CD3
CD4
FoxP3
CK

- Add 2nd set of fluorescent probes to bind different targets
- Image sample

Composite

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Multiplex Made Simple - same day staining to result

Same Day – Staining to Results

1. Automated Staining

2. Whole Slide Imaging

3. Image Analysis

Staining 5.5 hrs manually or automated

15 min or less per slide

ZEISS Axio Scan.Z1 — Akoya Biosciences Polaris — Olympus VS-120/200

User preference for software analysis

Leica BOND RX | Leica BOND RX^M

Hamamatsu NanoZoomer S60 - Leica Aperio VERSA — 3DHISTECH P250 — RareCyte CyteFinder HT

Indica Labs – Visiopharm

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FixVUE I/O T-act Kit

Determine cell proliferation and T-cell activation
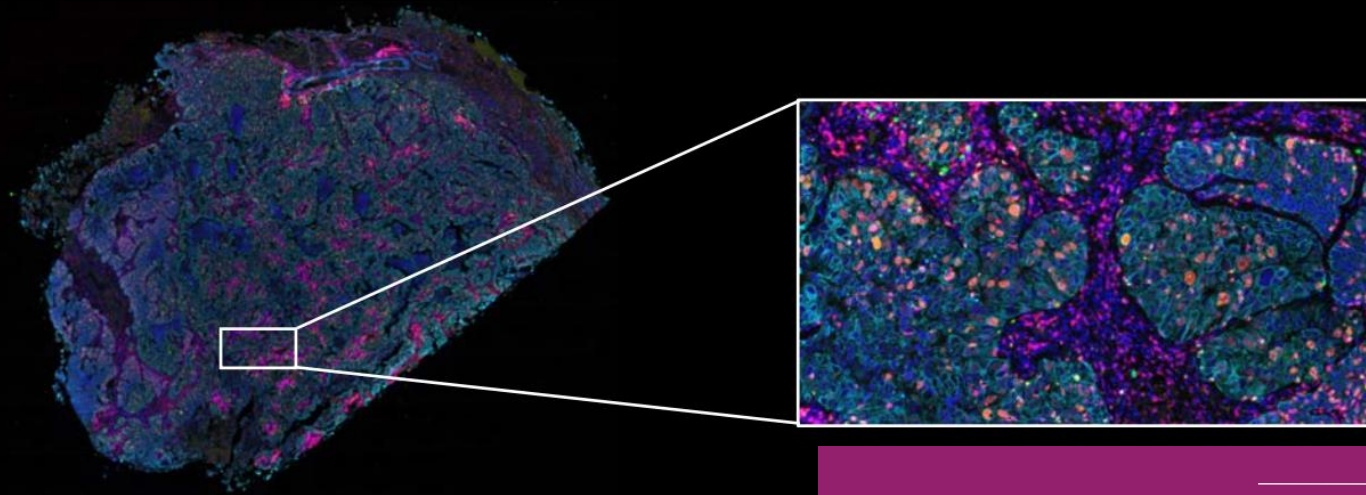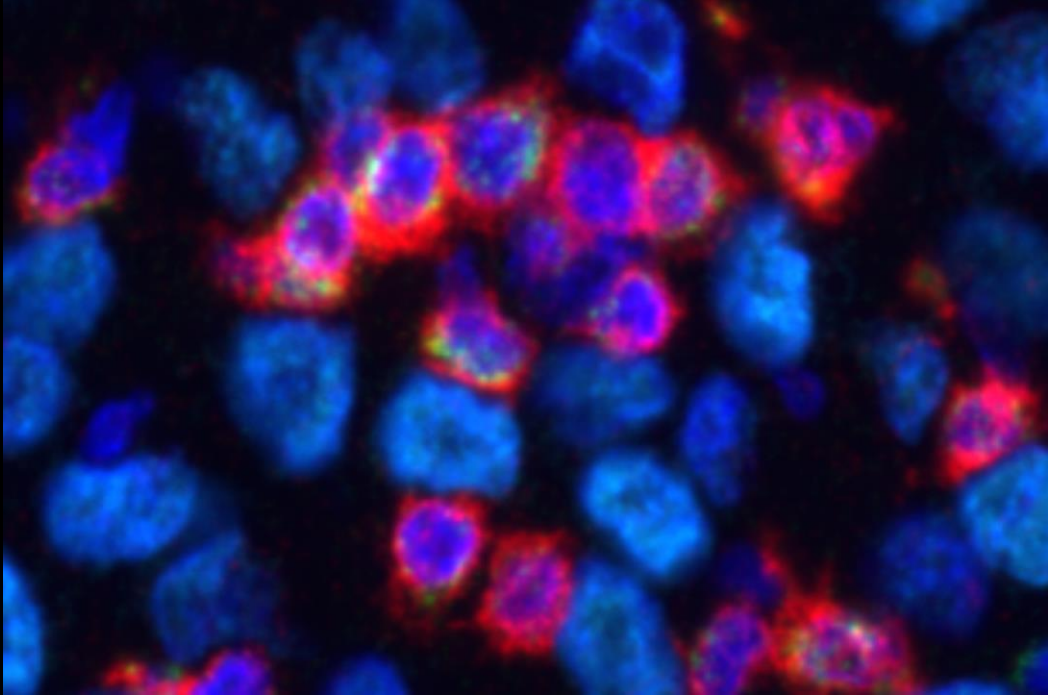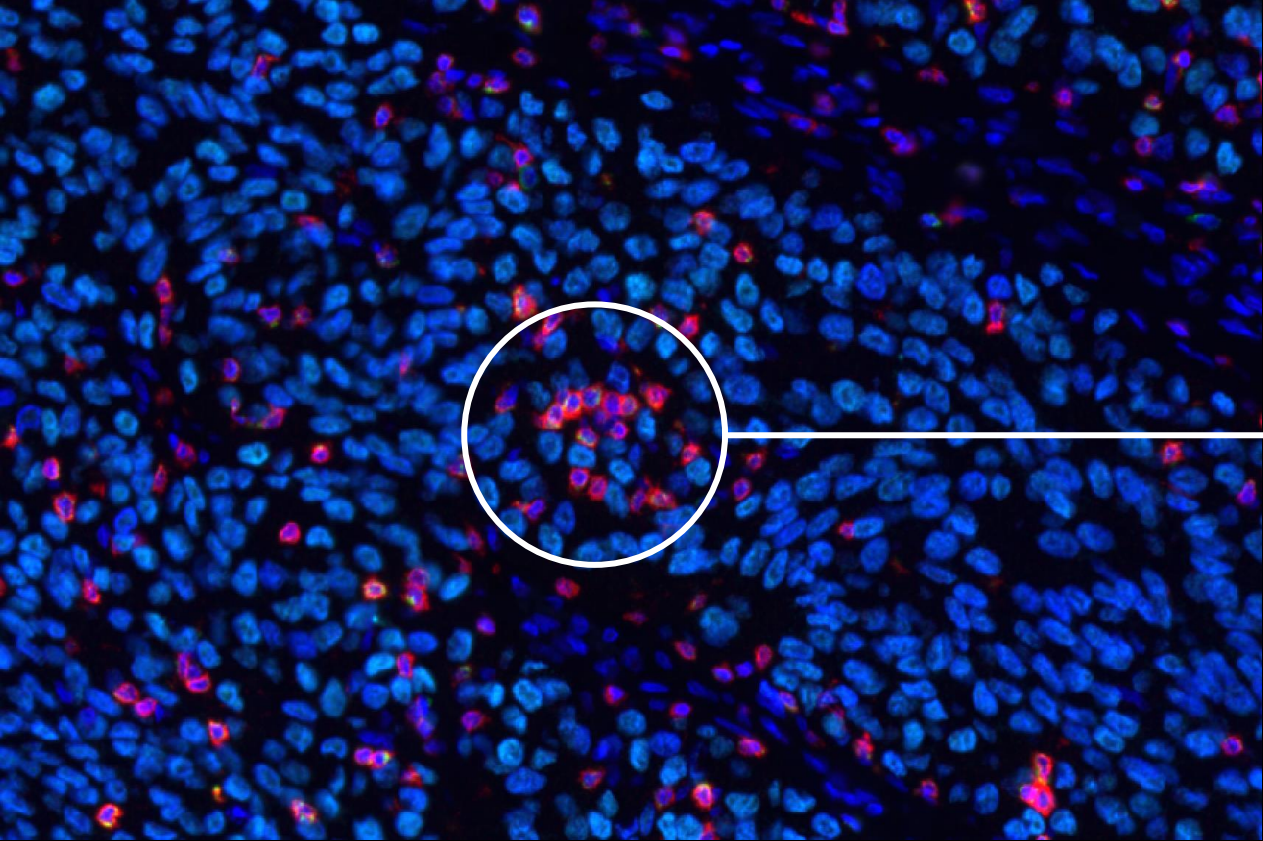
FixVUE I/O T-act kit staining
non-small cell lung cancer tissue.

Markers: CD3, Granzyme B, Ki67, panCK, DAPI

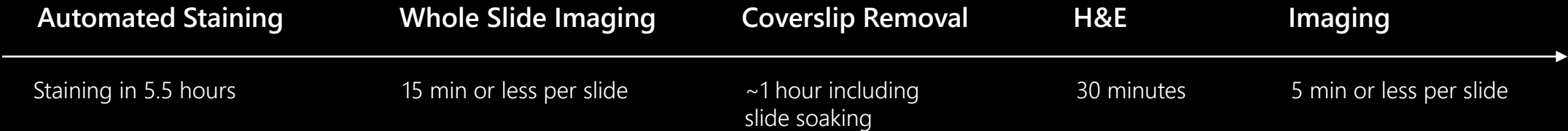| PHENOTYPE	MARKERS			
	CD3	Granzyme B	Ki67	panCK/SOX10
T cells	X			
Cytotoxic cells		X		
Proliferating cells			X	
Carcinoma (panCK) or Melanoma (SOX10)				X
Cytotoxic T cells	X	X		
Proliferating T cells	X		X	
Proliferating Cytotoxic cells		X	X	
Proliferating Cytotoxic T cells	X	X	X	
Proliferating tumor cell			X	X

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Cytotoxic T-Cell Mediated Melanoma Tumor Cell Death

See Biology in action!

DAPI Granzyme B CD3 Sox10

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Preservation of tissue architecture and cellular morphology

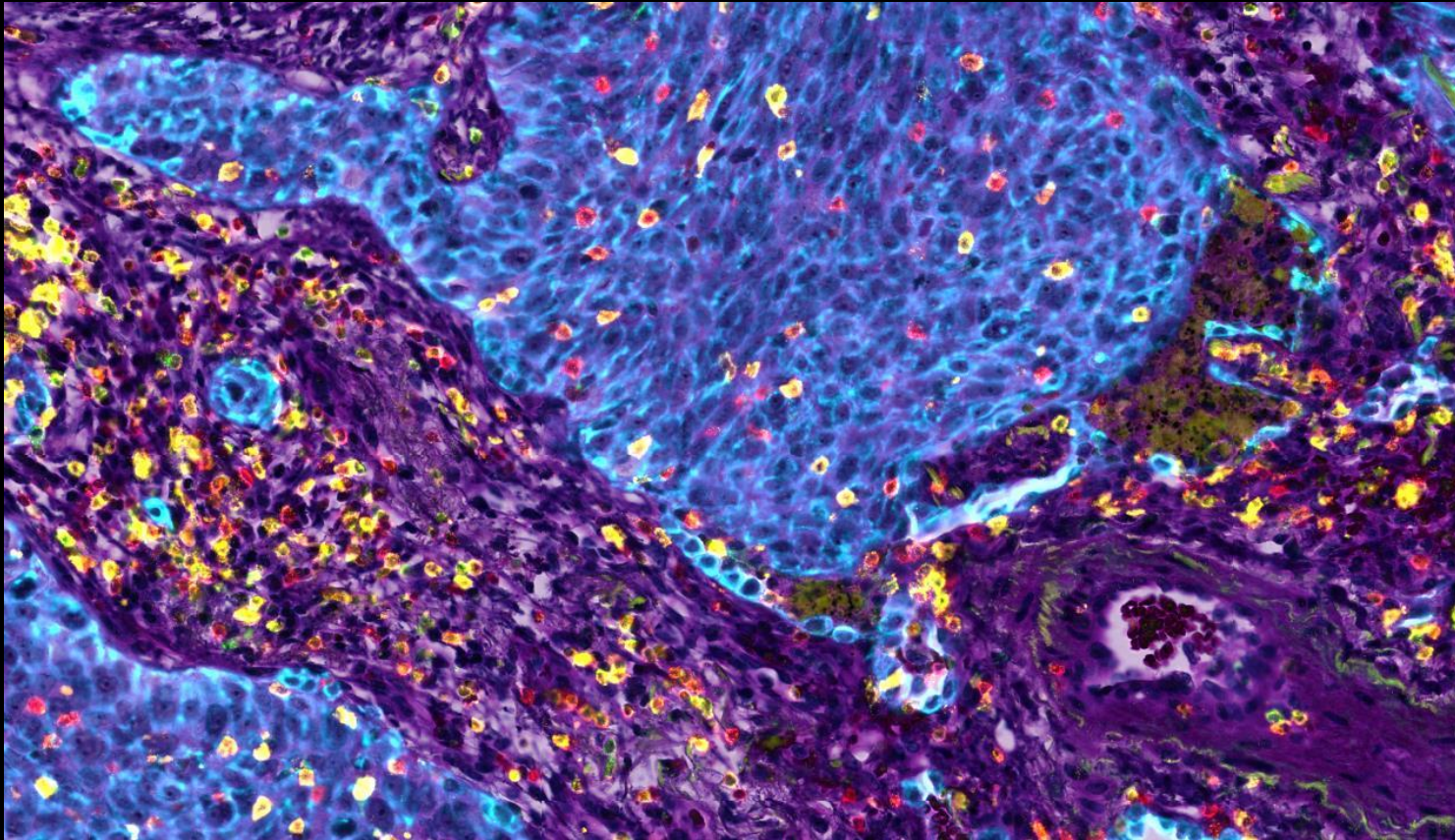| Automated Staining	Whole Slide Imaging	Coverslip Removal	H&E	Imaging
Staining in 5.5 hours	15 min or less per slide	~1 hour including slide soaking	30 minutes	5 min or less per slide

CD8
CD68
PD-L1
panCK
DAPI

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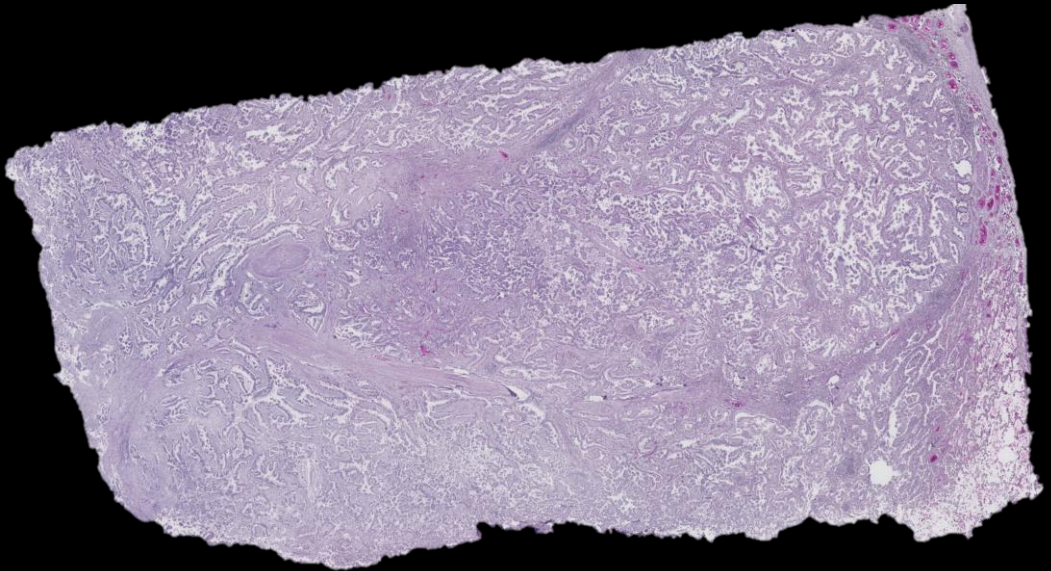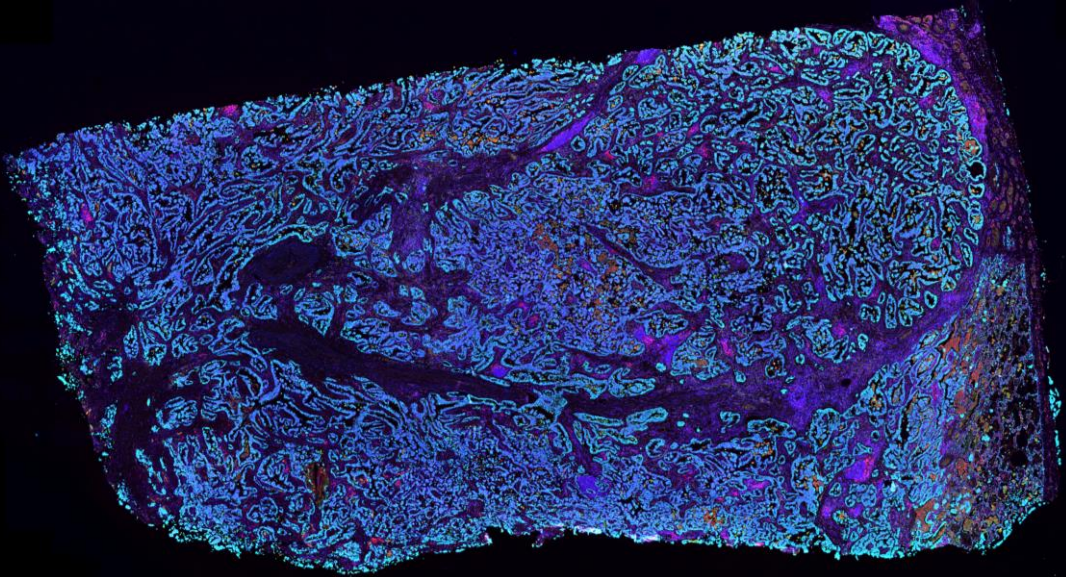
Fluorescent and H&E Images on the Same Slide

With a single round of antigen retrieval, tissue architecture and cellular morphology are preserved!


Multiplex IHC and HE

- PD1 UltiMapper Kit on NSCLC
  - CD3
  - CD45RO
  - PD-1
  - CK
- Followed by H&E Stain

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FixVUE I/O Immuno8™ panel

Development of a multiplex IF panel using InSituPlex® technology to investigate key immune phenotypes across multiple tumor types:

- CD3 (clone BC33)
- CD4 (clone SP35)
- CD8 (clone C8/144B)
- CD68 (clone KP-1)

- FoxP3 (clone 236A/E7)
- PD-1 (clone CAL20)
- PD-L1 (clone 73-10)
- pan-CK (clone AE1/AE3)

| PHENOTYPE	MARKERS							
	CD3	CD4	CD8	CD68	FoxP3	PD-1	PD-L1	CK
T cell	X							
T helper cell	X	X						
Cytotoxic lymphoid cell			X					
Cytotoxic T cell	X		X					
FoxP3+ T cell	X				X			
Exhausted T cell	X					X		
T-reg	X	X			X			
CD4/CD8 T cell	X	X	X					
CD8+ T-reg	X		X		X			
Exhausted, cytotoxic T cell	X		X			X		
Macrophage				X				
Immunosuppressive Macrophage				X			X	
Tumor cell								X
Immuno-evading tumor cell							X	X
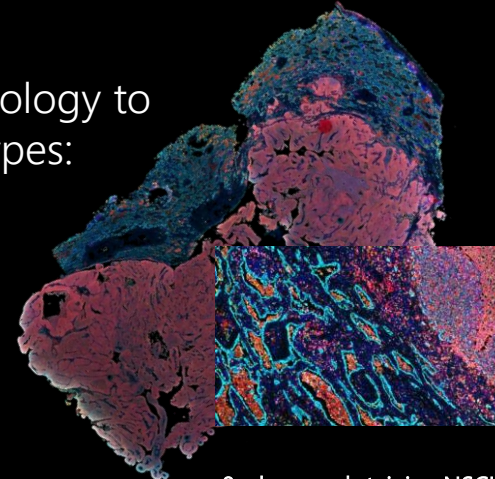
8-plex panel staining NSCLC tissue.
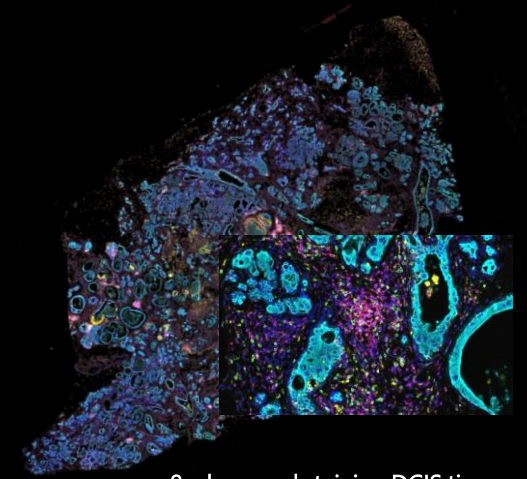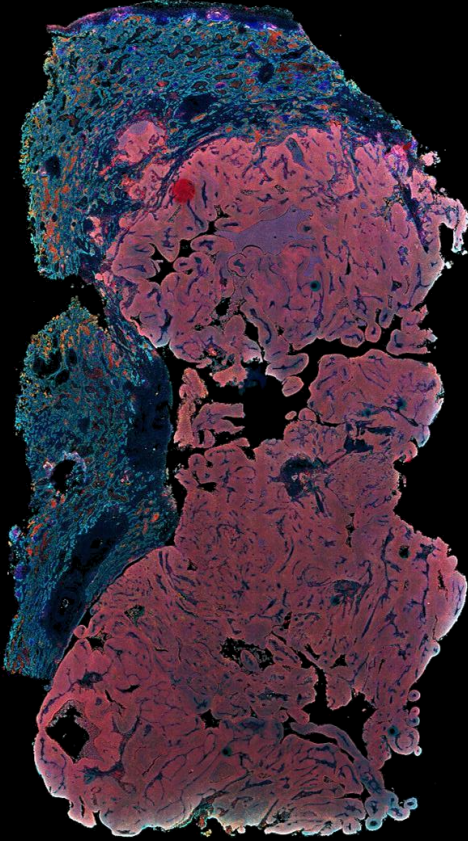

8-plex panel staining DCIS tissue.
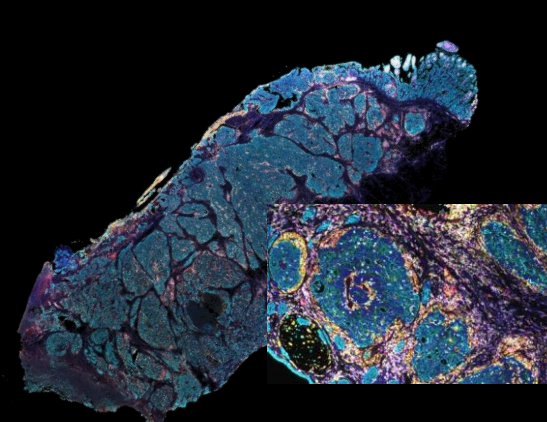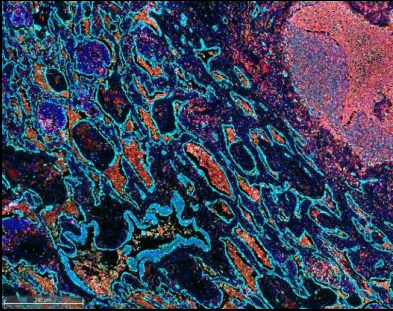

8-plex panel staining CRC tissue.
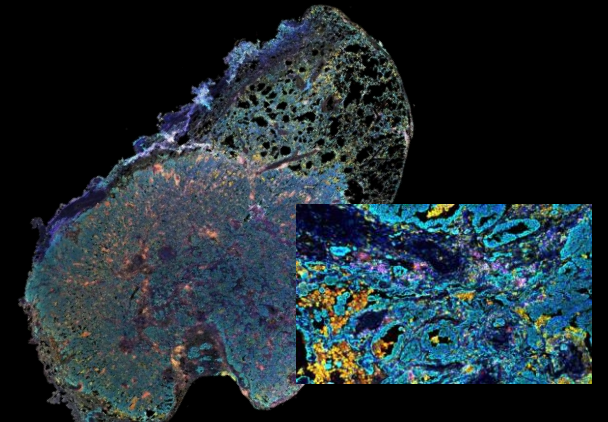
8-plex panel staining NSCLC tissue.

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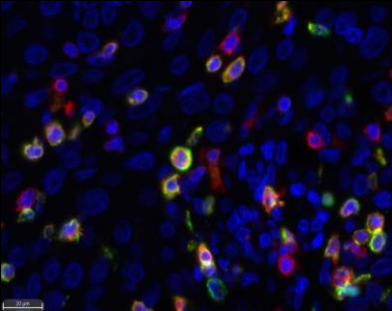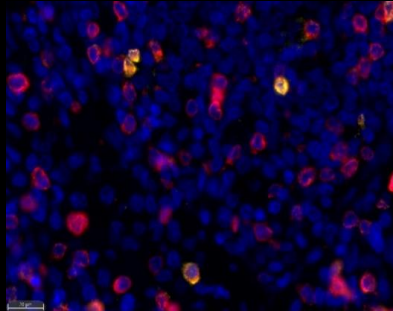
Immuno8™ panel– Regions of Interest

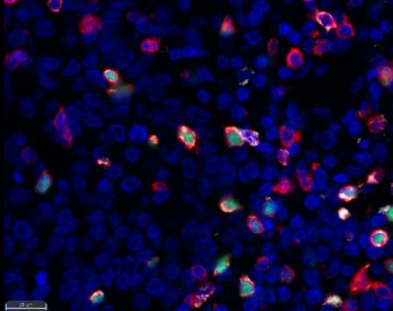Immuno8™ panel staining NSCLC tissue.

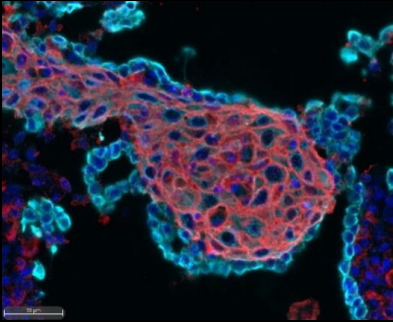Multiplex Zoom (all markers)

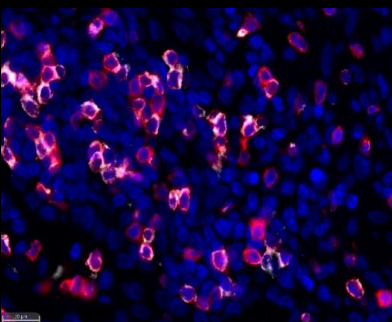Cytotoxic T cells (CD3+ CD8+)

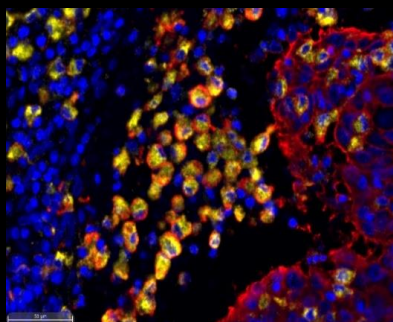
Exhausted T cells (CD3+ PD-1+)

T-regs (CD3+ CD4+ FoxP3+)
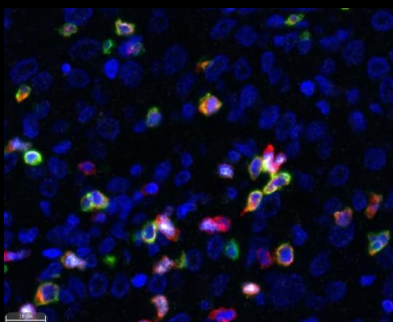

Immune-evading tumor
(CK+ PD-L1+)

T helper cells (CD3+ CD4+)

Immunosuppressive Macrophages
(CD68+ PD-L1+)

CD8+ T-regs
(CD3+ CD8+ FoxP3+)

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Ultivue offering

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Rapid Deployment of tissue multiplex assays in clinical trials

Off-the-Shelf Assays

Same day staining
to analysis for
any panel

- FixVUE PD-L1   CD8, CD68, PD-L1, CK/SOX10
- FixVUE PD-1   CD3, CD45RO, PD-1, CK/SOX10
- FixVUE APC   CD11c, CD20, CD68/CD163, MHCII
- FixVUE T-Act   CD3, Granzyme B, Ki-67, CK/SOX10
- FixVUE T-Reg   CD4, CD8, FoxP3, CK/SOX10
- FixVUE MDSC   CD11b, CD14, CD15, HLA-DR
- FixVUE Immuno8   CD3, CD4, CD8, CD68, FoxP3, PD-1, PD-L1, CK/SOX10
- FlexVUE Immuno8   CD3, CD4, CD8, CD68, FoxP3, PD-1, PD-L1, CK/SOX10
                        GrZB, CD45RO, HLA-DR, CD56, CD20, CD11C, CD163,
                        CD206, CD14, CD15, CD11B, MHCII

Custom Assay Development (8-13 weeks total)
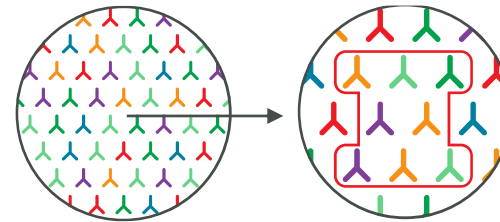
Develop your own plex
panel, only up to 8 plex,
from >70 available
biomarkers

Assay Development

- Screened antibody clones
- Consultative image review
- Optimized assay conditions
    - Conjugation
    - Panel configuration
    - Assay dilutions

Manufacturing

- Kit manufacturing
    - Antibody-conjugates
    - Ancillary buffers
- Documentation
    - Datasheet
    - Protocol

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Custom U-VUE kit

Develop your own plex panel, only up to 8 plex, from >70 available biomarkers

U-VUE Biomarker

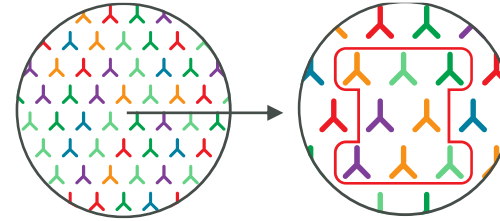| Checkpoints	TAMs	Exhaustion	Tertiary Lymphoid	Stromal	T cell Regulation	Dendritic	TILs
CD226	CD39	Arg1	CD138	Alpha-SMA	CD27	CD11c	CD11c
CD27	CD68	CD11c	CD20	CD299	CD3	Clec9a	CD20
CD28	CD8	CD163	CD3	CD31	CD38	Clec10a	CD3
CD40	CTLA4	CD206	CD4	FAP	CD4	CD138	CD38
CD70	LAG3	CD68	CD56	PDGFR	CD45RO	CD20	CD4
CD73	PD1	CD86	CD68		CD70	CD3	CD45RO
CD80	TIGIT	HLA-DR	DC-Lamp	**B cells**	CD8	CD4	CD56
CTLA4		INOS		BCMA	FoxP3	CD56	CD68
GITR	**Cell Signaling**			CD138	GrxB	CD68	CD8
IDO1	Bcl-2	**Functional**		CD19	Lag3	DC-Lamp	
LAG3	Bcl-xl	CK		CD20	PD1		
PD1	Beta-Catenin	Ki67		CD22	PD-L1		
PD-L1	Bim	Sox10		CD27			
TIGIT	Ki67			CD38			
	Mcl-1			MHC Class II			
				Pax5			

download overview

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Introducing FlexVUE

The ability to swap in 1 to 2 of the following 13 markers into an 8-plex kit, no additional development or assay cost

Immuno-8

CD8	PD-1	PD-L1	CD68
CD3	CD4	FOXP3	CK/SOX-10

- Ki67
- GrZB
- CD45RO
- HLA-DR
- CD56
- CD20
- CD11C
- CD163
- CD206
- CD14
- CD15
- CD11B
- MHCII

IMMUNE REACTIVITY

Ki67	GrzB	PD-L1	CD68
CD3	CD4	FOXP3	CK/SOX-10

TERTIARY LYMPHOID

CD8	CD20	CD11C	CD68
CD3	CD4	FOXP3	CK/SOX-10

TIL

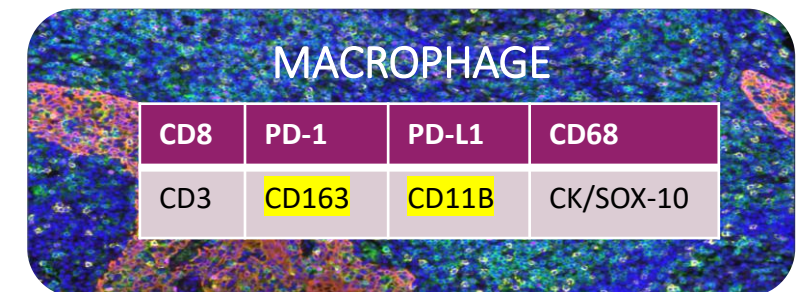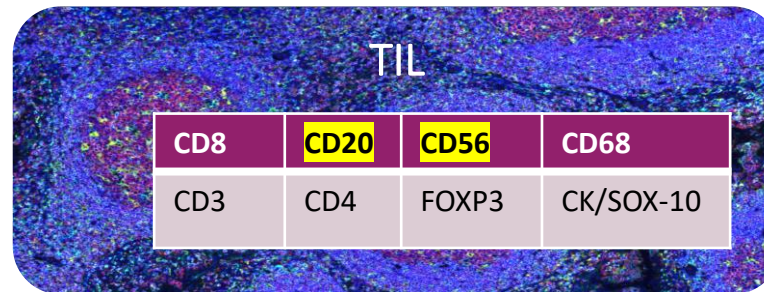| CD8	CD20	CD56	CD68
CD3	CD4	FOXP3	CK/SOX-10

MACROPHAGE

CD8	PD-1	PD-L1	CD68
CD3	CD163	CD11B	CK/SOX-10
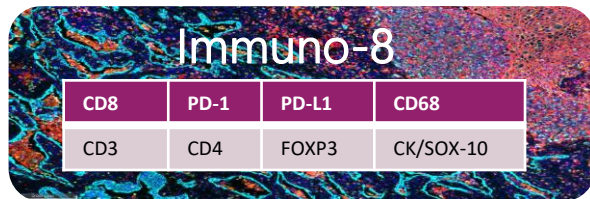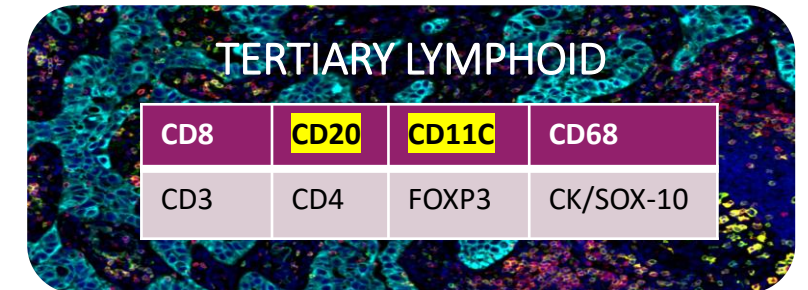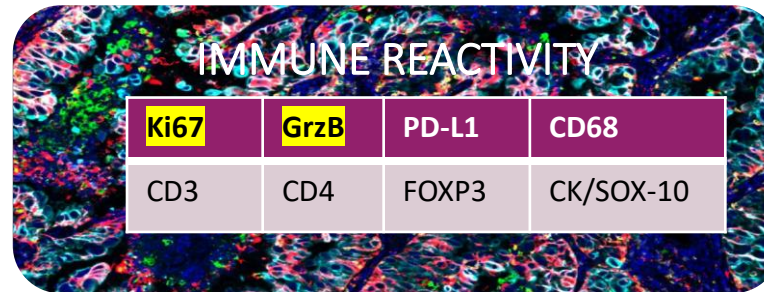
*Panels represented are suggestions only. Customers may swap in any of the 13 markers listed to the left to create their own 8-FLEX panel.

ULTIVUE

Ultivue's Unique Value Proposition and Differentiation

InSituPlex®

Standardized Multiplexing In Situ
Reproducibility of plug-and-play biomarker panels across cancer types and instruments without the need for reoptimization

Sample fully preserved
Complete retention of sample antigenicity and minimization of antibody steric hinderance in the mIF staining process

Compatible with conventional Pathology
Co-registration of mIF and H&E images acquired from the same slide

Amenable to high-throughput
Minimal 'Sample-to-Answer' time with whole slide imaging

Scalable Implementation
Transferable biomarker panels to CRO outsourcing partners for clinical research roll-out

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