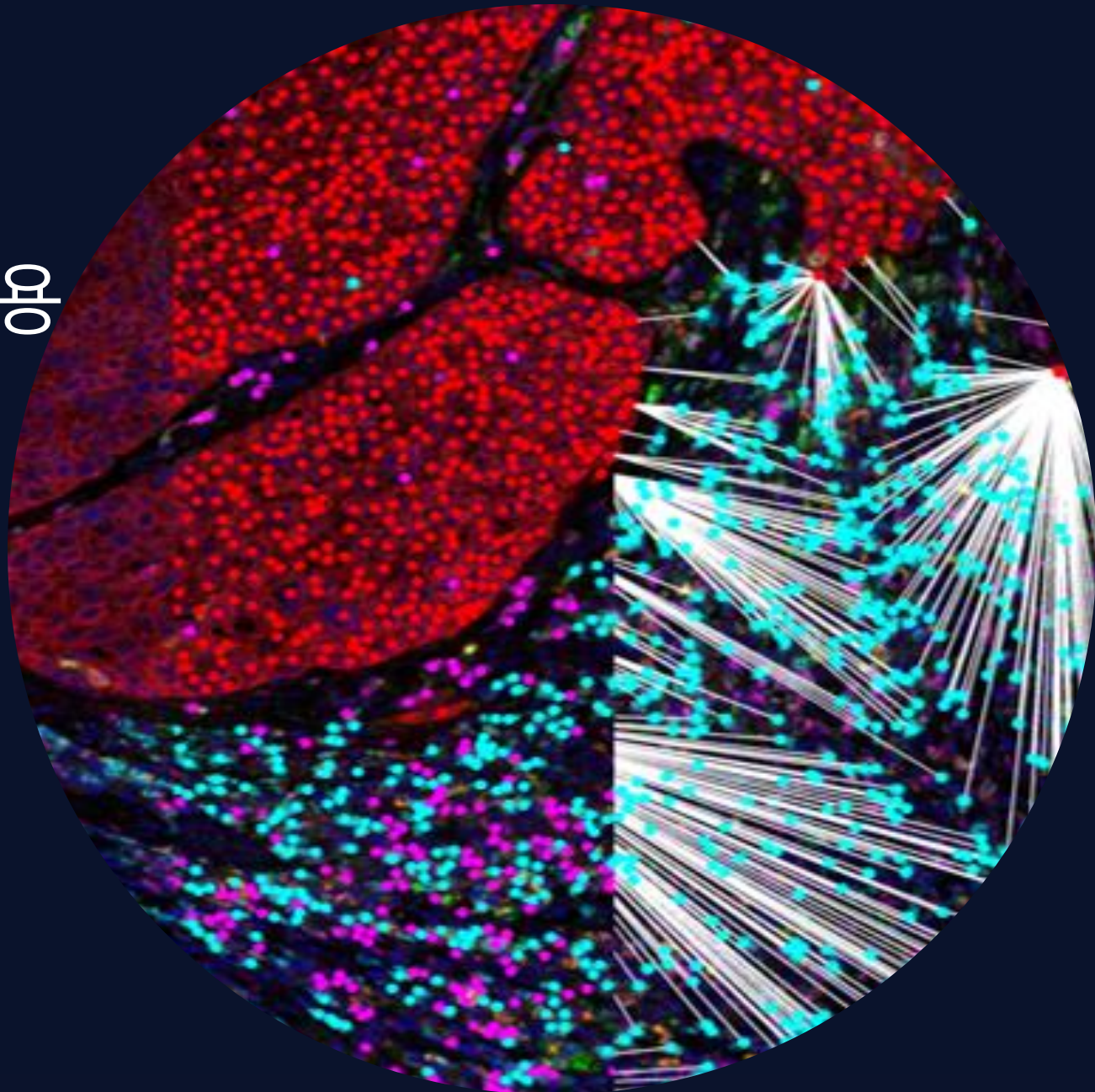


# PIHT (구 Polaris)의 활용 Spatial Signatures at Scale

---

이길제

Sr. manager, Akoya Biosciences



# PIHT workflow



**MOTiF Panel Kits**  
Quality reagents



**Bond Rx**  
Optimized protocol



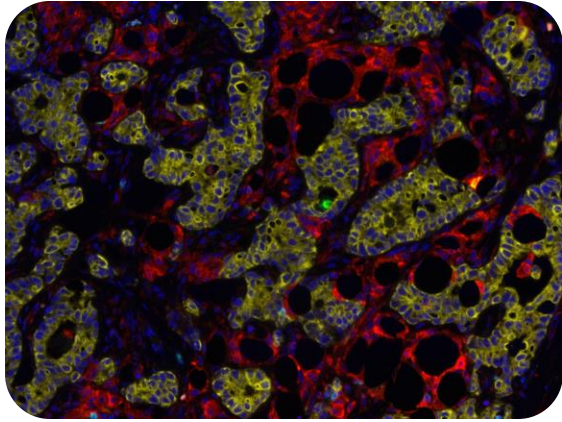
**PIHT**  
Standardized imaging  
protocol



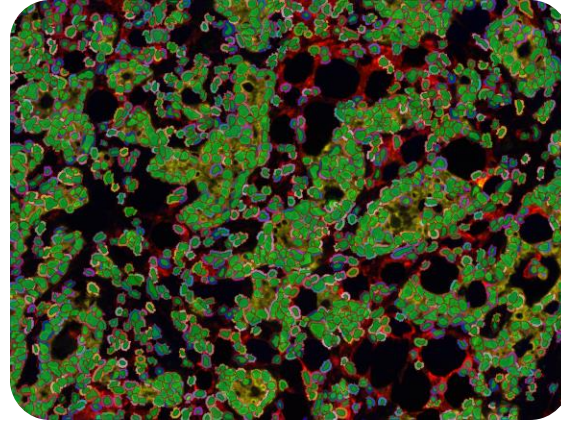
**inForm software**



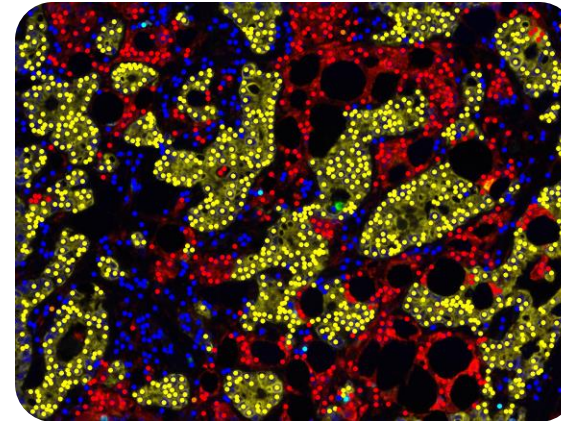
# From Images to Phenotypes to Signatures



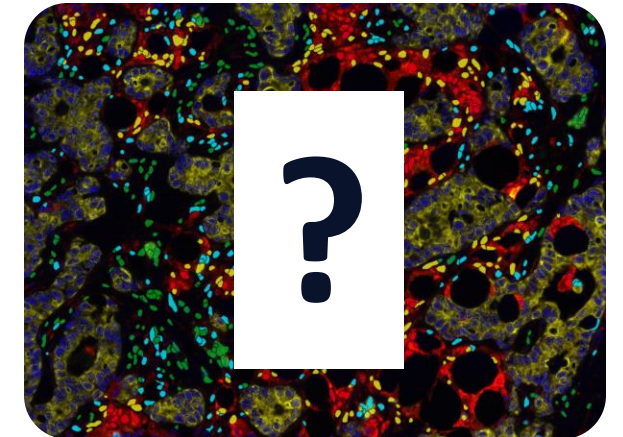
 Spectral Unmixing  
*PhenoChart & inForm*



 Tissue and Cell  
Segmentation  
*inForm*



 Cell Phenotyping  
*inForm*



 Advanced Analysis  
*Phenoptr, phenoptrReports*

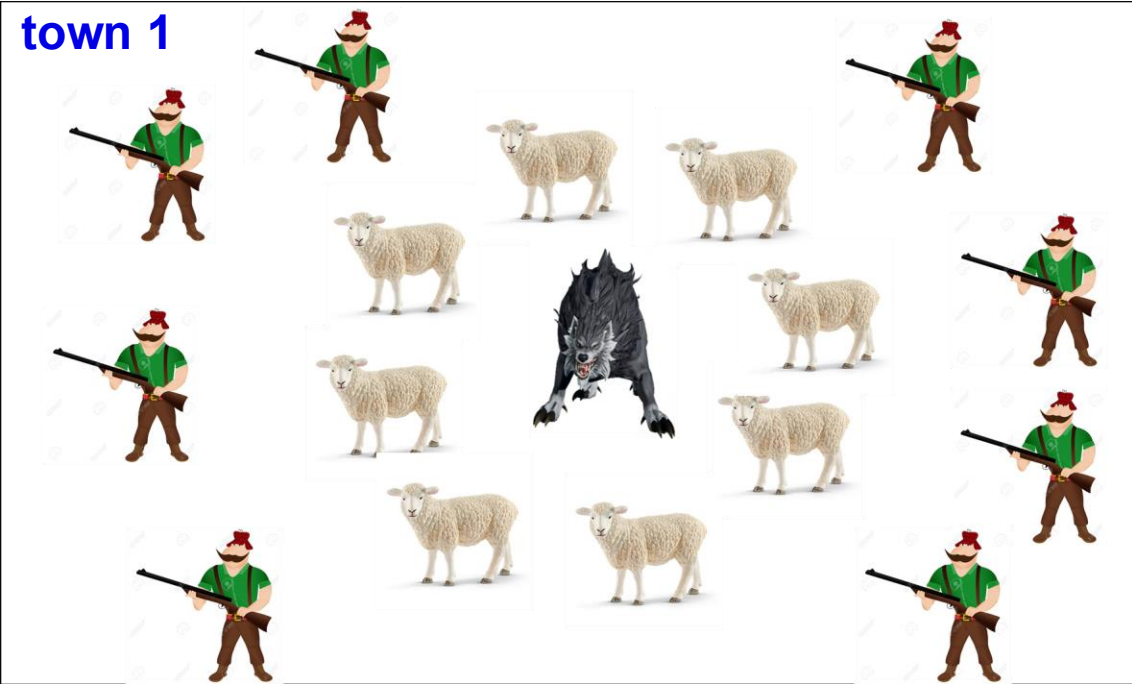
그래서....

다중형광이미지로부터 무엇을 분석해야 하는지?

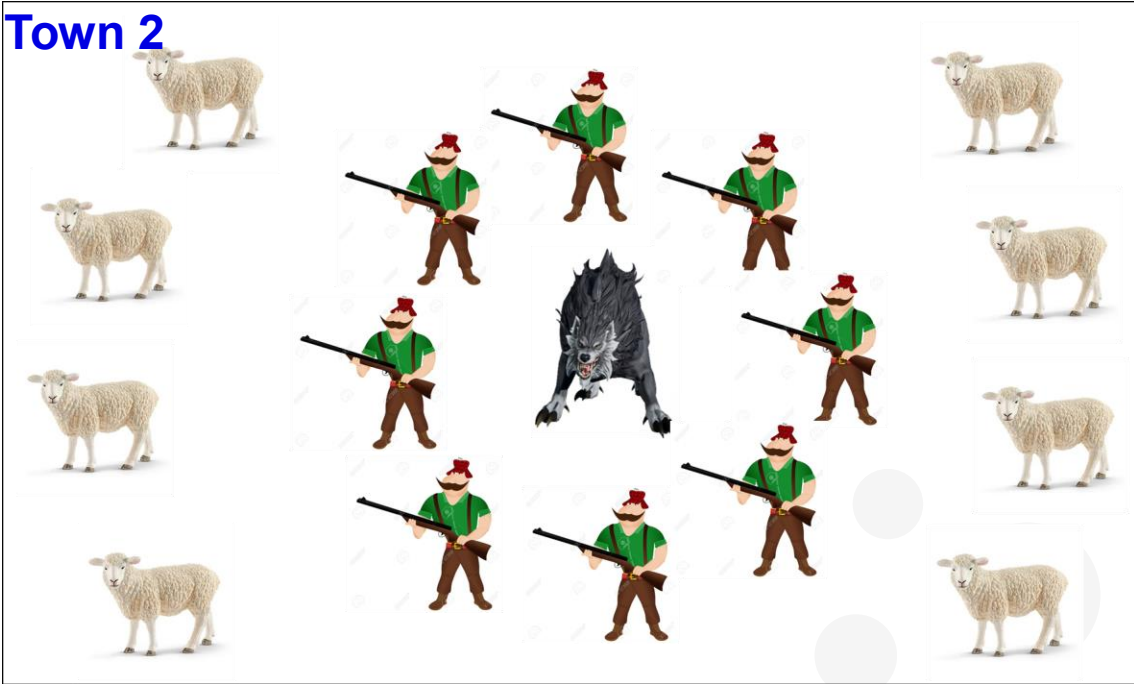
**Spatial analysis!!!**  
**공간해석**  
**(spatial signature)**

# What is different information from NGS/flow cytometry

town 1



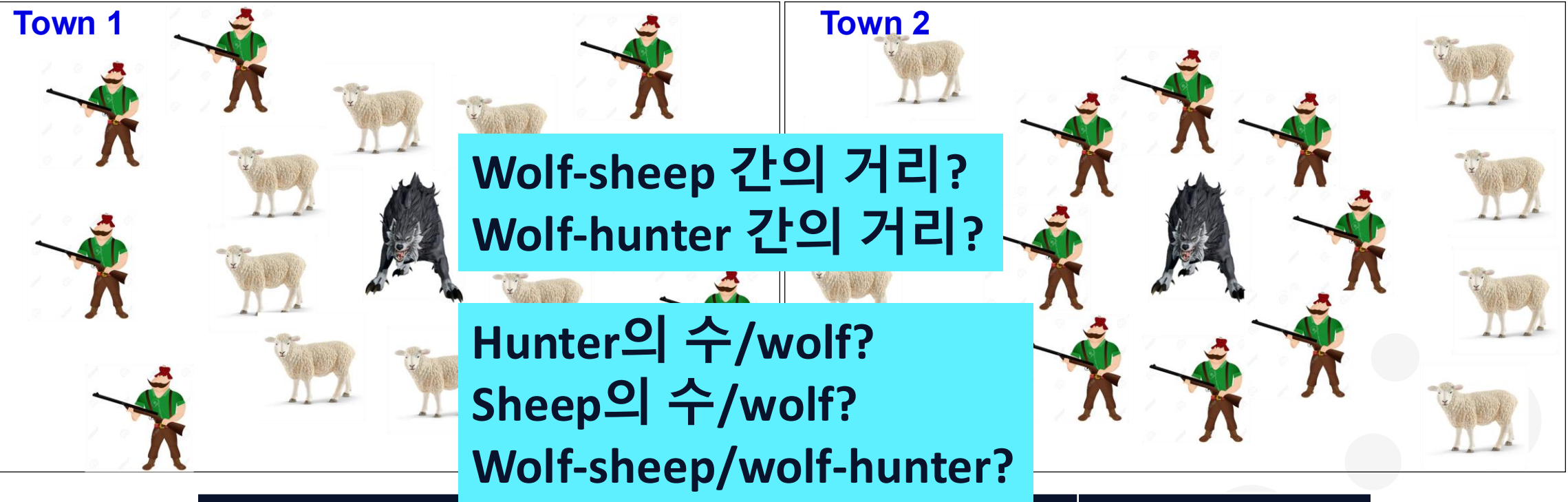
Town 2



	wolf	sheep	hunter
Town 1	1	8	8
Town 2	1	8	8

No difference between town1 and town 2 without IMAGE (positional information)

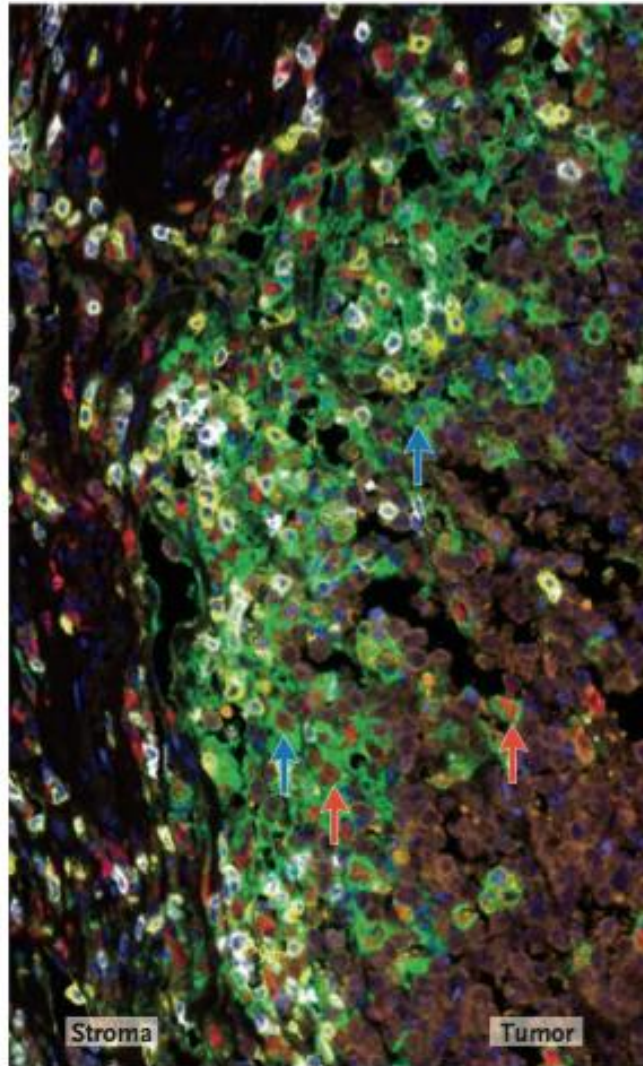
# What Spatial Biology can do



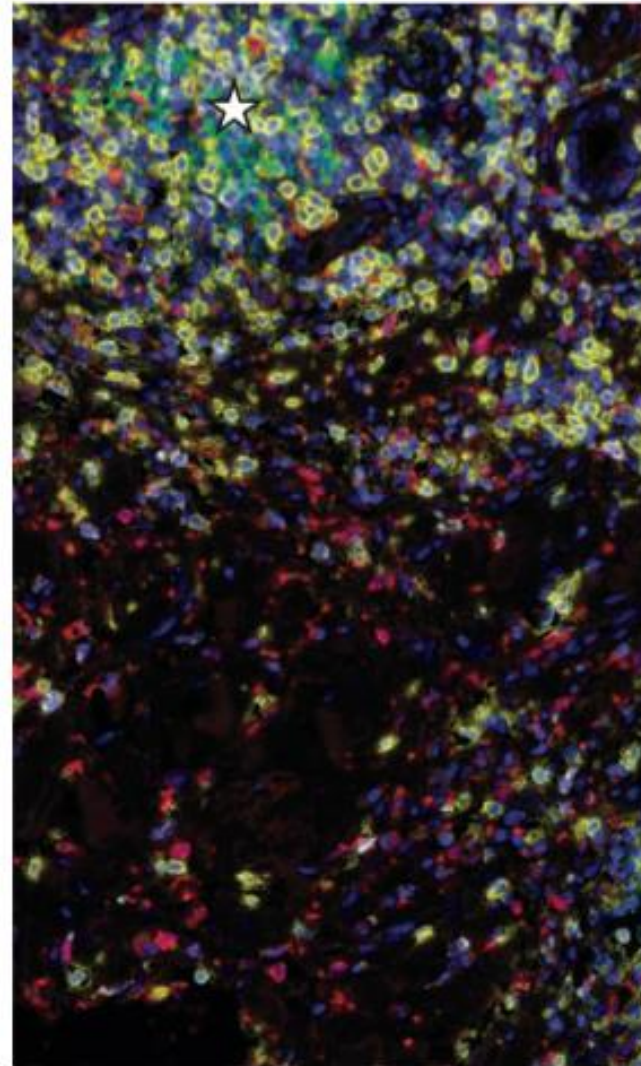
	wolf	sheep	hunter
Town 1	1	8	8
Town 2	1	8	8
distance	Wolf-sheep	Wolf-hunter	Sheep-hunter
Town 1	1 m	10 m	9 m
Town 2	10 m	1 m	9 m



# 더 복잡한 조직에서는 무엇을 보아야 하는지?



Archival Biopsy Specimen  
of Primary Merkel-Cell Carcinoma

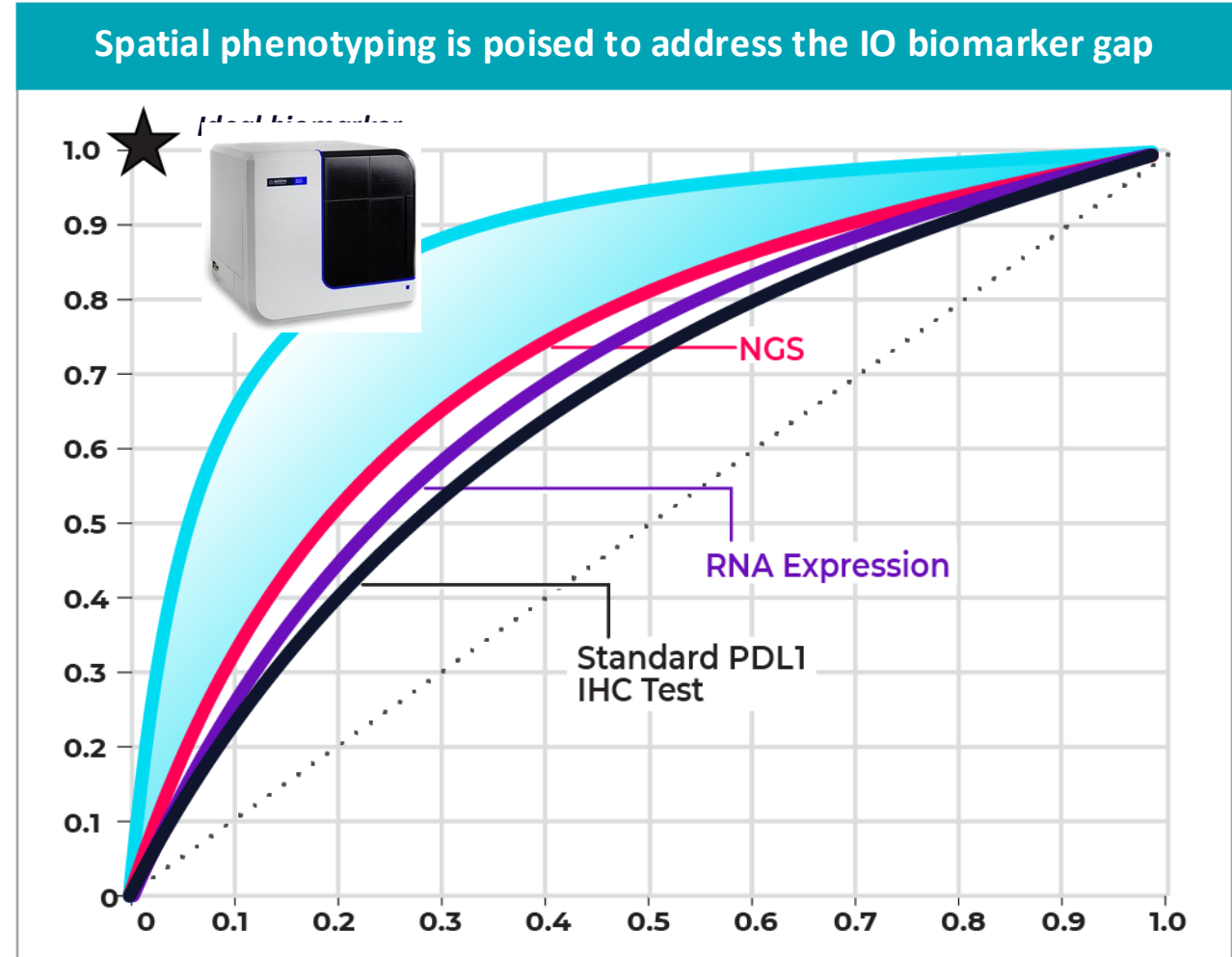
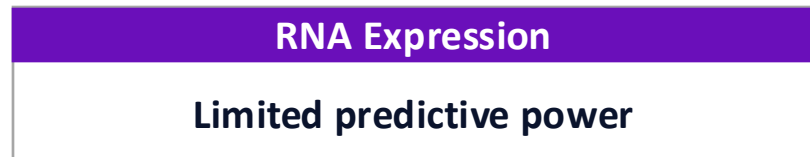
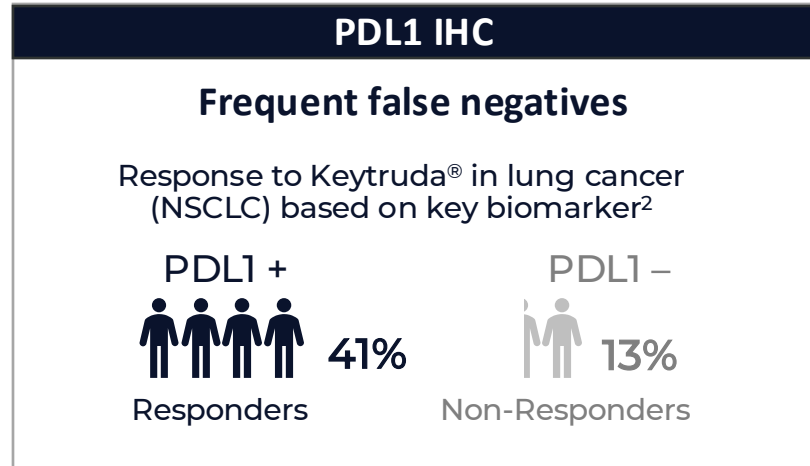


Post-treatment Biopsy Specimen  
of Subcutaneous Metastasis

Orange: Merkel  
Yellow: CD8+ T cells  
Red: CD68+ macrophages  
White: PD-1  
Green: the PD-1 ligand PD-L1  
Blue: nuclear

# Protein Spatial phenotyping을 이용한 예시

JAMA publication highlights spatial phenotyping as a superior approach<sup>1</sup>



<sup>1</sup> Lu S, Stein JE, Rimm DL, et al, JAMA Oncology 2019;5(8):1195–1204

<sup>2</sup> Diggs and Hsueh Biomarker Research (2017) 5:12



# Much more information from publications

Google Scholar

perkinelmer vectra polaris opal

Articles About 271 results (0.05 sec)

Any time  
Since 2023  
Since 2022  
Since 2019  
Custom range...

Sort by relevance

akoya vectra polaris opal

About 416 results (0.03 sec)

akoya phenoimager opal

About 17 results (0.03 sec)

51 A novel cross-site 1/PD-L1 immunohistochemistry microsatellite ...  
S Pollan, B Remeniuk, A ...  
... -1/PD-L1 cross-site at using the **Vectra Polaris**  
☆ Save Cite Relate

A robust multiplex characterization of ...  
A Viratham Pulsawatdi S

149 Complementary PhenoCode signature panels comprehensively map cell interactions and identify spatial phenotypic signatures in the tumor microenvironment  
B Remeniuk, B Hopkins, N Monteiro, D Locke - 2022 - jtc.bmj.com  
... from **Akoya's** PhenoCycler® platform integrated with the signal amplification capabilities of **Opal** chemistry from **Akoya's Phenomager®** ... Slides were imaged on a **Phenoimager** HT ...  
☆ Save Cite

160 Novel, high-plex, and flexible biomarker panels for rapid development of spatial signatures to improve stratification of response to combination therapies  
..., J Circelli, O Perez, L Liu, M McLane, YZ **Akoya** - J ..., 2022 - scholar.archive.org  
... step followed by amplified detection using **Opal** fluorescent dye technology. These panels ...  
... Multispectral imaging was performed on the **Phenomager®** platform, and image analysis ...  
☆ Save Cite All 2 versions

# 1. Multiple marker+ 매우 specific 한 cell type의 밀도측정

EBioMedicine – The Lancet Vol. 57 (2020)



F Cell phenotyping

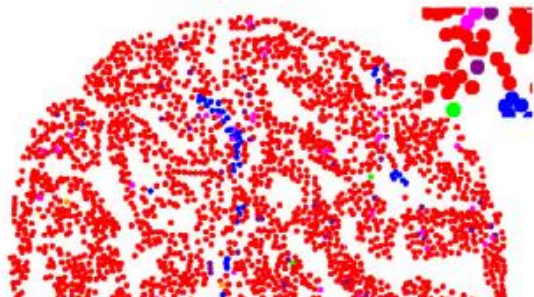


Table 2  
Correlation between intraepithelial and stromal T-cell densities and tumour budding at invasive front.

		Tumour budding <sup>a</sup>
Intraepithelial region		
CD3 <sup>+</sup> cells		$r = -0.10, P = 0.003$
CD3 <sup>+</sup> CD4 <sup>+</sup> cells		N.S.
CD3 <sup>+</sup> CD8 <sup>+</sup> cells		$r = -0.14, P < 0.001$
CD3 <sup>+</sup> CD4 <sup>+</sup> FOXP3 <sup>+</sup> cells		N.S.
CD3 <sup>+</sup> CD4 <sup>+</sup> CD45RO <sup>+</sup> cells		N.S.

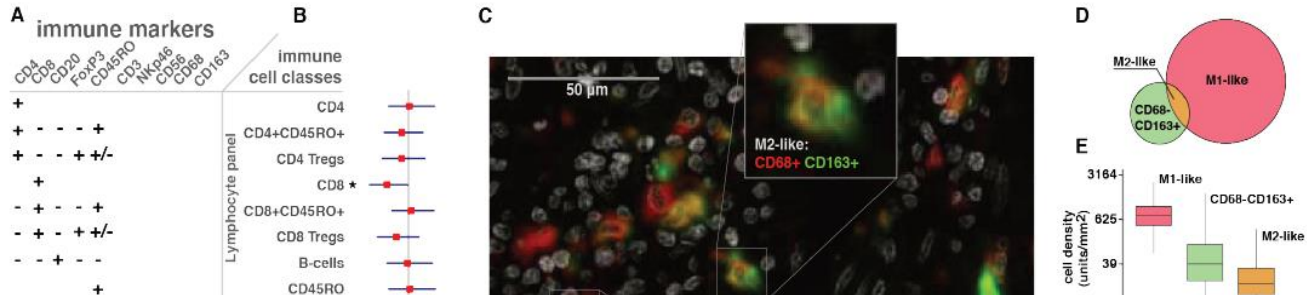
Slide ID	Tissue Category	Tissue Area (mm2)	Cell Densities (cells/mm2)					
			CD8+	CD8+/PD1+	CD8+/PD1+/FoxP3+	CD8+/PD1-/FoxP3+	CD8+/PD1+/FoxP3-	Total Cells
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	stroma	0.92	1543	291	48	108	243	6628
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	tumor	1.43	331	186	14	15	172	5937
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	Total	2.35	805	227	27	51	200	6207

4420 colorectal cancer cases and molecular pathological epidemiology

Slide ID	Tissue Category	Percentage of Total Cells					
		CD8+	CD8+/PD1+	CD8+/PD1+/FoxP3+	CD8+/PD1-/FoxP3+	CD8+/PD1+/FoxP3-	Total Cells
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	stroma	23%	4%	1%	2%	4%	100%
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	tumor	6%	3%	0%	0%	3%	100%
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	Total	13%	4%	0%	1%	3%	100%

# 2. 다른 기능을 하는 두 세포타입 간의 비율

The ratio of cytotoxic lymphocytes to M2-like macrophages is prognostic in immunogenic tumors

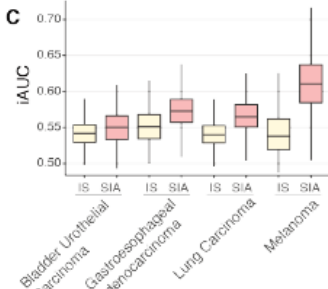
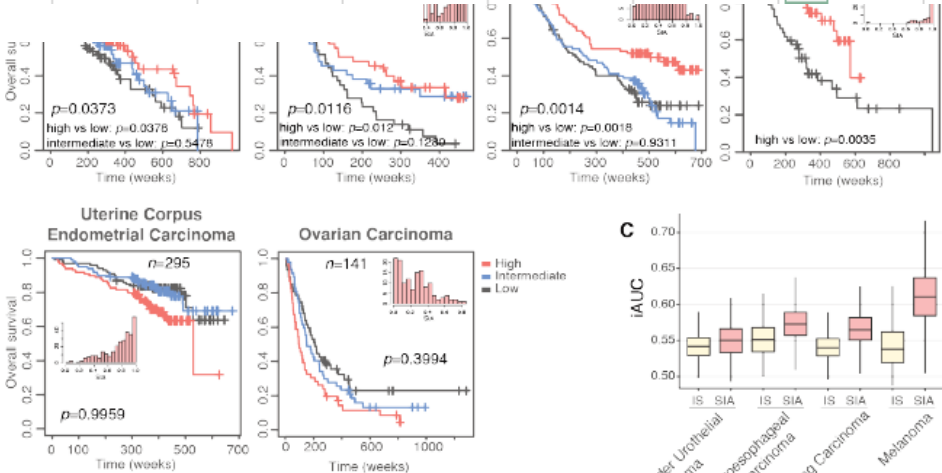


Slide ID	Tissue Category	Tissue Area (mm2)	Cell Densities (cells/mm2)									
			CD8+	PD1+	CD8+/PD1+	tumor+	FoxP3+	CD8+/FoxP3+	CD8+/PD1+/FoxP3+	CD68+	PD-L1+	Total Cells
0190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	stroma	0.92	1543	453	291	30	618	155	48	1443	1445	6628
0190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	tumor	1.43	331	269	186	5078	143	29	14	266	1318	5937
0190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	Total	2.35	805	341	227	3105	329	79	27	726	1368	6207

immune scoring system can

prognostic factor in at least five tumor types

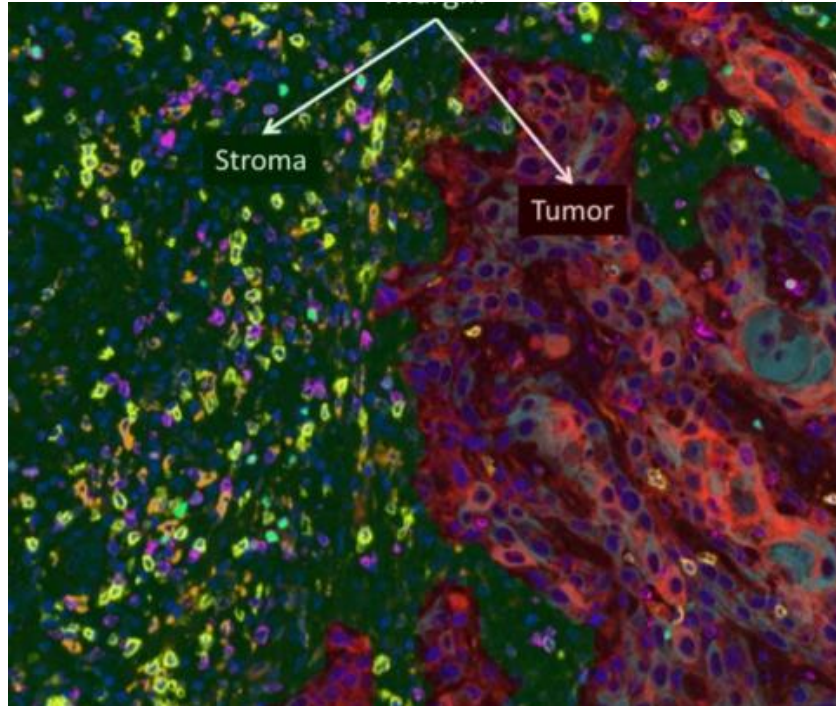
- Phenoptics contributes to identify various lymphocytes in single image and calculate immune cell ratio like SIA and IS.



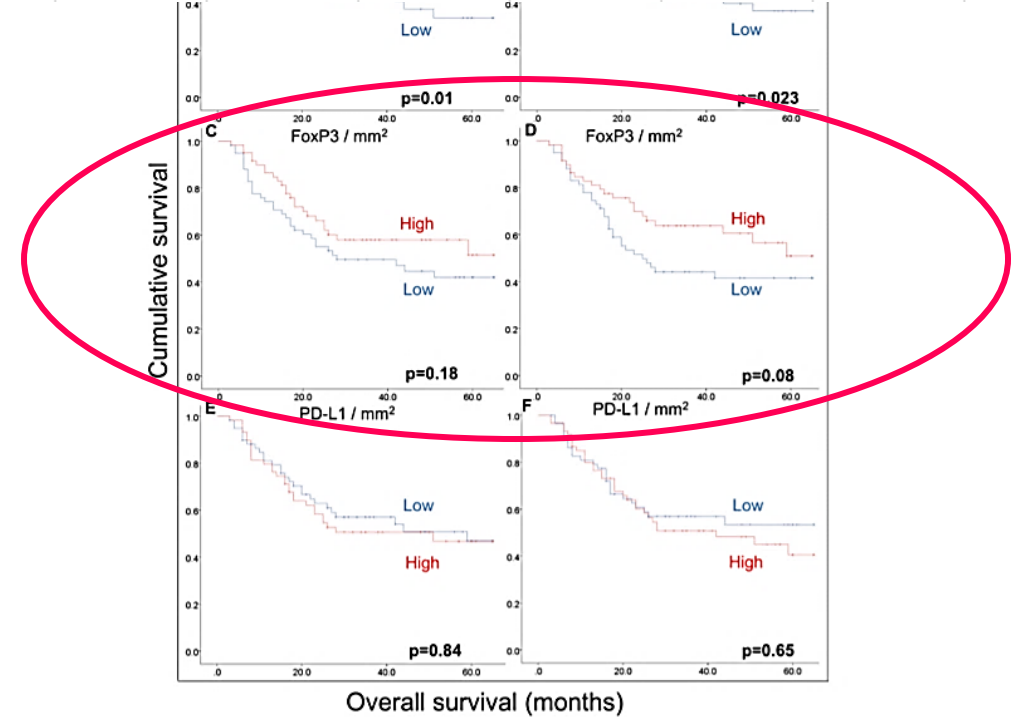


### 3. 특정 조직에서의 marker+ cell의 밀도 정량

A	B	C	D	E	F	G	H	I	J	K	L	M
Slide ID	Tissue Category	Tissue Area (mm2)	Cell Densities (cells/mm2)									
			CD8+	PD1+	CD8+/PD1+	tumor+	FoxP3+	CD8+/FoxP3 +	CD8+/PD1-	PD1+/FoxP3 +	CD68+	Total Cells
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	stroma	0.92	1543	453	291	30	618	155	1252	86	1443	6628
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	tumor	1.43	331	269	186	5078	143	29	145	27	266	5937
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	Total	2.35	805	341	227	3105	329	79	578	50	726	6207



PD-L1  
 FoxP3  
 CD8  
 CD3  
 CD163  
 Cytokeratin  
 DAPI



## 4. Marker A+ 반경 30um 이내의 marker B/C +세포의 수

CD8+ T cell 반경 30 um 이내의 FoxP3+ cell의 수와 PD-L1+cell의 수

Slide ID	Tissue Category	Count of cells within the specified radius						
		From	To	Radius	From count	To count	From with	Within mean
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	stroma	CD8+	CD8+	30	1420	1420	1368	8.929577465
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	tumor	CD8+	CD8+	30	475	475	355	1.743157895
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	All	CD8+	CD8+	30	1895	1895	1743	7.516622691
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	stroma	CD8+	PD1+	30	1420	417	1054	2.401408451
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	tumor	CD8+	PD1+	30	475	386	280	1.147368421
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	All	CD8+	PD1+	30	1895	803	1391	2.265963061
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	stroma	CD8+	CD8+/PD1+	30	1420	268	921	1.721126761
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	tumor	CD8+	CD8+/PD1+	30	475	267	249	0.928421053
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	All	CD8+	CD8+/PD1+	30	1895	535	1223	1.627440633
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	stroma	CD8+	tumor+	30	1420	28	120	0.121830986
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	tumor	CD8+	tumor+	30	475	7283	467	12.32210526
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	All	CD8+	tumor+	30	1895	7311	834	3.627440633
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	stroma	CD8+	FoxP3+	30	1420	569	1066	1.964084507
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	tumor	CD8+	FoxP3+	30	475	205	143	0.454736842
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	All	CD8+	FoxP3+	30	1895	774	1274	1.765171504
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	stroma	CD8+	CD8+/FoxP3+	30	1420	143	685	0.853521127
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	tumor	CD8+	CD8+/FoxP3+	30	475	42	59	0.136842105
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	All	CD8+	CD8+/FoxP3+	30	1895	185	800	0.74353562
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	stroma	CD8+	CD8+/PD1+/FoxP3+	30	1420	44	292	0.282394366
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	tumor	CD8+	CD8+/PD1+/FoxP3+	30	475	20	27	0.065263158
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	All	CD8+	CD8+/PD1+/FoxP3+	30	1895	64	361	0.257519789
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	stroma	CD8+	CD68+	30	1420	1328	1237	7.098591549
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	tumor	CD8+	CD68+	30	475	381	178	1.035789474
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	All	CD8+	CD68+	30	1895	1709	1441	6.036939314
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	stroma	CD8+	PD-L1+	30	1420	1330	1285	4.73943662
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	tumor	CD8+	PD-L1+	30	475	1890	395	3.305263158
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	All	CD8+	PD-L1+	30	1895	3220	1724	5.022691293
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	stroma	CD8+	Total Cells	30	1420	6141	1420	19.82957746
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	tumor	CD8+	Total Cells	30	475	8570	475	14.88421053
20190904-B-6-plex-DF-LuCa44483_Scan1_seriesnum_162	All	CD8+	Total Cells	30	1895	14711	1895	19.9979919

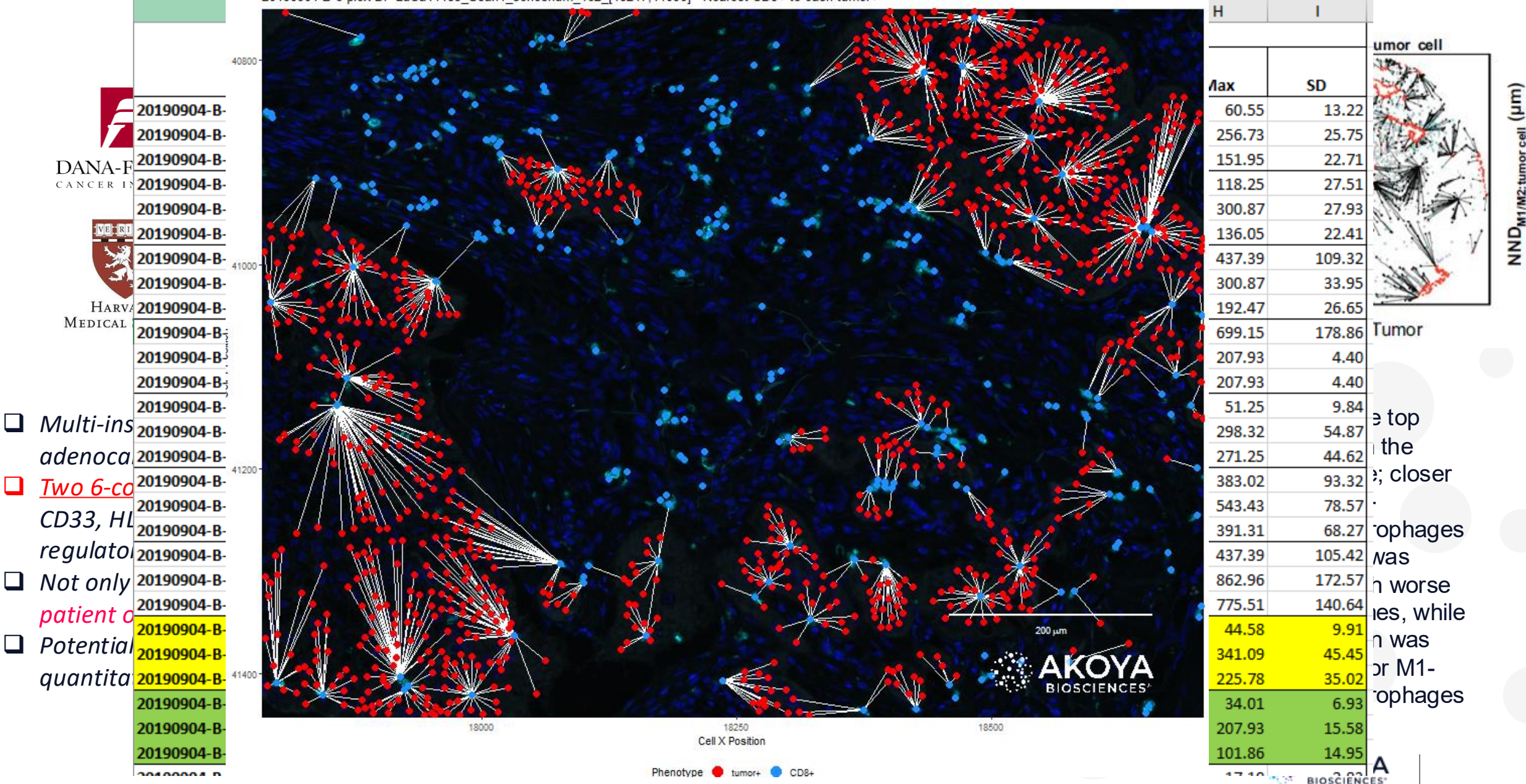
CD8<sup>+</sup> T cells and PD-L1<sup>+</sup> cells outcome in HPV- OSCC

CD8n	Suppression Index (SI)	Overall Survival (OS)
	High	Low
	Intermediate	Med
	Intermediate	Med
	Low	High

nts in the top 50% or lower 50% for their cohort



5. Tumor로부터 특정 세포까지의 적대 거리 전략으로 계급화



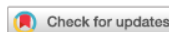


# 6. Spatial Score: 세포간 상대적 거리 정량

ARTICLE

<https://doi.org/10.1038/s41467-021-26974-6>

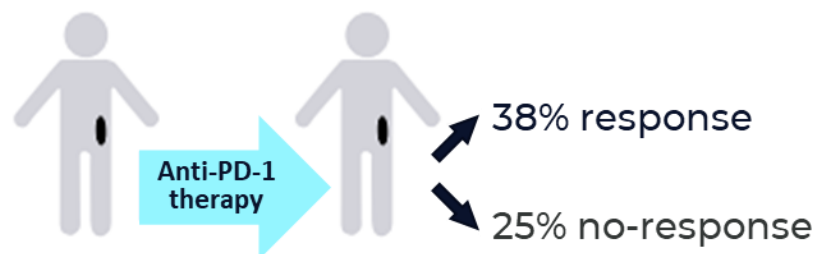
OPEN



## Immune cell topography predicts response to PD-1 blockade in cutaneous T cell lymphoma

Darci Phillips<sup>1,2,3,9</sup>, Magdalena Matusiak<sup>3,9</sup>, Belén Rivero Gutierrez<sup>3</sup>, Salil S. Bhate<sup>1,3,4</sup>, Graham L. Barlow<sup>1,3</sup>, Sizun Jiang<sup>1,3,5</sup>, Janos Demeter<sup>1</sup>, Kimberly S. Smythe<sup>6</sup>, Robert H. Pierce<sup>6</sup>, Steven P. Fling<sup>6</sup>, Nirasha Ramchurren<sup>6</sup>, Martin A. Cheever<sup>6</sup>, Yuri Goltsev<sup>1,3</sup>, Robert B. West<sup>3</sup>, Michael S. Khodadoust<sup>7,10</sup>, Youn H. Kim<sup>2,7,10</sup>, Christian M. Schürch<sup>1,3,8,10</sup> & Garry P. Nolan<sup>1,3,10</sup>

pembrolizumab clinical trial (NCT02243579)



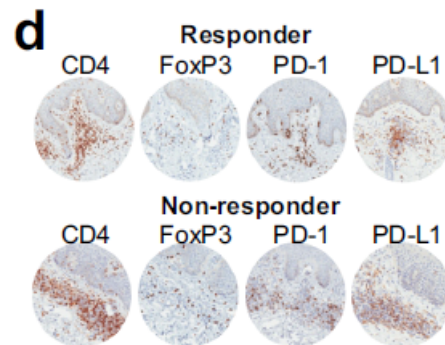
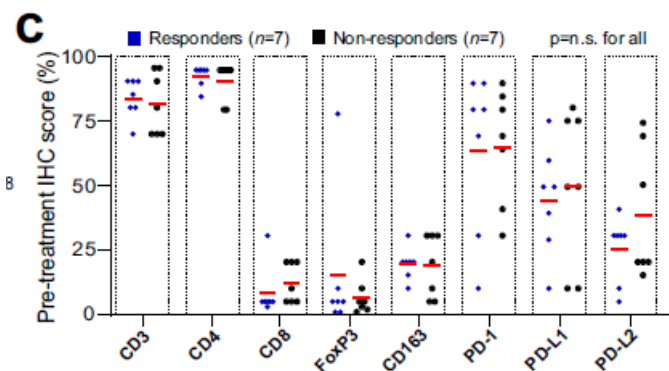
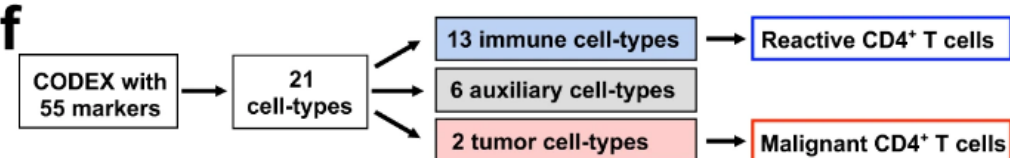
## 56-plex IO Panel

e

Tumor & Immune		Functional	Stromal
<i>T cell &amp; tumor cell</i>	<i>Macrophages</i>	<i>Proliferation &amp; activation</i>	<i>Epithelia</i>
	CD11b	Granzyme B	Cytokeratin
	CD2	ICOS	MUC-1
	CD3	Ki-67	<i>Blood vessels</i>
	CD4	MMP-9	CD31
<i>NK cells</i>	CD5	<i>Checkpoint &amp; inhibition</i>	CD34
	CD7		<i>Lymphatics</i>
	CD8		
	CD25		<i>Extracellular matrix</i>
	CD30		
<i>B &amp; plasma cells</i>	CD69	LAG-3	<i>Cytoplasm</i>
	CD162	PD-1	
	CD164	PD-L1	<i>Nuclei</i>
	CD194	VISTA	
	FoxP3	<i>Multifunctional</i>	<i>Cytoplasm</i>
<i>Granulocytes</i>	GATA3		
	MMP-12		
	T-bet		
	p53		
<i>Lymphocytes</i>	<i>Dendritic cells</i>	β-catenin	<i>Nuclei</i>
		BCL-2	
		CD71	
		EGFR	
		HLA-DR	
<i>Dendritic cells</i>	<i>Dendritic cells</i>	IDO-1	
<i>Dendritic cells</i>	<i>Dendritic cells</i>		

## Identification of 21 cell types by clustering

f



# 6. Spatial Score: 세포간 상대적 거리 정량

Spatial Signature stratifies responders vs. non-responders prior to treatment

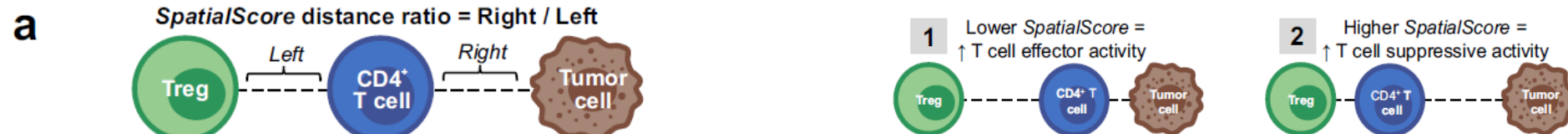
ARTICLE

<https://doi.org/10.1038/s41467-021-26974-6> OPEN

Immune cell topography predicts response to PD-1 blockade in cutaneous T cell lymphoma

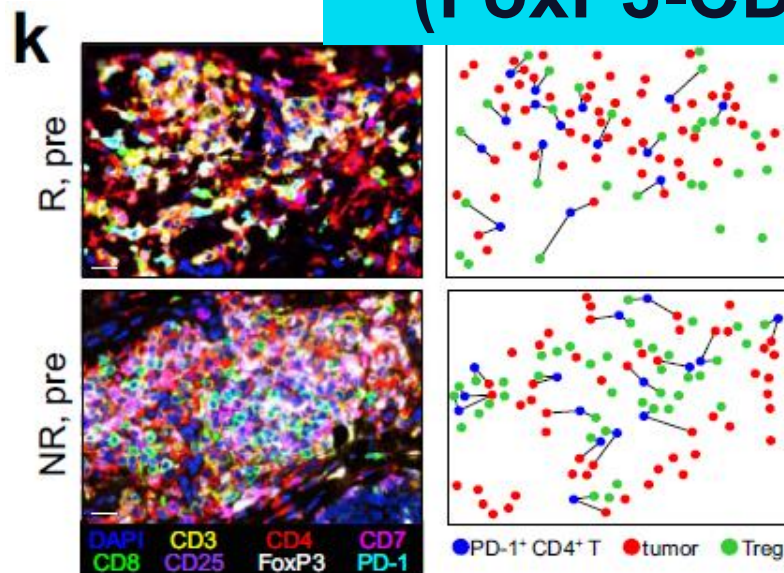
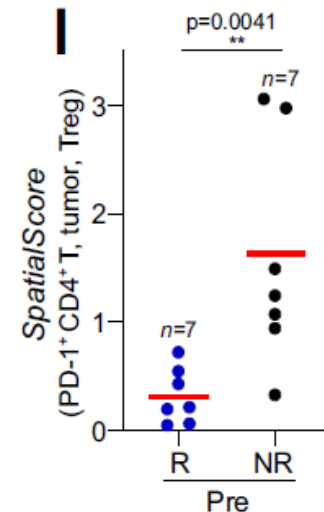
Darci Phillips<sup>12,3,9</sup>, Magdalena Matusiak<sup>3,9</sup>, Belén Rivero Gutierrez<sup>3</sup>, Salil S. Bhate<sup>13,4</sup>, Graham L. Barlow<sup>1,3</sup>, Sizun Jiang<sup>1,3,5</sup>, Janos Demeter<sup>1</sup>, Kimberly S. Smythe<sup>6</sup>, Robert H. Pierce<sup>6</sup>, Steven P. Fling<sup>6</sup>, Nirasha Ramchurren<sup>6</sup>, Martin A. Cheever<sup>6</sup>, Yury Goltsev<sup>1,3</sup>, Robert B. West<sup>3</sup>, Michael S. Khodadoust<sup>7,10</sup>, Youn H. Kim<sup>2,7,10</sup>, Christian M. Schürch<sup>1,3,8,10</sup> & Garry P. Nolan<sup>1,3,10</sup>

## SpatialScore



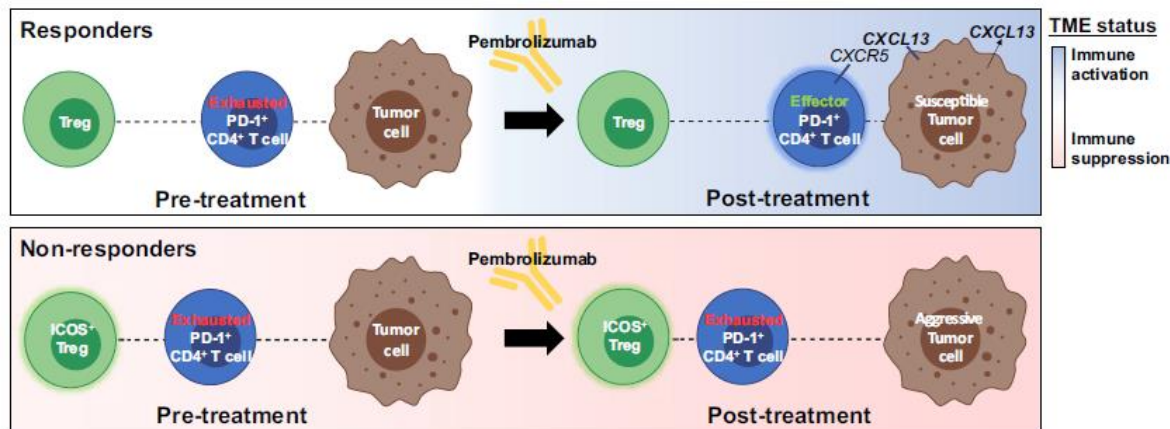
SpatialScore = T  
CD3  
FoxP3

$$\text{Spatial Score} = \frac{(\text{FoxP3-CD8})_{\text{um}}}{(\text{CD8-tumor})_{\text{um}}}$$



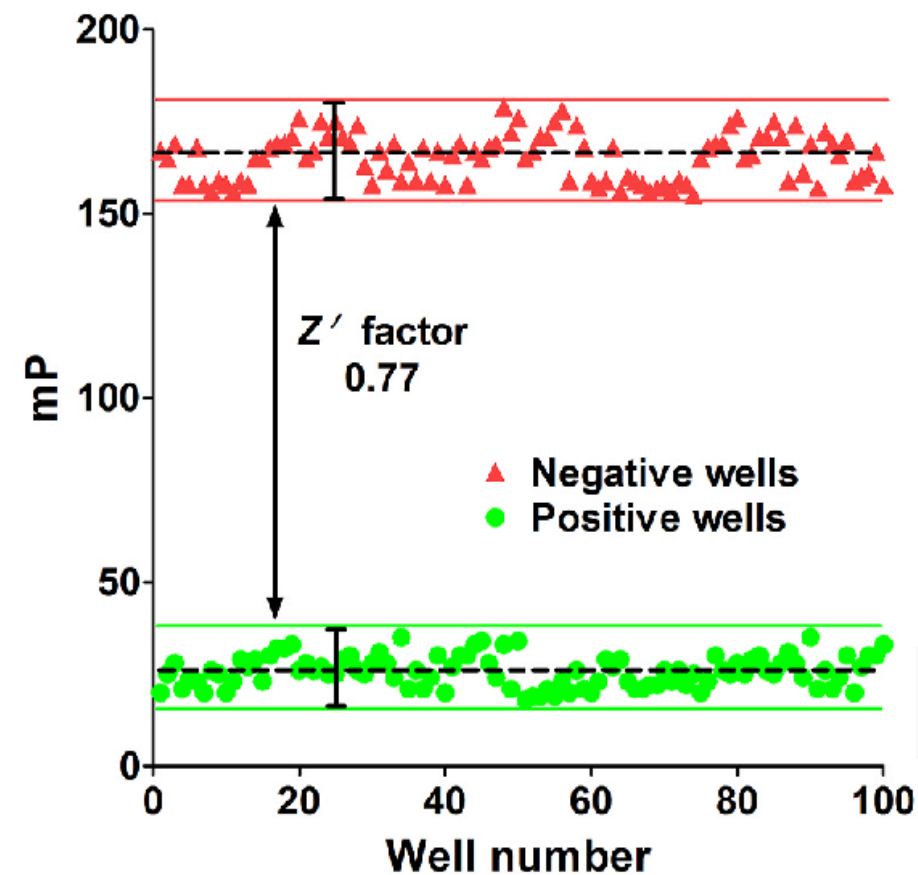
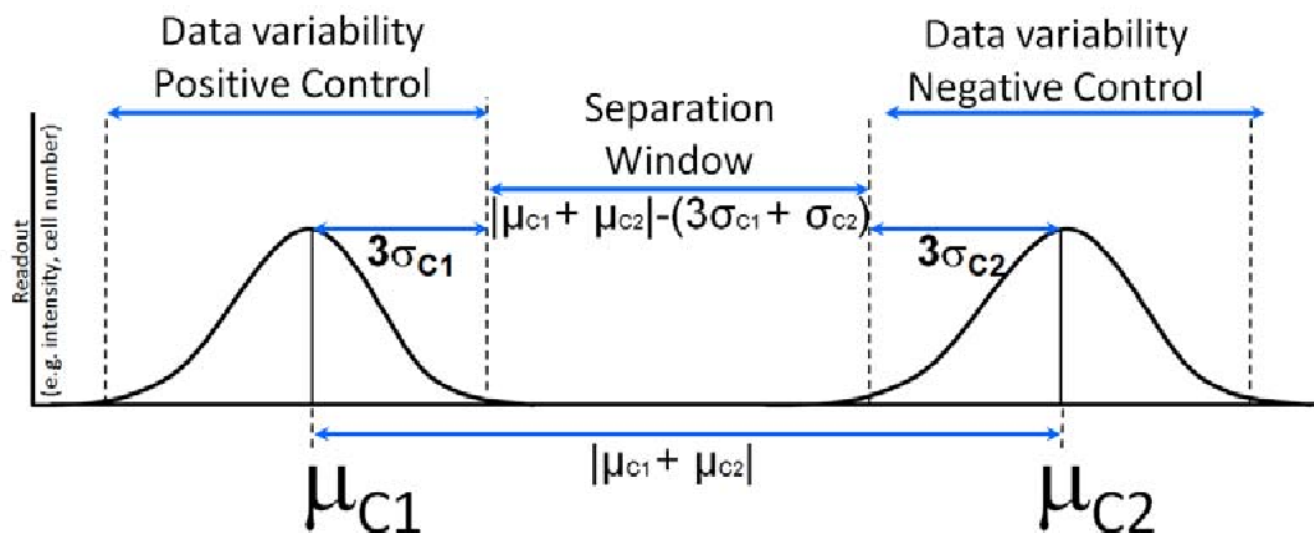
Representative image about SpatialScore

## Prediction of the effect of Immunotherapy



# 어떤 parameter (signature)를 선택할까?

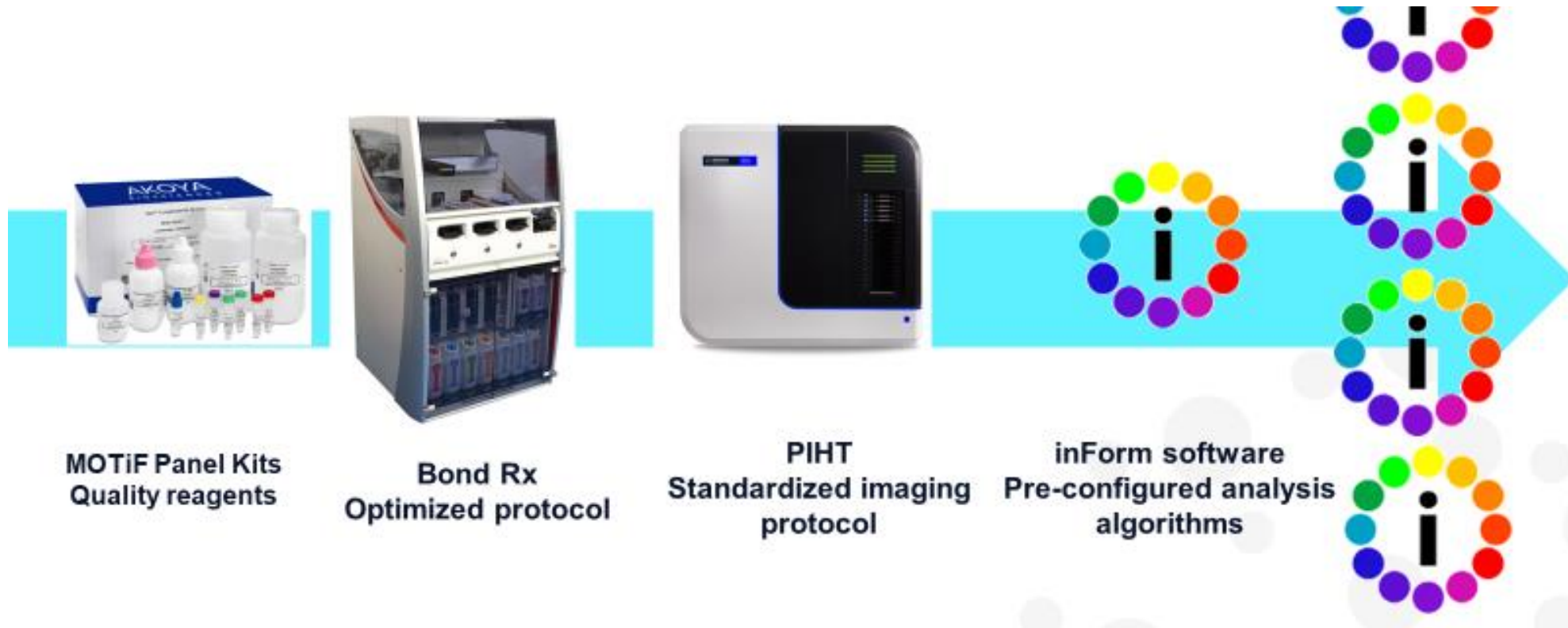
Z factor





# PIHT + inForm + PhenoptR, phenoptrReport

- Spatial analysis에 필요한 모든 tool을 제공
- Tip: **inForm**에서 정확한 tissue segmentation, cell segmentation, 마커별 phenotyping을 정확히 한다.



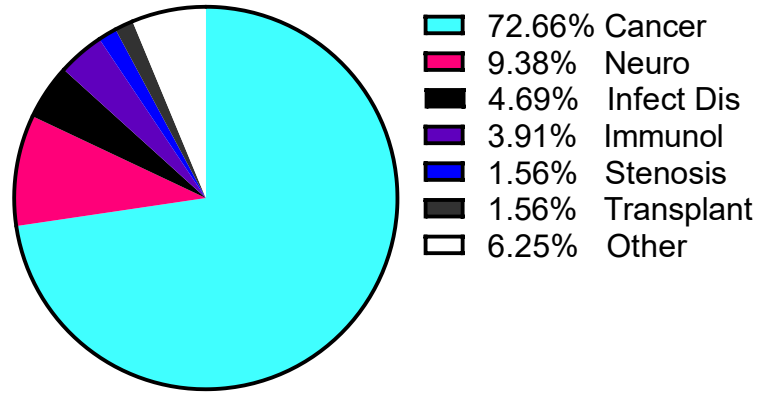
그래서....

## 다중형광이미징의 마커는 무엇을 선정해야 하는지?

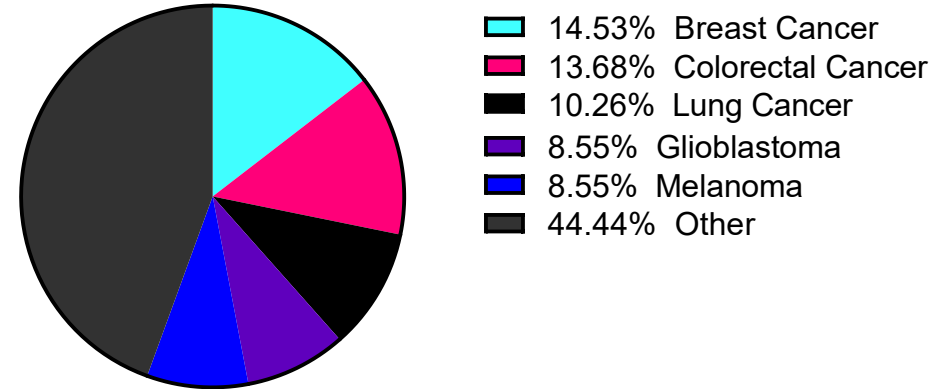
- 다른 기술방법 (NGS, FACS etc. ) 과 연계
- 참고문헌 연구

# 사용자 분석

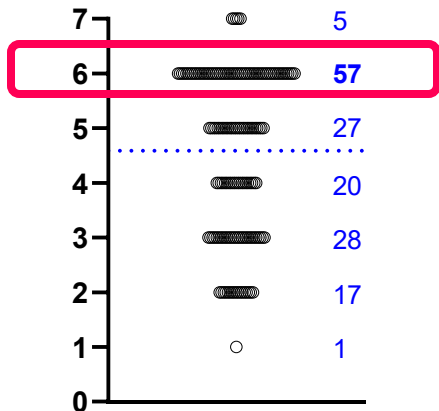
User Discipline



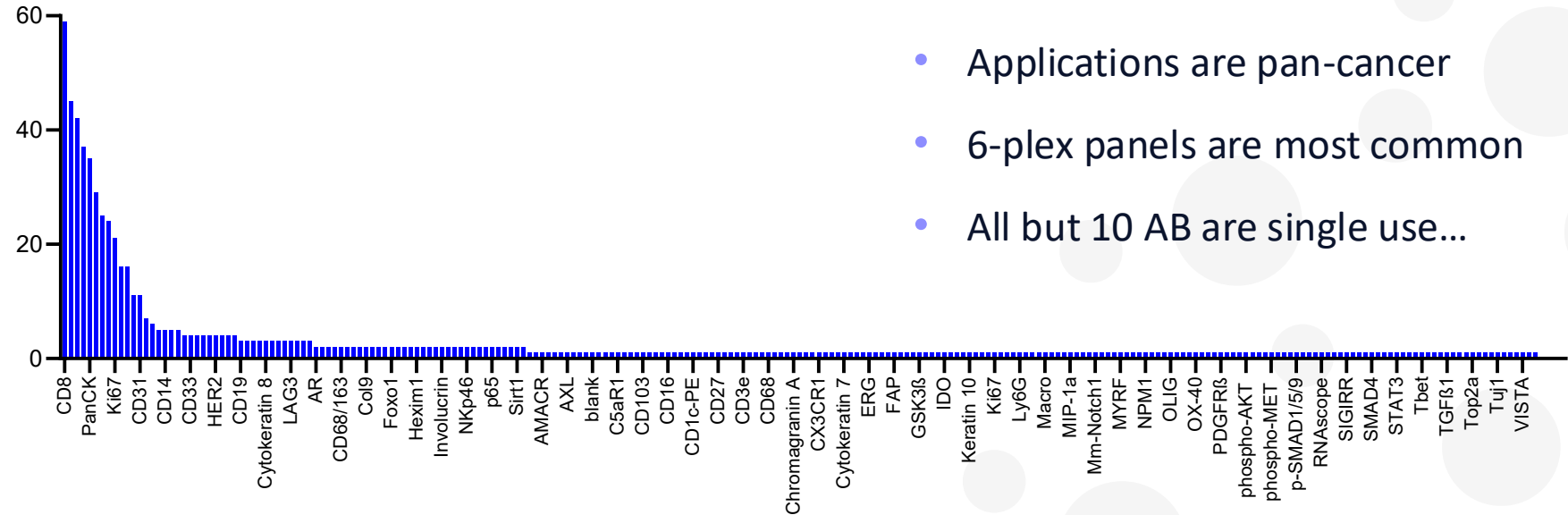
Pan-Cancer Focus



Average Panel Size



Few Frequent-use Antibodies



- Cancer research dominates user base
- Applications are pan-cancer
- 6-plex panels are most common
- All but 10 AB are single use...



# 10+ Ab이 사용되는 대표적인 5가지 질문

Comprehensively phenotype the tumor microenvironment for better stratification

Is the tumor “hot” or “cold”?

Where are the immune cells located in the TME?

## Immuno- contexture Panel

CD8, CD68,  
PD-L1,  
FoxP3,  
PanCK

## Immune Profile

CD8,  
CD68,  
CD3,  
CD20,  
PanCK

## Activated TIL Status

CD8, CD3, Ki67, Grz B,  
PanCK

Are they activated?  
Is the tumor proliferating?

## Exhaustion

CD8, CD4, CD20, FoxP3,  
PD-1

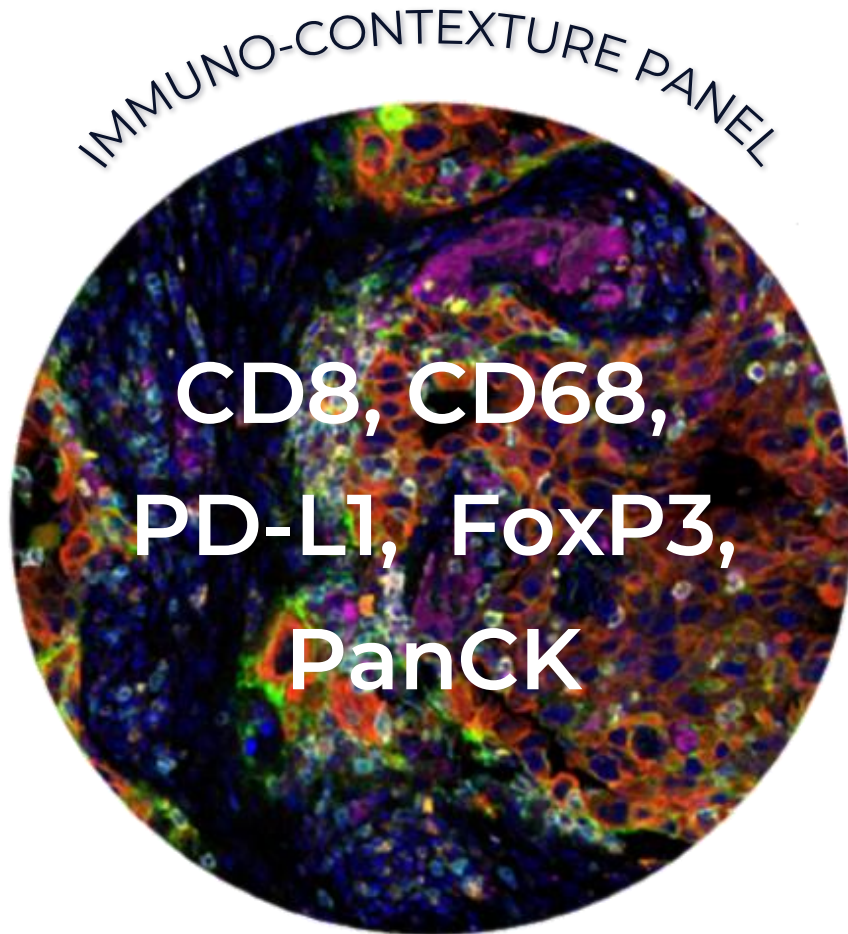
Where are the Tregs?  
Are the T cell exhausted?

## M1/M2 Polarization

CD8, CD68, PD-L1, PD-1,  
CD163

Are there TAMs?  
Are they M1 or M2?

# Immune contexture + 1 marker



CD4

Where are the Helper T cells?

PD-1

Are the T cells exhausted?

Ki67

Which cell types are proliferating?

CD20

Where are the B cells?

GrzB

Where are the activated immune cells?

?

Marker of choice for specific research question

# Much more information from publications

Google Scholar

perkinelmer vectra polaris opal

Articles About 271 results (0.05 sec)

Any time  
Since 2023  
Since 2022  
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akoya phenoimager opal

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51 A novel cross-site 1/PD-L1 immunohistochemistry microsatellite ...  
S Pollan, B Remeniuk, A ... -1/PD-L1 cross-site at using the **Vectra Polaris**  
☆ Save Cite Relate

A robust multiplex characterization of ...  
A Viratham Pulsawatdi S

149 Complementary PhenoCode signature panels comprehensively map cell interactions and identify spatial phenotypic signatures in the tumor microenvironment  
B Remeniuk, B Hopkins, N Monteiro, D Locke - 2022 - jtc.bmj.com  
... from **Akoya's** PhenoCycler® platform integrated with the signal amplification capabilities of **Opal** chemistry from **Akoya's Phenolmager®** ... Slides were imaged on a **Phenoimager** HT ...  
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160 Novel, high-plex, and flexible biomarker panels for rapid development of spatial signatures to improve stratification of response to combination therapies  
..., J Circelli, O Perez, L Liu, M McLane, YZ **Akoya** - J ..., 2022 - scholar.archive.org  
... step followed by amplified detection using **Opal** fluorescent dye technology. These panels ...  
... Multispectral imaging was performed on the **Phenolmager®** platform, and image analysis ...  
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