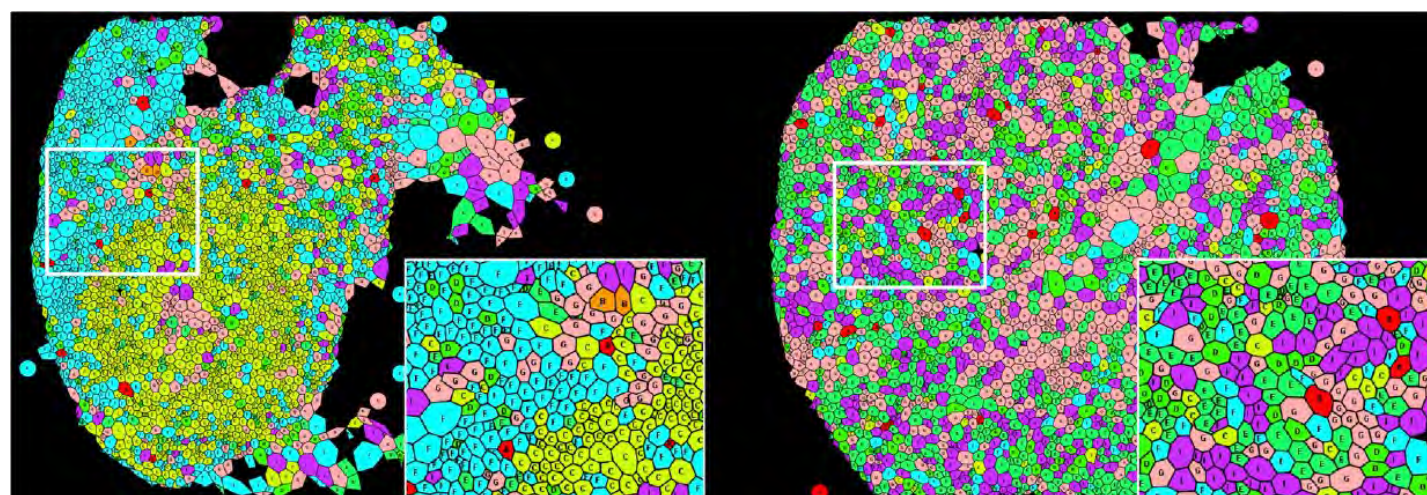
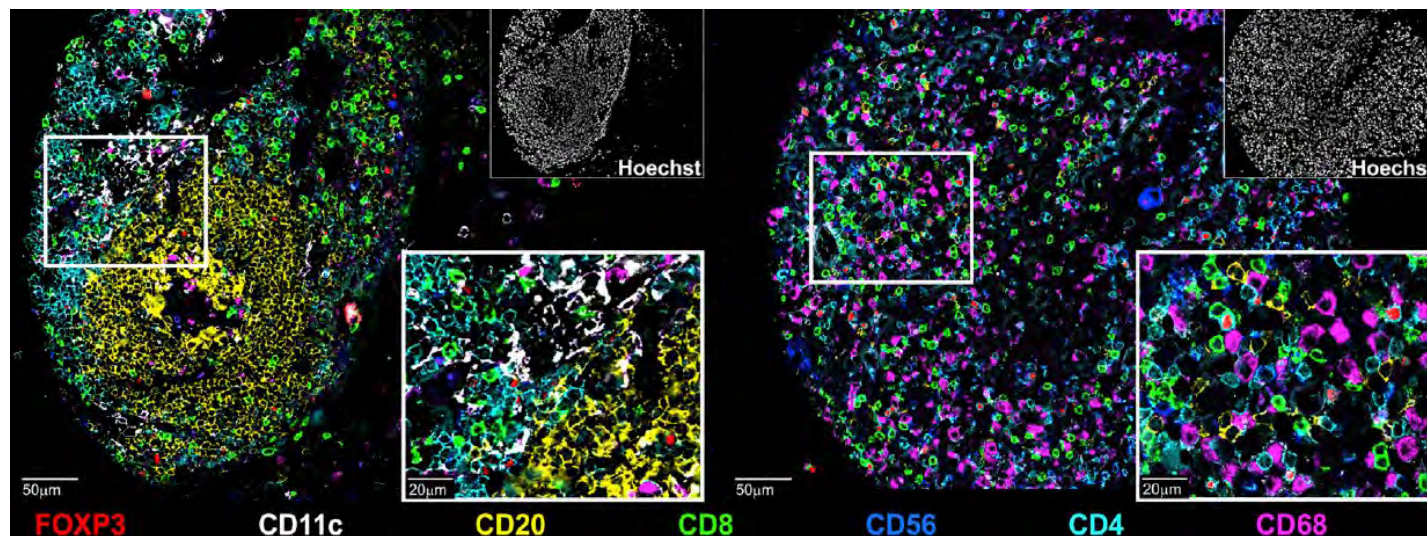


組織微小環境における空間的シングルセル解析の最新技術 co-detection by indexing (CODEX) 技術を用いた高度な(~60) 多重免疫蛍光染色による ”切片を用いたシングルセル解析”



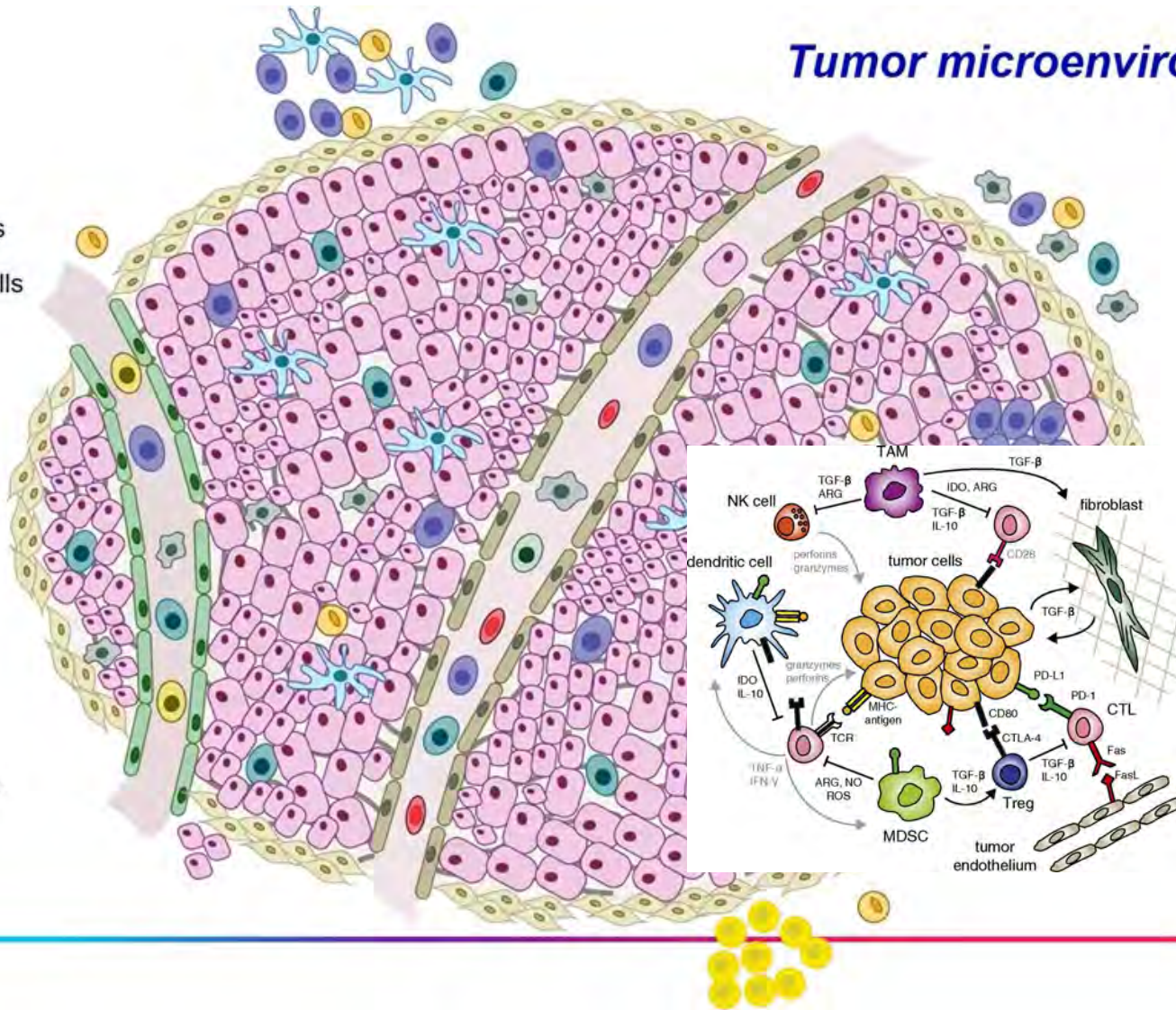
バイオストリーム株式会社 細野直哉

本日の内容

- 空間的シングルセル解析の重要性
- CODEX技術の原理とワークフロー
- CODEX技術をベースにしたPhenoCyclerシステムの特徴
- 空間的シングルセルデータ解析
- 空間的シングルセル解析例
- 空間的マルチオミックス解析に向けて

空間的シングルセル解析の重要性

- Malignant cells
- ECM
- CAFs
- Vascular endothelial cells
- Lymphatic endothelial cells
- Helper T cells
- Killer T cells
- B cells
- NK cells
- DC cells
- Macrophages
- Red blood cells
- Adipocytes
- Mesenchymal stem cells



Tumor microenvironment

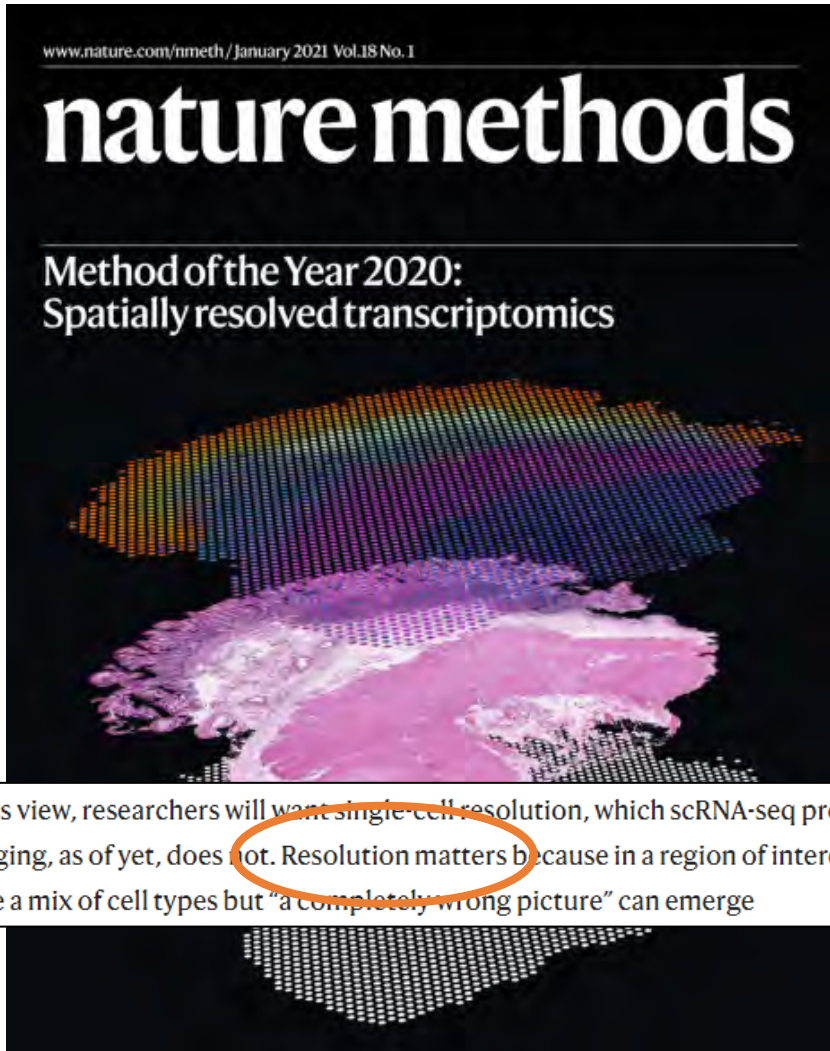
組織は個々の細胞の相互作用で成り立っており、サイトメトリーやscRNAseqに代表される細胞サスペンションの解析ではその情報が失われてしまう。

がんや様々な組織の微小環境の解析には空間情報（位置情報）を保持した切片でのシングルセル解析が重要。

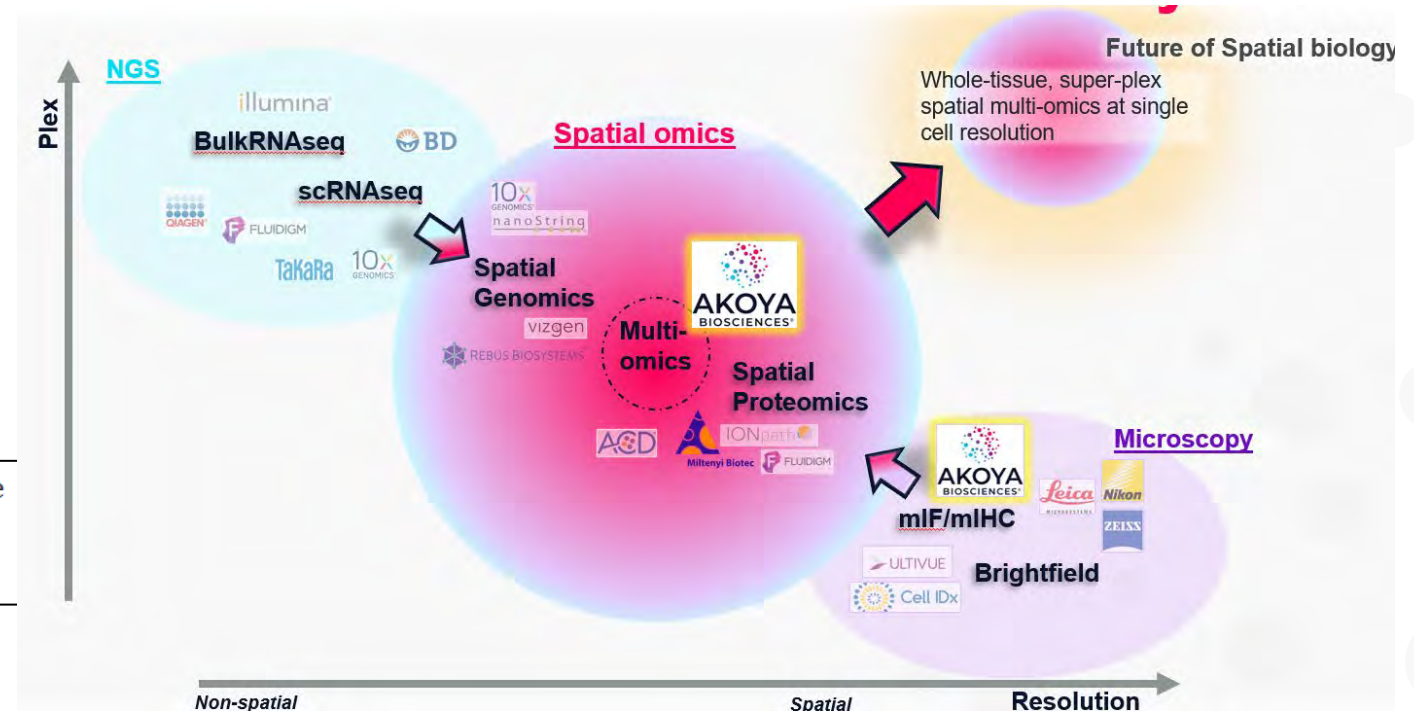
しかし、現状の数マーカー免疫染色では、微小環境に存在する個々の細胞のセルタイプやその状態を把握することはできない。

細胞表面マーカーや機能マーカーも含め包括的に解析できる高度な（～100）多重免疫染色の解析システムが必要。

Spatial Omics is Hot, Rapidly emerged field



Spatial Whole Transcriptome解析は微小環境解析の有力なディスカバリーツールとして普及しつつあるが、解像度と検出力が問題（55um）。高解像度で検出力の高いSpatial single cell Proteomicsでのvalidationやデータ統合によるマルチオミックス解析がトレンドになりつつある。
Spatial Whole Transcriptome自体の高解像度化も進みつつある。



In his view, researchers will want single-cell resolution, which scRNA-seq provides but tissue imaging, as of yet, does not. Resolution matters because in a region of interest tissues can have a mix of cell types but "a completely wrong picture" can emerge

CODEX is the leading Spatial Phenotyping solution for Global Cell Atlas Efforts

Global program to create cell atlas

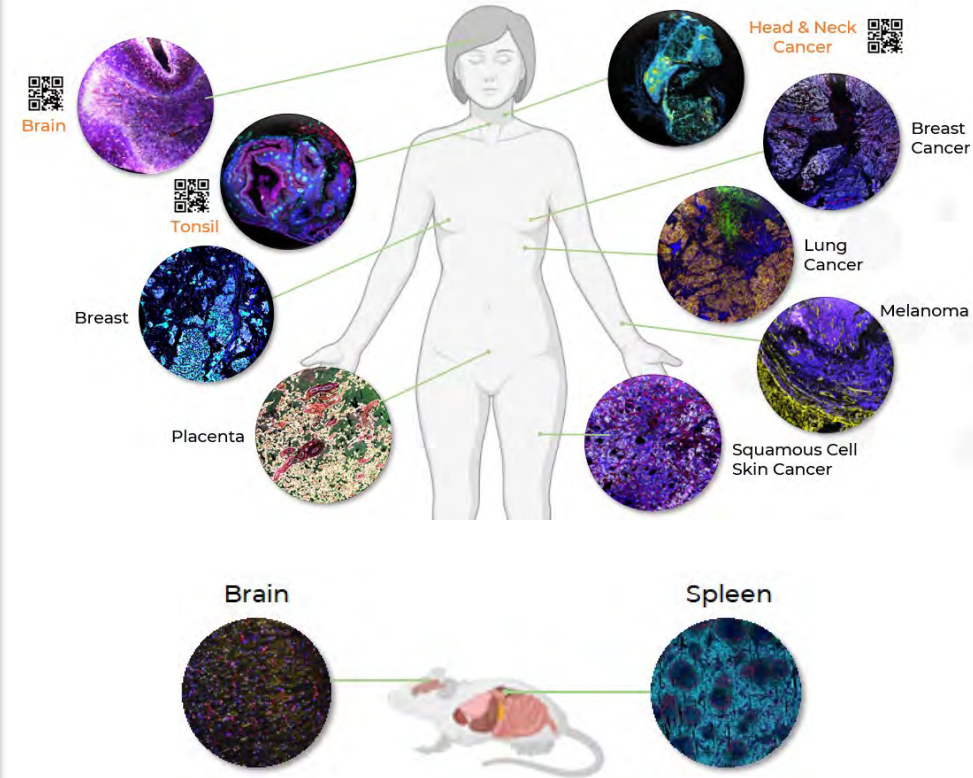


Top tier institute participation



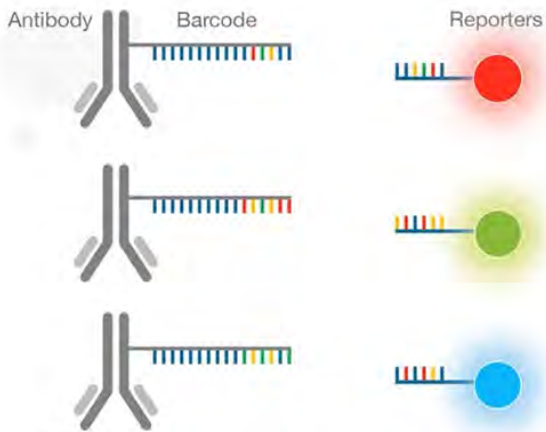
Pacific Northwest
NATIONAL LABORATORY

UC San Diego



Nearly 100 datasets contributed to public domain
Over 50 publications

co-detection by indexing (CODEX)の原理とワークフロー



- Unique Barcode on every antibody
- Complementary oligo attached to each dye enables highly specific detection of Reporters to Barcodes
- Spectrally distinct dyes avoid signal overlap

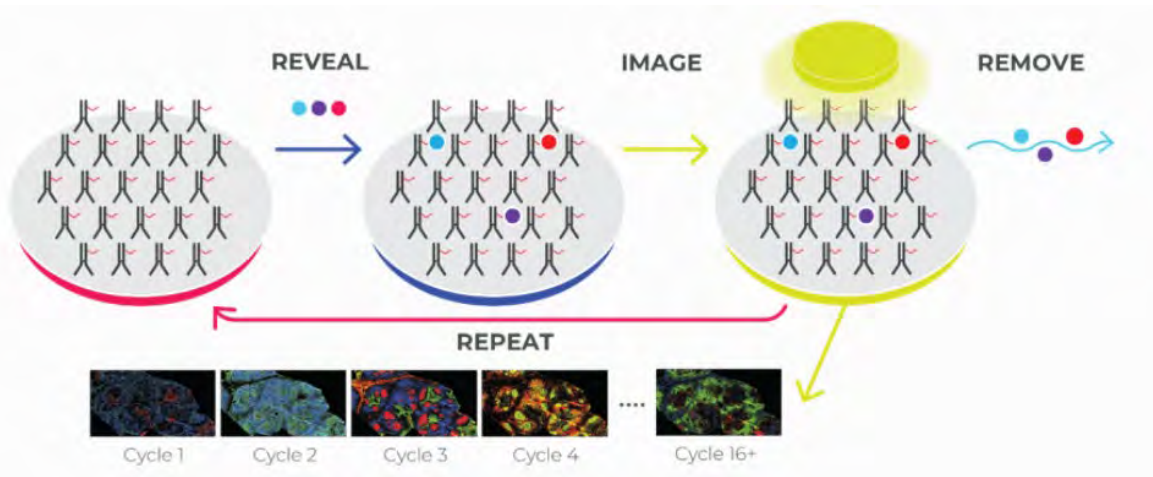
フィルターセット

Filter	Excitation (nm)	Emission (nm)	凍結切片	FFPE
DAPI Filter – Nuclear Stain	358	461	✓	✓
AF488/FITC/GFP	492	517	✓	✗
Atto550/TRITC	560	575	✓	✓
Cy5	648	668	✓	✓
AF750 (Cy7)	753	775	✗	✓

CODEX ANTIBODY PANEL



TISSUE STAIN



- 抗体にリンクしているDNAバーコードと蛍光プローブのハイブリによりタンパク質を検出
- ハイブリと変性（室温）を繰り返し、3色の蛍光をサイクルで検出することで高度な多重染色を実現（3色X20サイクルで60ターゲットなど。35サイクル以上安定）
- 切片の再ランも可能
- バックグラウンド減算で自家蛍光の影響を最小化（PhotoBleachingで自家蛍光を実験的に減少させるプロトコールも公開）
- 抗体はバーコード付きでready-to-useの108種類とカスタムコンジュゲーションが可能（400種類以上のバリデート済み抗体）

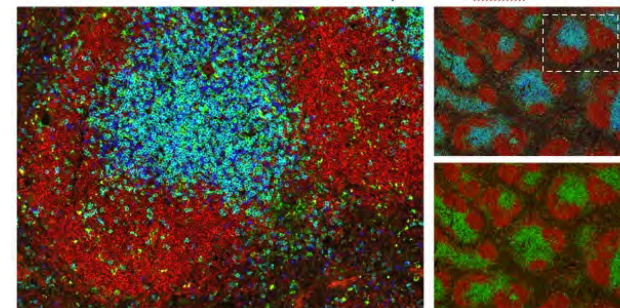
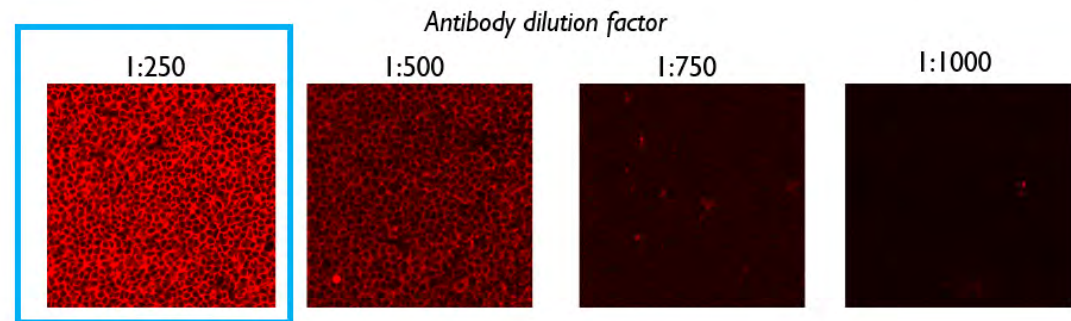
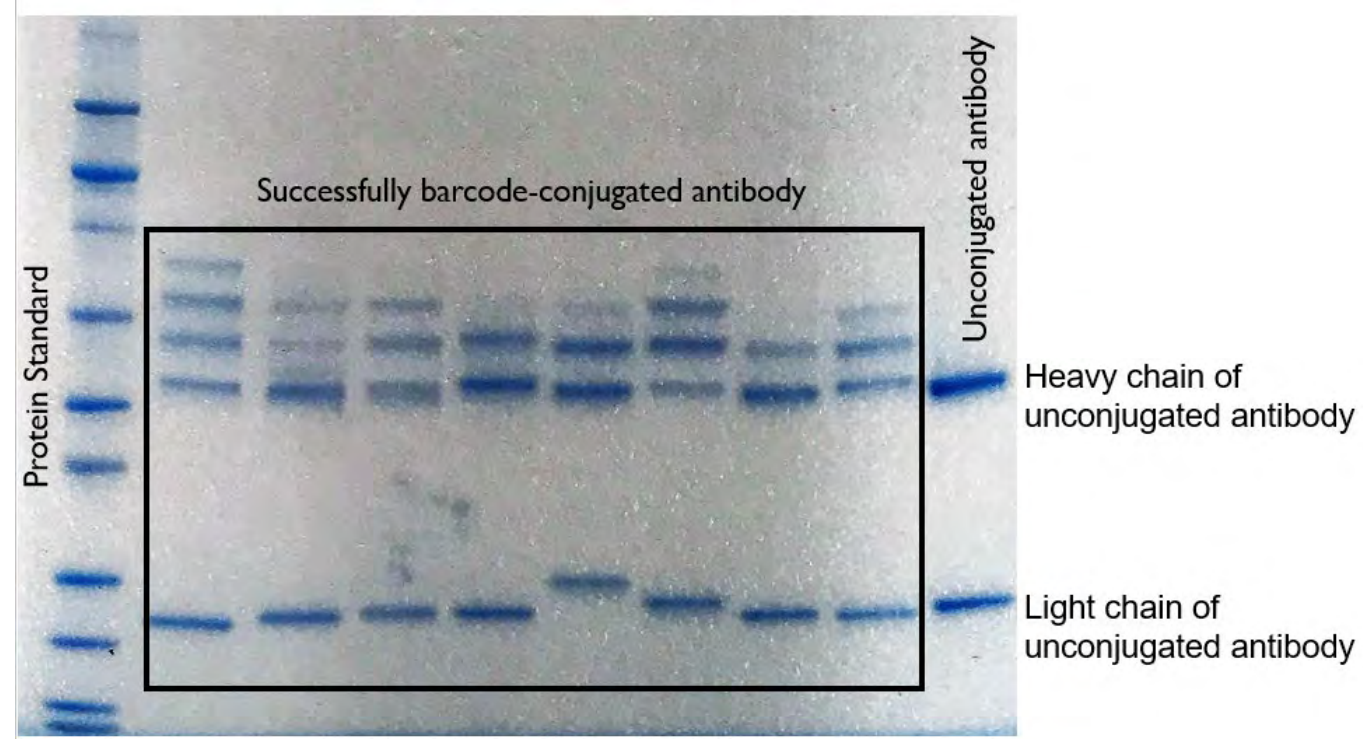
PhenoCycler抗体リスト (バーコードコンジュゲート済み-108)

ヒトFFPE用		ヒトFF用	マウスFF用
Anti-Hu ATM(AKYP0122)-BX083	Anti-Hu ER(AKYP0105)-BX084	Anti-Hu CD2(AKYP0009)-BX002	Anti-Mu CD3(AKYP0035)-BX021
Anti-Hu b-Catenin1(AKYP0068)-BX020	Anti-Hu FOXP3(AKYP0102)-BX031	Anti-Hu CD3(AKYP0027)-BX015	Anti-Mu CD4(AKYP0041)-BX026
Anti-Hu Bcl-2(AKYP0120)-BX085	Anti-Hu GATA3(AKYP0116)-BX049	Anti-Hu CD4(AKYP0034)-BX021	Anti-Mu CD5(AKYP0016)-BX017
Anti-Hu Beta-actin(AKYP0072)-BX010	Anti-Hu Granzyme B(AKYP0086)-BX041	Anti-Hu CD8(AKYP0012)-BX004	Anti-Mu CD8a(AKYP0044)-BX029
Anti-Hu Caveolin(AKYP0115)-BX086	Anti-Hu Histone H3 Pho(AKYP0060)-BX030	Anti-Hu CD11c(AKYP0042)-BX027	Anti-Mu CD11b(AKYP0040)-BX025
Anti-Hu CD3e(AKYP0062)-BX045	Anti-Hu HLA-A(AKYP0078)-BX004	Anti-Hu CD19(AKYP0010)-BX003	Anti-Mu CD11c(AKYP0045)-BX030
Anti-Hu CD4(AKYP0048)-BX003	Anti-Hu HLA-DR(AKYP0063)-BX033	Anti-Hu CD21(AKYP0022)-BX013	Anti-Mu CD19(AKYP0033)-BX020
Anti-Hu CD8(AKYP0028)-BX026	Anti-Hu HLA-E(AKYP0096)-BX034	Anti-Hu CD31(AKYP0029)-BX032	Anti-Mu CD21/35(AKYP0037)-BX023
Anti-Hu CD11c(AKYP0051)-BX024	Anti-Hu ICOS(AKYP0090)-BX054	Anti-Hu CD34(AKYP0056)-BX035	Anti-Mu CD24(AKYP0036)-BX022
Anti-Hu CD14(AKYP0079)-BX037	Anti-Hu IDO1(AKYP0084)-BX027	Anti-Hu CD38(AKYP0020)-BX007	Anti-Mu CD31(AKYP0002)-BX002
Anti-Hu CD20(AKYP0049)-BX007	Anti-Hu IFNG(AKYP0093)-BX020	Anti-Hu CD45(AKYP0008)-BX001	Anti-Mu CD38(AKYP0032)-BX019
Anti-Hu CD21(AKYP0061)-BX032	Anti-Hu iNOS(AKYP0104)-BX023	Anti-Hu CD45RO(AKYP0059)-BX017	Anti-Hu/Mu CD44(AKYP0003)-BX005
Anti-Hu CD31(AKYP0047)-BX001	Anti-Hu Keratin 8/18(AKYP0112)-BX081	Anti-Hu/Mu CD49f(AKYP0018)-BX033	Anti-Mu CD45(AKYP0005)-BX007
Anti-Hu CD34(AKYP0088)-BX025	Anti-Hu Keratin 14(AKYP0064)-BX002	Anti-Hu CD69(AKYP0058)-BX041	Anti-Mu CD45R/B220(AKYP0014)-BX010
Anti-Hu CD38(AKYP0110)-BX089	Anti-Hu/Mu Ki67(AKYP0052)-BX047	Anti-Hu CD90(AKYP0054)-BX022	Anti-Hu/Mu CD49f(AKYP0018)-BX033
Anti-Hu CD39(AKYP0107)-BX099	Anti-Hu LAG3(AKYP0089)-BX055	Anti-Hu CD104(AKYP0019)-BX005	Anti-Hu/Mu CD49f(AKYP0018)-BX033
Anti-Hu CD40(AKYP0095)-BX010	Anti-Hu LIF(AKYP0065)-BX006	Anti-Hu CD138(AKYP0021)-BX010	Anti-Mu CD71(AKYP0043)-BX027
Anti-Hu CD44(AKYP0073)-BX005	Anti-Hu Mac2/Galectin-3(AKYP0067)-BX035	Anti-Hu CD278(AKYP0031)-BX017	Anti-Mu CD90.2(AKYP0001)-BX001
Anti-Hu CD45(AKYP0074)-BX021	Anti-Hu MPO(AKYP0113)-BX098	Anti-Hu HLA-DR(AKYP0017)-BX026	Anti-Mu CD169(AKYP0015)-BX015
Anti-Hu CD45RO(AKYP0059)-BX017	Anti-Hu Pan-Cytokeratin(AKYP0053)-BX019	Anti-Hu/Mu Ki67(AKYP0052)-BX047	Anti-Mu IgD(AKYP0030)-BX016
Anti-Hu CD66(AKYP0080)-BX016	Anti-Hu PCNA(AKYP0085)-BX036	Anti-Hu Pan-Cytokeratin(AKYP0053)-BX019	Anti-Mu IgM(AKYP0025)-BX013
Anti-Hu CD68(AKYP0050)-BX015	Anti-Hu PD-1(AKYP0070)-BX046	Anti-Hu PD-1(AKYP0026)-BX014	Anti-Hu/Mu Ki67(AKYP0052)-BX047
Anti-Hu CD79a(AKYP0109)-BX090	Anti-Hu PD-L1(AKYP0103)-BX043	Anti-Hu Podoplanin(AKYP0007)-BX023	Anti-Mu Ly6g(AKYP0039)-BX024
Anti-Hu CD107a(AKYP0004)-BX006	Anti-Hu Podoplanin(AKYP0007)-BX023		Anti-Mu MHC II(AKYP0006)-BX014
Anti-Hu CD141(AKYP0124)-BX087	Anti-Hu SMA(AKYP0081)-BX013		Anti-Mu TCRb(AKYP0011)-BX003
Anti-Hu CD163(AKYP0114)-BX069	Anti-Hu TFAM(AKYP0066)-BX029		Anti-Mu Ter119(AKYP0013)-BX004
Anti-Hu Collagen IV(AKYP0083)-BX042	Anti-Hu TIGIT(AKYP0092)-BX002		
Anti-Hu E-cadherin(AKYP0057)-BX014	Anti-Hu TP63(AKYP0111)-BX093		
Anti-Hu EpCAM(AKYP0119)-BX091	Anti-Hu Vimentin(AKYP0082)-BX022		
	Anti-Hu VISTA(AKYP0094)-BX040		

カスタムでのコンジュゲートも可能。カスタムコンジュゲートで確認されているものも含めると~400種類 (HubMAPなどのプロジェクトで確認された抗体リストも順次アップデートされている)

カスタムコンジュゲート

- ❖ 免染で実績のある抗体を使用する。
- ❖ 抗体は50ugを使用
- ❖ 原則としてPBSに懸濁された精製抗体を使用する
- ❖ BSA、ゼラチン等のキャリアータンパク質、グリセロールなどが含まれるものは使用しない
- ❖ 抗体を部分的に還元してHeavy chainにDNAバーコードをコンジュゲート



CODEX技術を利用したAkoya PhenoCycler-システム

PhenoCycler-Fusion (PCF)システム



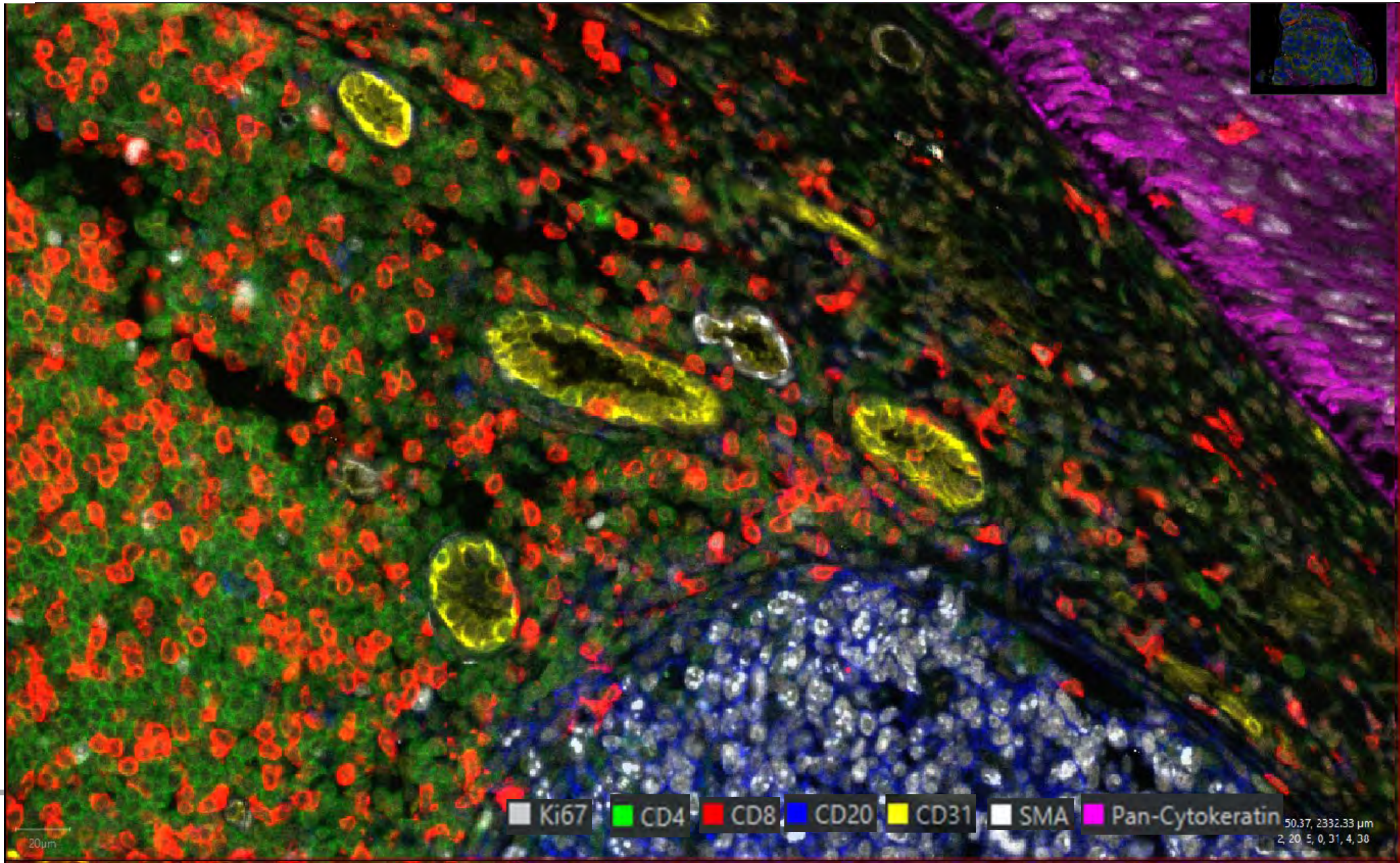
- 凍結切片、FFPE切片を用いて～60種類のタンパク質をシングルセルの解像度でイメージング
(解像度は0.5um/0.37um/0.33um)
- Akoya Fusionシステム、キーエンスBZX-700/800シリーズ、またはZEISS Axio Observer7と組み合わせて使用
- 切片の厚みは5-10umでPCFでは高速でスライドガラスの全面スキャンが可能。PCOはカバーガラスで領域ベース。
(PCF-18x34mm/PCO-15mmx15mm)

PhenoCycler-Open (PCO)システム



- 無償の解析ツールQupath/CytoMapが利用可能。
イメージデータから個々の細胞をセグメント化し、次元削減、クラスタリング解析や細胞間距離を指標にした細胞間相互作用、CellularNeighborhood解析が可能。
- PCFはTSAを用いたイメージングが可能
PCOでは通常の顕微鏡として利用する際に切り替えが容易

Human Tonsil (24plex) 12.2mm x8.9mm, >1M cells



空間的シングルセル解析の流れ

1次解析(QuPathなど)

イメージデータの取得



解析領域の指定



Cell Segmentation



Cell Classification / Clustering
—教師あり機械学習ベースの分類
—教師無しクラスタリング

2次解析(CytoMAPなど)

細胞レベルの解析

次元削減 (UMAPなど)



フェノタイプマッピング



細胞間相互作用の解析

Cellular Neighborhood (CN)レベルの解析

CNの検出



CNの領域へのマッピング



CN間相互作用の解析

核シグナルによる細胞のセグメンテーション

Watershed Algorithm

DAPI Image



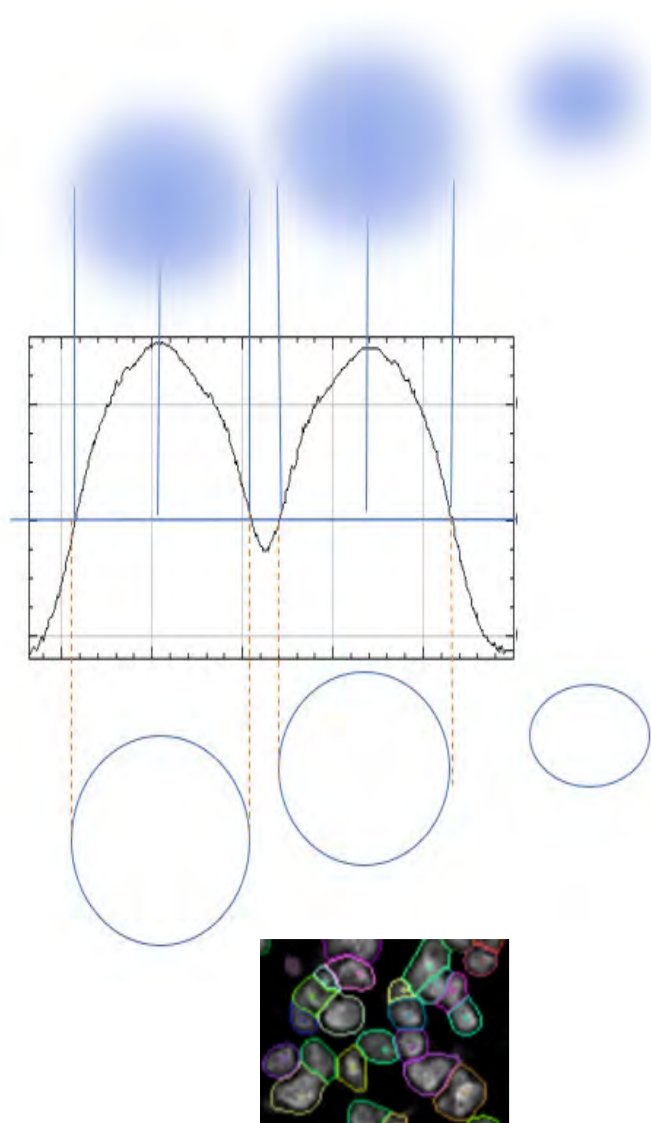
Intensity Profile

極大値の探索
と領域拡張

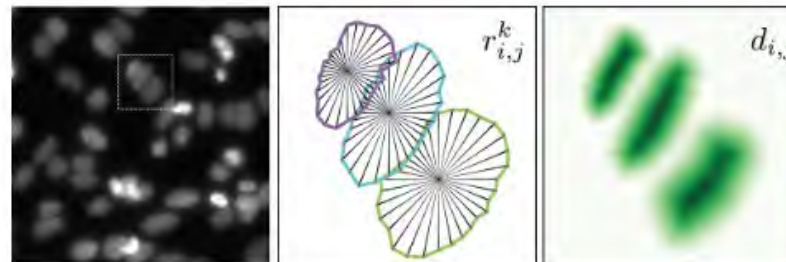


距離、近隣シグナル
ベースで細胞の境界
を推定

Cells Outline



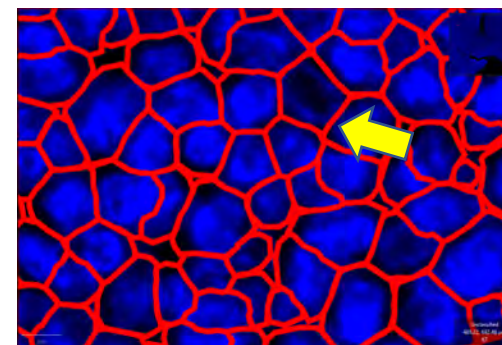
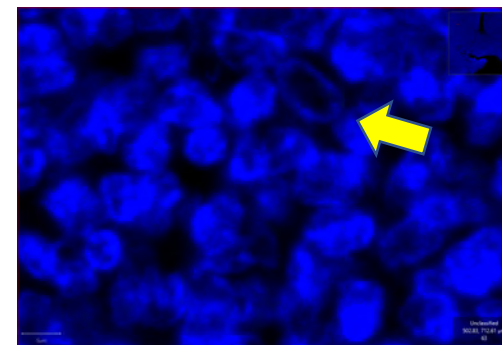
StarDist Algorithm (HubMap) (<https://github.com/stardist/stardist>)



事前学習されたモデルを利用した深層学習で各ピクセルのシグナル、バックグラウンドを判別し核の境界をポリゴンで予測

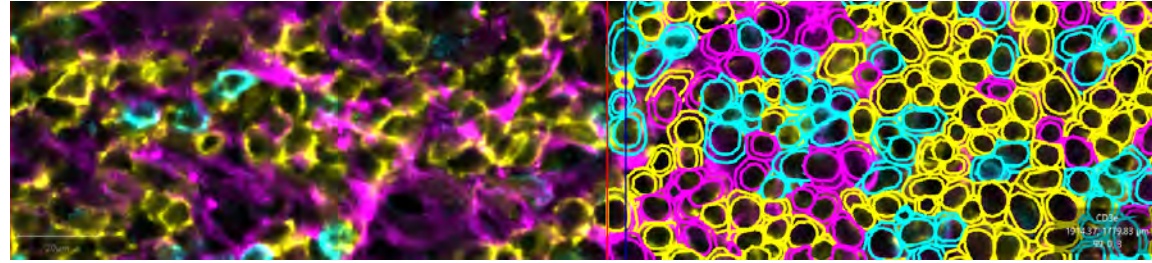


距離、近隣シグナル
ベースで細胞の境界
を推定



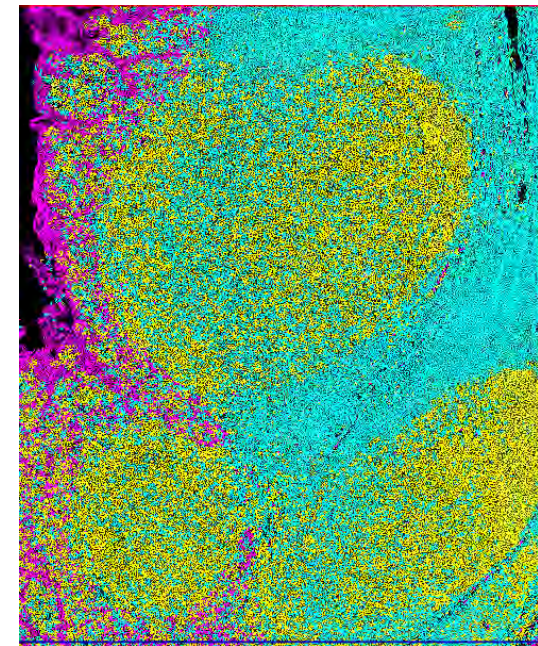
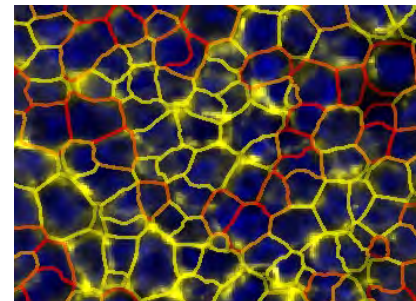
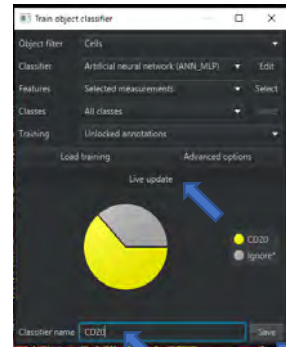
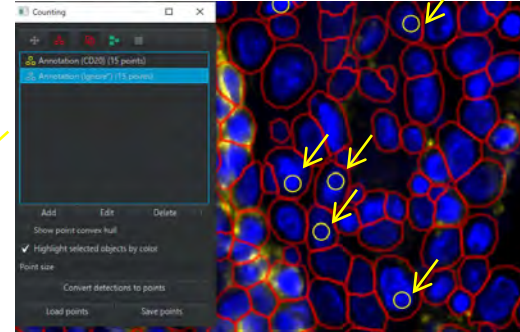
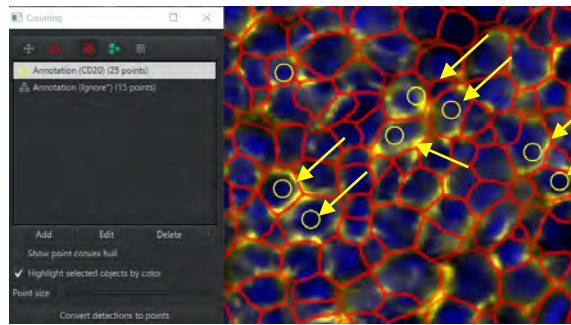
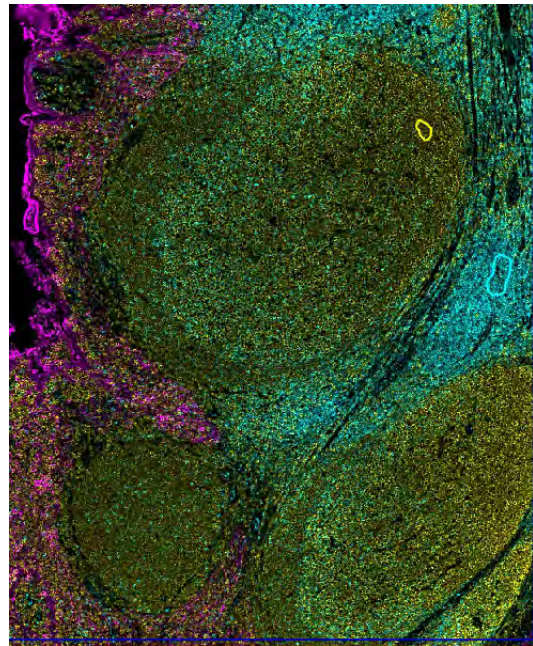
QuPath (無償のツール) による細胞のセグメンテーションと分類

Cell Segmentation (QuPath original & StarDist)
DAPIを利用したクラスタリング



Cell Classification

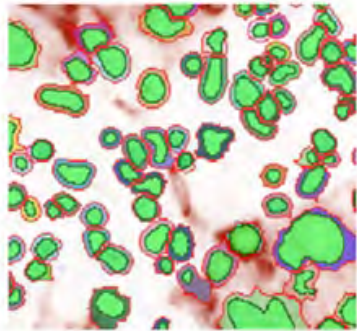
(教師付き機械学習でトレーニングされた分類子でテストデータの予測を行う)



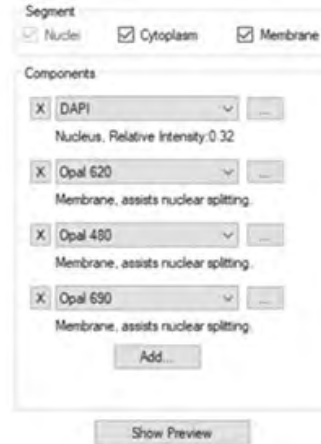
核、細胞質、細胞膜のマーカなどマルチパラメーターを利用した Cell Segmentation & Cell Classificationの解析ツール

Commercial

Akoya Informソフトウェア &
PhenoptrReport



More flexible in cell
segmentation options →



Indicalabs HALOソフトウェア



VISIOPHARMソフトウェア



Public open source

bioRxiv

Evaluation of cell segmentation methods without reference segmentations

Haoran Chen, Robert F. Murphy

doi: <https://doi.org/10.1101/2021.09.17.460800>

Table 3 Segmentation methods evaluated

Method	Inputs					Output
	Cytoplasm	Cell Membrane	Nucleus	Dimension	Requires scale	Nuclear mask
DeepCell 0.6.0		X	X	2D	No	Yes
DeepCell 0.6.0	X		X	2D	No	Yes
DeepCell 0.9.0 (new)		X	X	2D	No	Yes
DeepCell 0.9.0 (new)	X		X	2D	No	Yes
Cellpose 0.0.3.1	X		X	2D/3D	No	Yes
Cellpose 0.6.1 (new)	X		X	2D/3D	No	Yes
CellSegm		X		2D/3D	Yes	No
CellIX		X		2D	Yes	No
CellProfiler		X	X	2D	Yes	No
AICS (classic)		X	X	2D/3D	Yes	No
Voronoi			X	2D	No	Yes

論文ではDeepCell、Cellposeのクオリティスコアが高くなっている

空間的シングルセル解析の流れ

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CNの検出



CNの領域へのマッピング

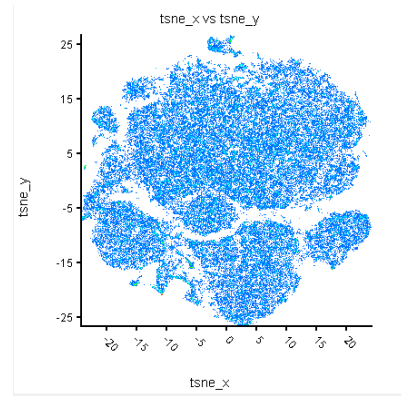


CN間相互作用の解析

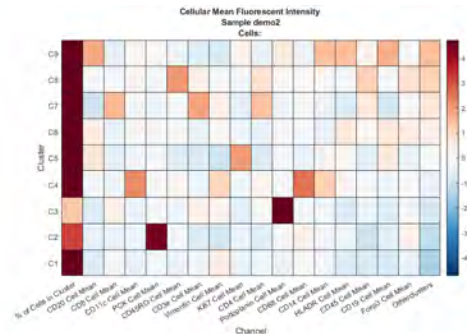
細胞レベルの解析 (無償の解析ツールCytoMAP)

フェノタイプマッピング

tsNE/UMAP(次元削減)

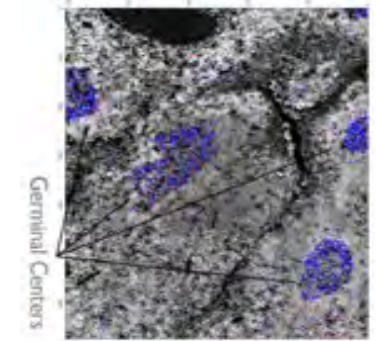
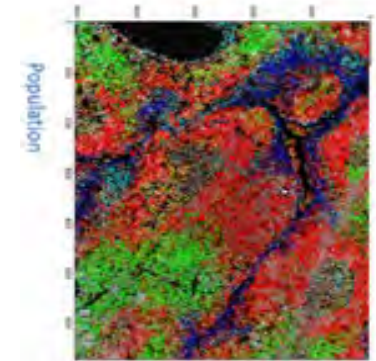


教師なしクラスタリング

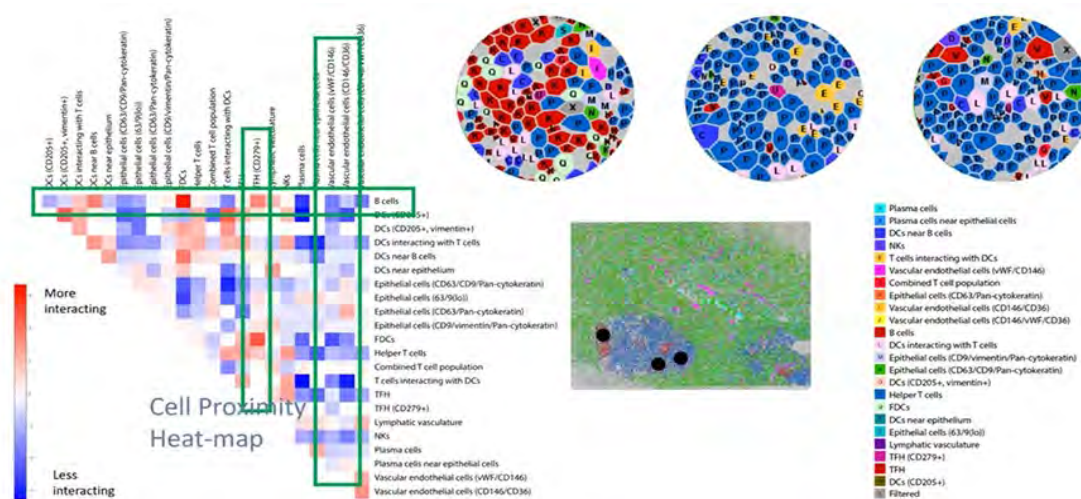


アノテーション (マニュアル)

Phenotype Assignment	Population	Positive Markers (All CD45+)
Stromal Cells	3414	CD90+, CD9+, HLA-DR+, Cytokeratin+
Stromal Cells in connective regions	807	CD31+, CD90+, Collagen IV+
T _H Cells	328	CD3+, CD4+, CD7+, CD278+, CD279+
Activated T _H Cells	1459	CD3+, CD4+, CD40+, CD278+, CD279+
Activated Helper T cells	11866	CD3+, CD4+, CD7(+)
T Cells near connective regions	222	CD3+, CD29+, CD90(+), Collagen IV(+)
Proliferating Th-1 cells	2411	CD4+, Ki67+
Dendritic Cells	135	CD4+, HLA-DR+
Dendritic Cells	366	CD4+, CD11c+
Dendritic Cells	429	CD11c+, HLA-DR+
NK cells	126	CD3-, CD7+
Plasma Cells	2180	CD31+, CD38+, CD40+
Lymphatic Vasculature Cells	1336	CD9+, Podoplanin+
Mature, proliferating B cells near follicular matrix	960	CD19+, CD22+, CD21+, CD90+, Ki67+
Mature B cells	32840	CD19+, CD22+



細胞間相互作用の解析

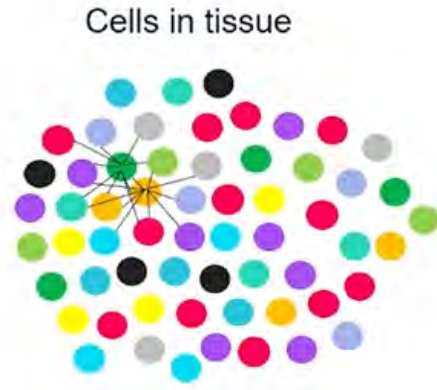


- Plasma cells
- Plasma cells near epithelial cells
- DCs near B cells
- NKs
- T cells interacting with DCs
- Vascular endothelial cells (vWF/CD146)
- Combined T cell population
- Epithelial cells (CD63/Pan-cytokeratin)
- Vascular endothelial cells (CD146/CD36)
- Vascular endothelial cells (CD146/vWF/CD36)
- B cells
- DCs interacting with T cells
- Epithelial cells (CD9/vimentin/Pan-cytokeratin)
- Epithelial cells (CD63/CD9/Pan-cytokeratin)
- T cells interacting with DCs
- Helper T cells
- Combined T cell population
- T cells interacting with DCs
- TH1 (CD279+)
- Lymphatic vasculature
- NKs
- Plasma cells
- Plasma cells near epithelial cells
- Vascular endothelial cells (vWF/CD146)
- Vascular endothelial cells (CD146/CD36)
- Filtered

Cellular Neighborhood解析 (CytoMAP)

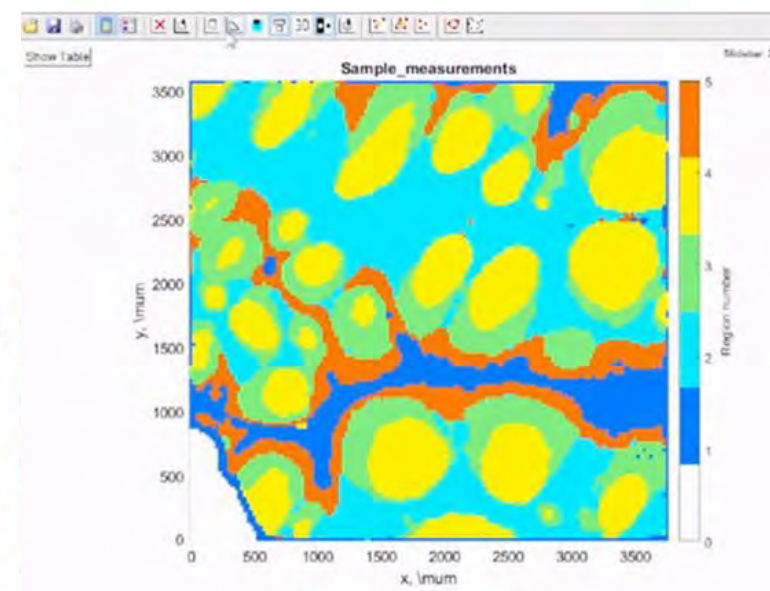
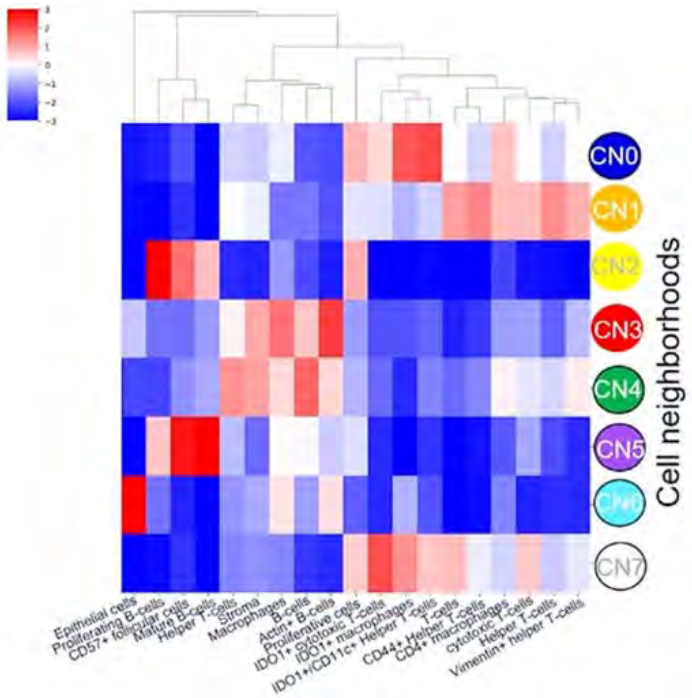
<https://www.akoyabio.com/webinar/codex-with-seurat-a-high-dimensional-approach-to-unlocking-spatial-insights/>

Cellular Neighborhood解析



Cluster spatial windows to find common patterns of proximity, i.e., cell neighborhoods

- ● ● ● ● ● ● ● ● ● Cell 1 neighbors (window 1)
- ● ● ● ● ● ● ● ● ● Cell 2 neighbors (window 2)
- ⋮



Cellular Neighborhood Interaction



解析可能なツール
Neighborhood Coordination
CytoMAP,
VisioPharm

MAV, QuPathの使い方はYoutubeにて公開しております。

<https://www.youtube.com/channel/UCKgB6yUz-cVef-l-3U4siNQ/videos>



バイオストリーム株式会社

チャンネル登録者数 25人

登録済み



ホーム

動画

再生リスト

チャンネル

概要



アップロード済み

並べ替え



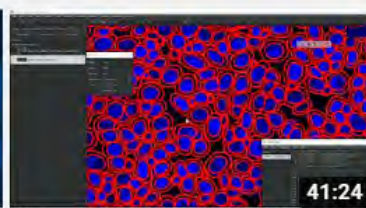
20分でわかる！Akoya PhenoCycler-Fusion (旧名...

24回視聴・3週間前



PhenoCyclerのデータ解析：QuPath後のCytoMapによる...

48回視聴・1か月前



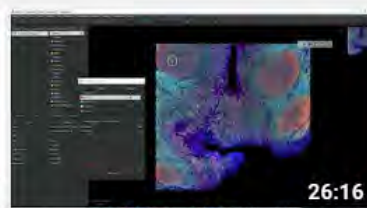
PhenoCycler (旧名CODEX)データのQuPathでのCell...

48回視聴・1か月前



Akoya PhenoCycler (旧名CODEX)の抗体のcustom...

14回視聴・1か月前



PhenoCyclerデータのQuPathでの解析。データのインポ...

73回視聴・1か月前



PhenoCycler-FusionシステムデータのPhenochartソフト...

17回視聴・1か月前



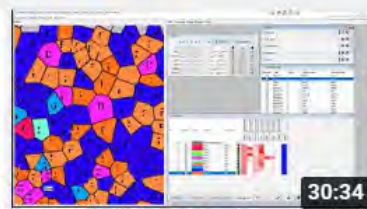
PhenoCycler (旧名CODEX)データのQupahおよび...

58回視聴・1か月前



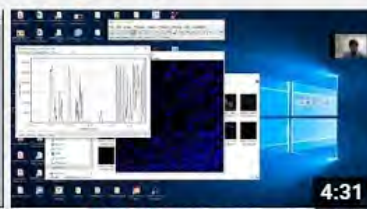
Akoya PhenoCycler (旧名CODEX)システムのご紹介...

213回視聴・1か月前



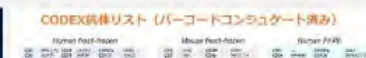
Akoya CODEX MAV(イメージビュー、Spatial解析など) ...

121回視聴・10か月前



ImageJでCODEXデータのSNを確認する方法

19回視聴・11か月前



組織マイクロアレイを用いた効率的な大腸がんの微小環境の解析

Cell

Resource

関連論文

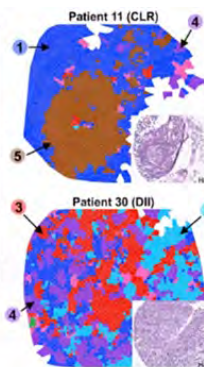
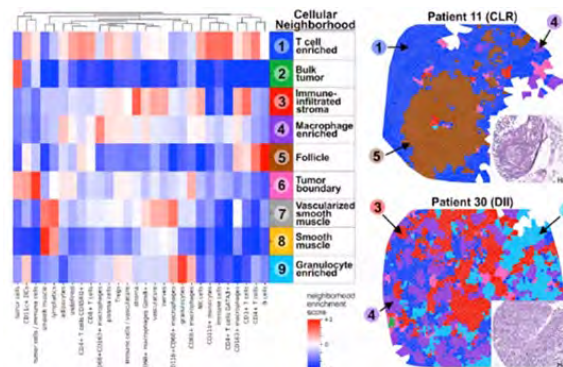
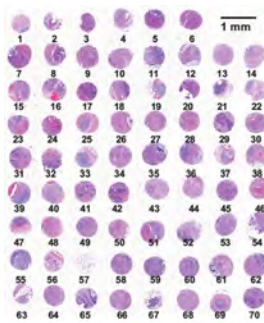
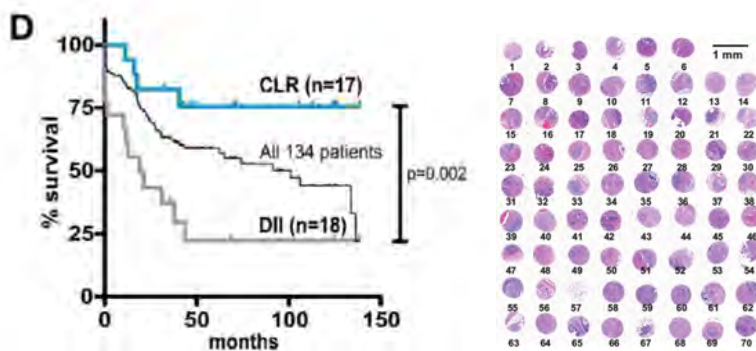
Coordinated Cellular Neighborhoods Orchestrate Antitumoral Immunity at the Colorectal Cancer Invasive Front

Cell. 2020 Oct 29;183(3):838.

nature protocols PROTOCOL
<https://doi.org/10.1038/s41594-021-00556-8>

CODEX multiplexed tissue imaging with DNA-conjugated antibodies

Sarah Black^{1,2,6}, Darci Phillips^{1,2,6}, John W. Hickey^{1,2,6}, Julia Kennedy-Darling^{1,4}, Vishal G. Venkataraman^{1,2}, Nikolay Samusik^{1,5}, Yury Goltsev^{1,2}, Christian M. Schürch^{1,2,3,7,8,9} and Garry P. Nolan^{1,2,7,8,9}

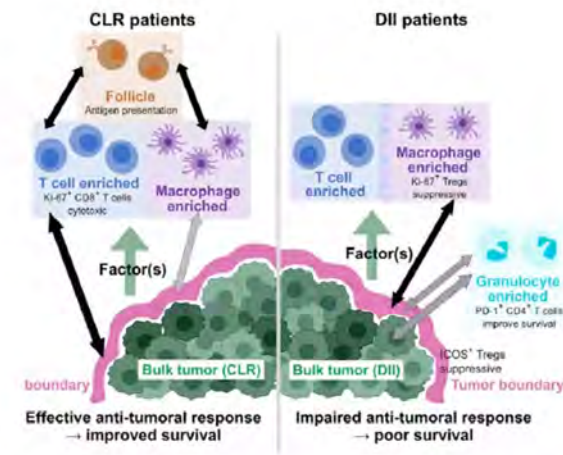
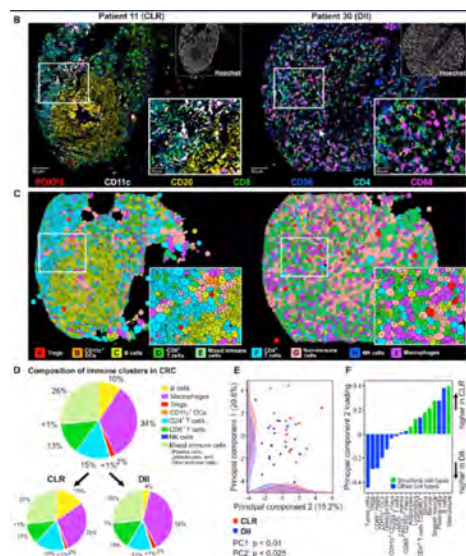


- PhenoCyclerシステムの原理、方法を詳述
- 組織マイクロアレイとの組み合わせでハイスループット解析を実現

• 2枚のカバースリップに140組織スポット (0.6mm)をマウントし、大腸がん35検体 (17 CLR vs 18 DII)を解析。

• 56タンパク質を同時に解析して28のセルタイプを同定し、シングルセルレベルの細胞間相互作用を解析。

• 細胞間相互作用だけでなく領域としての特徴づけを行い (CN: Cellular Neighborhood)、領域間の相互作用解析により予後に関連する微小環境の特徴を同定。



Model of differences in the IME between CLR and DII patients with respect to CN organization, cellular function, and inter-CN communication

New Biomarkers are Needed to Better Predict Patient Response



ARTICLE

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

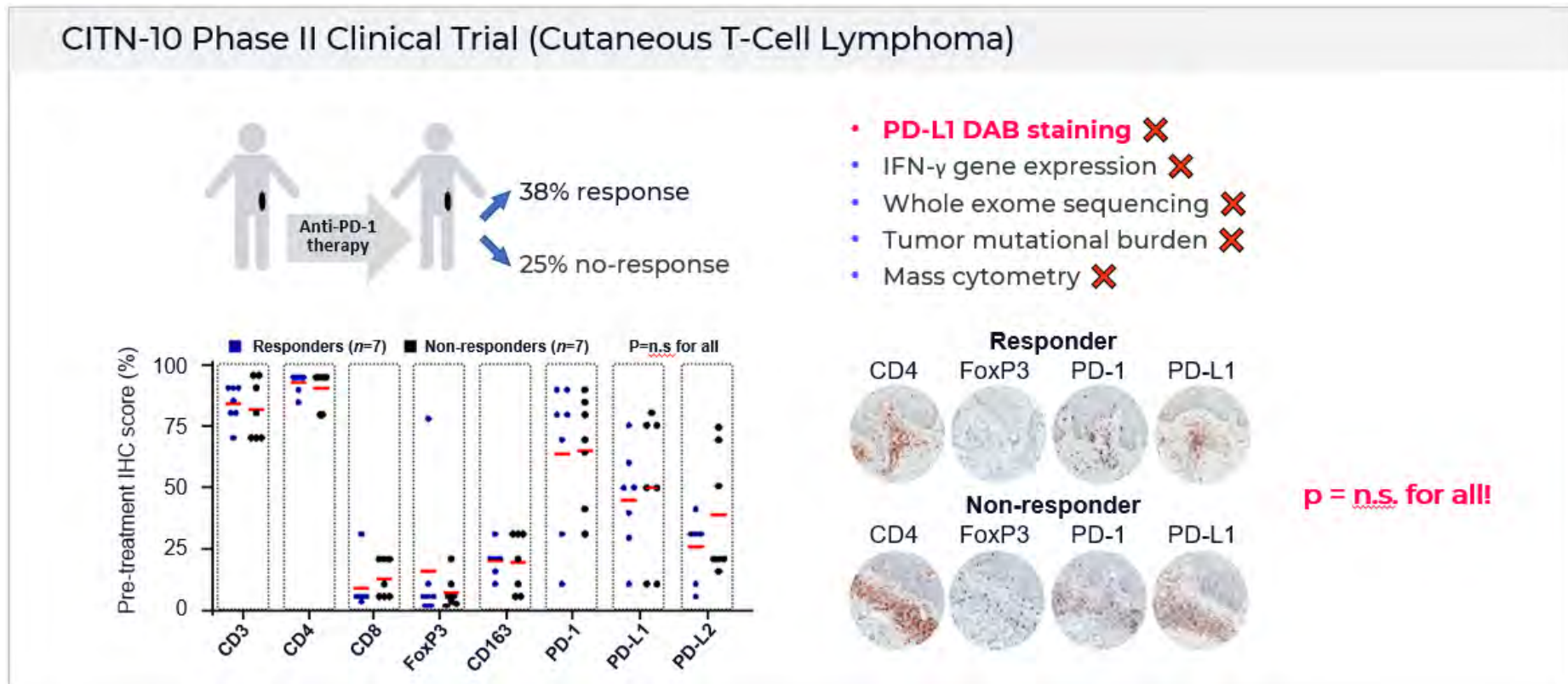
OPEN

Check for updates

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

Darci Phillips^{1,2,3,9}, Magdalena Matusiak^{3,9}, Belén Rivero Gutierrez³, Salil S. Bhate^{1,3,4}, Graham L. Barlow^{1,3}, Sizun Jiang^{1,3,5}, Janos Demeter¹, Kimberly S. Smythe⁶, Robert H. Pierce⁶, Steven P. Fling⁶, Nirasha Ramchurren⁶, Martin A. Cheever⁶, Yury Goltsev^{1,3}, Robert B. West³, Michael S. Khodadoust^{7,10}, Youn H. Kim^{2,7,10}, Christian M. Schürch^{1,3,8,10} & Garry P. Nolan^{1,3,10}

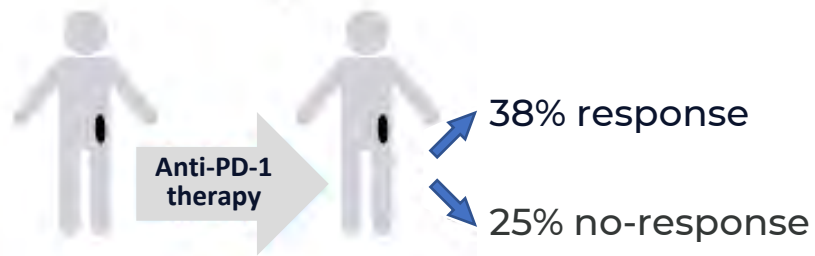
Conventional Biomarkers Fail to Predict Outcomes in Lymphoma



Context Matters: Spatial Neighborhoods Predict Response

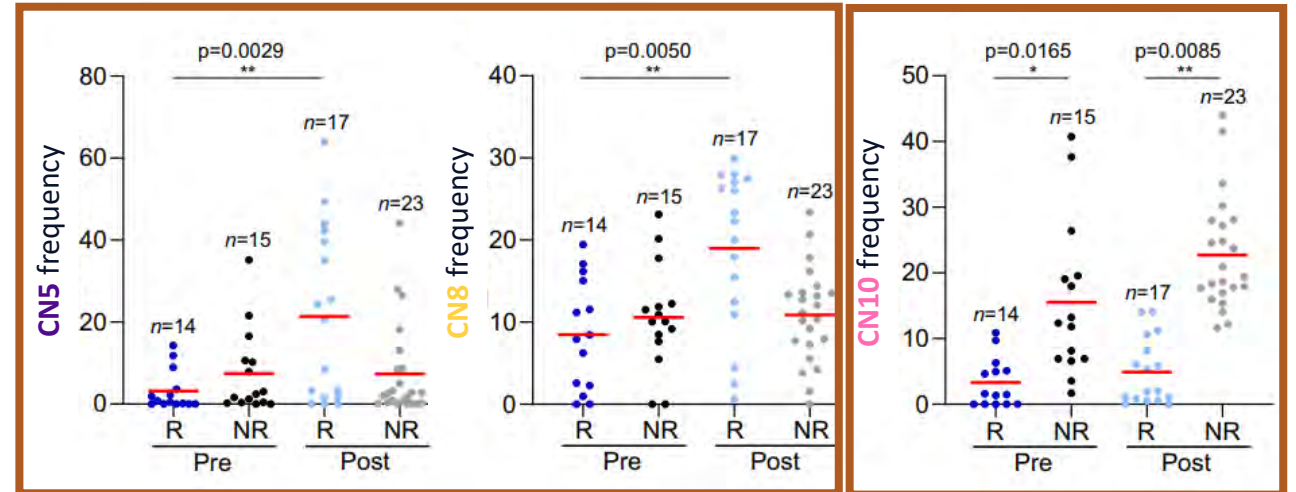
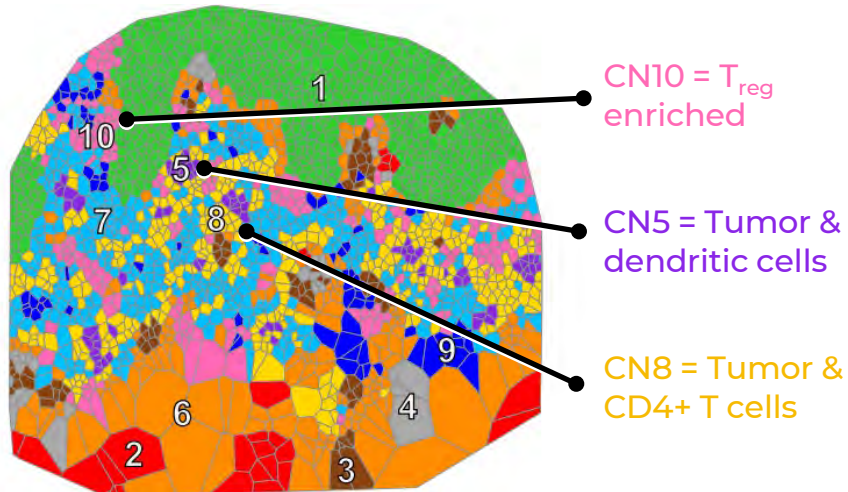
Discovery Uncovers Predictive Potential of Spatial Neighborhoods

CITN-10 Phase II Clinical Trial (Cutaneous T-Cell Lymphoma)



- 56-plex IO Panel
- Multiple spatial neighborhoods differentiate responders vs. non-responders!

Spatial Neighborhood Map Responder



Spatial Neighborhoods in Today's Literature

Rapid Adoption of SNs as Potential Biomarkers Across the Biomedical Literature

Cell Resource

Coordinated Cellular Neighborhoods Orchestrate Antitumoral Immunity at the Colorectal Cancer Invasive Front

Graphical Abstract

Technology
CODEX + Optimization for Formalin Fixed Paraffin Embedded Tissue (FFPE)

Data Collection
Colorectal Cancer (High Risk, Low Risk) → FFPE → Tissue Microarray

Computation
Cell Types, Cell Neighborhoods
① Coupling
② Functional State
③ Communication

Authors
Christian M. Schürch, Salil S. Bhate, Graham L. Barlow, ..., Nikolay Samusik, Yury Goltsev, Garry P. Nolan

Correspondence
christian.m.schuerch@gmail.com (C.M.S.), gnolan@stanford.edu (G.P.N.)

In Brief
A multiplexed tissue imaging and computational analysis framework applied to colorectal cancer allows interrogation of how spatial organization of the immune tumor microenvironment is linked to clinical outcomes.

Highlights

- FFPE-CODEX multiplexed tissue imaging of 56 markers in 140 tissues of 35 CRC patients
- Cellular neighborhoods reveal spatial organization of the tumor microenvironment

nature COMMUNICATIONS

ARTICLE

<https://doi.org/10.1038/s41467-021-29303-7> OPEN

An N-Cadherin 2 expressing epithelial cell subpopulation predicts response to surgery, chemotherapy and immunotherapy in bladder cancer

Kenneth H. Gouin III^{1,2,8}, Nathan Ing^{1,2,8}, Jasmine T. Plummer^{1,2}, Charles J. Rosser^{3,4}, Bassam Ben Cheikh^{1,2}, Catherine Oh^{1,2}, Stephanie S. Chen^{1,2}, Keith Syson Chan^{4,5}, Hideki Furuya^{3,4}, Warren G. Tourtellotte^{1,4,5,6,7}, Simon R. V. Knott^{1,2,4,8} & Dan Theodorescu^{3,4,5,8}

Cell Systems Article

Tissue schematics map the specialization of immune tissue motifs and their appropriation by tumors

Graphical abstract

Tissue Schematics
Cellular Neighborhoods (CNs): regions with characteristic local processes

Spatial Contexts
Fluctuations in cell states indicate local CN interactions

Assembly Rules
Align tissue types, Evaluate mutations (Good survival, Poor survival)

Authors
Salil S. Bhate, Graham L. Barlow, Christian M. Schürch, Garry P. Nolan

Correspondence
gnolan@stanford.edu

In brief
We present Tissue Schematics, a computational and conceptual framework for using high-parameter imaging data to map how tissues are formed by the assembly of cellular neighborhoods into tissue motifs. Applying our framework to human lymphoid tissues and the colorectal cancer immune-tumor microenvironment suggests mechanistic insights into the specific biology of these tissues, as well as general principles of tissue organization.

nature COMMUNICATIONS

ARTICLE

<https://doi.org/10.1038/s41467-021-26974-0> OPEN

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

Darci Phillips^{1,2,3,9}, Magdalena Matusiak^{3,9}, Belén Rivero Gutierrez³, Salil S. Bhate^{1,3,4}, Graham L. Barlow^{1,3}, Sizun Jiang^{1,3,5}, Janos Demeter¹, Kimberly S. Smythe⁶, Robert H. Pierce⁶, Steven P. Fling⁶, Nirasha Ramchurren⁶, Martin A. Cheever⁶, Yury Goltsev^{1,3}, Robert B. West³, Michael S. Khodadoust^{7,10}, Youn H. Kim^{2,7,10}, Christian M. Schürch^{1,3,8,10,8} & Garry P. Nolan^{1,3,10,8}

Blood Cancer Journal

ARTICLE OPEN

Lack of intrafollicular memory CD4+ T cells is predictive of early clinical failure in newly diagnosed follicular lymphoma

Patrizia Mondello^{1,2,11}, Angelo Fama^{1,11}, Melissa C. Larson¹, Andrew L. Feldman⁵, Jose C. Villasboas¹, Zhi-Zhang Yang¹, Ilya Galkin¹, Viktor Svelolikin¹, Ekaterina Postovalova¹, Alexander Bagaev¹, Pavel Ovcharov¹, Arina Varlamova¹, Sarah Huet¹, Bruno Tesson¹, Kaitlyn R. McGrath¹, Susan Slager¹, Brian K. Link¹, Sergei Syrbu¹, Anne J. Novak¹, Thomas M. Habermann¹, Thomas E. Witig¹, Grzegorz S. Nowakowski¹, Gilles Salles^{1,12}, James R. Cothan¹ and Stephen M. Ansell^{1,13}

CSH Cold Spring Harbor Laboratory bioRxiv

High Resolution Single Cell Maps Reveals Distinct Cell Organization and Function Across Different Regions of the Human Intestine

John W. Hickey^{1*}, Winston R. Becker^{2*}, Stephanie A. Nevins^{2*}, Aaron Horning², Almudena Espin Perez³, Roxanne Chiu², Derek C. Chen², Daniel Cotter², Edward D. Esplin², Annika K. Weimer², Chiara Caraccio¹, Vishal Venkataaraman¹, Christian M. Schürch^{1,7}, Sarah Black¹, Maria Brbic⁵, Kaidi Cao⁵, Jure Leskovec⁵, Zhengyan Zhang⁵, Shin Lin⁴, Teri Longacre¹, Sylvia K. Plevitis³, Yiing Lin⁵, Garry P. Nolan¹*, William J. Greenleaf²*, Michael Snyder²*

Footnotes:
¹ Department of Pathology, Stanford School of Medicine, Stanford, CA, 94305
² Department of Genetics, Stanford School of Medicine, Stanford, CA, 94305
³ Department of Biomedical Data Science, Stanford School of Medicine, Stanford, CA, 94305
⁴ Department of Medicine, University of Washington, Seattle, WA, 98195
⁵ Department of Surgery, Washington University, St Louis, MO 63110
⁶ Department of Computer Science, Stanford University, Stanford, CA 94305, USA
⁷ Department of Pathology and Neuro pathology, University Hospital and Comprehensive Cancer Center Tübingen, Tübingen, Germany

*These authors contributed equally
Correspondence should be addressed to: gnolan@stanford.edu, wjg@stanford.edu, mpsnyder@stanford.edu

Research Square

Preprints are preliminary reports that have not undergone peer review. They should not be considered conclusive, used to inform clinical practice, or referenced by the media as validated information.

A tissue atlas of ulcerative colitis to guide TNF inhibitor therapy

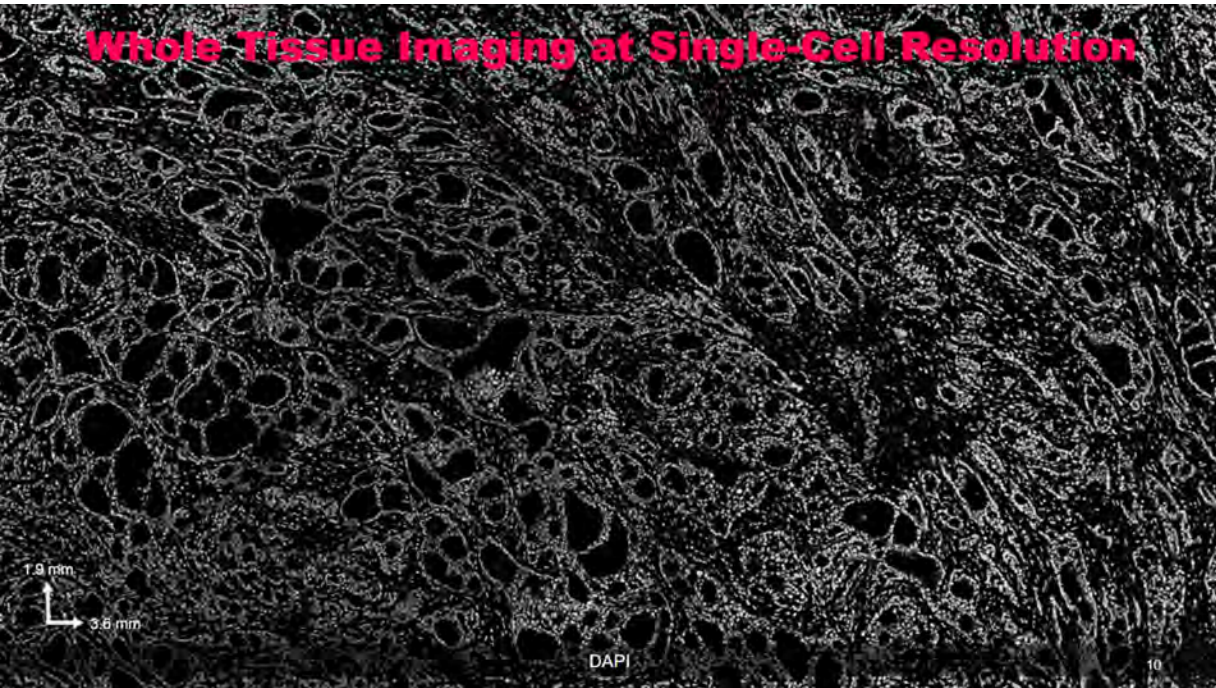
Aaron Mayer, Derek Holman, Utkarsh Tandon, Salil Bhate, Sunil Bodapati, Graham Barlow, Jeff Chang, Sarah Black, Erica Crenshaw, Brigid Boland, Sarah Streett, William Sandborn, John Chang, Sanjiv Gambhir, Garry Nolan, Christian Schürch, Stephan Rogalla

Abstract
Ulcerative colitis is a chronic-relapsing inflammatory disease of the large intestine with a complex, multifactorial pathogenesis. TNF inhibitors are widely used to suppress immune-mediated tissue damage in ulcerative colitis patients; however, therapy failures are common. Predicting TNF inhibitor response requires an understanding of the architectural features that underlie mucosal inflammation and those responsible for resistance. Here, we used highly multiplexed immunofluorescence to uncover the spatially resolved tissue architectures underlying disease progression and treatment response in 42 tissue regions from 34 individuals. We created a tissue atlas and performed spatial analysis to identify cell-cell contacts and cellular neighborhoods. We observed that cellular functional states depend on cellular neighborhood and that a subset of inflammatory cell types and cellular neighborhoods in ulcerative colitis patients persisted even during treatment with TNF inhibitor, indicating resistant niches. A computer vision model, with no *a priori* assumptions regarding cellular architectural features, was able to predict TNF inhibitor resistance. This spatial model significantly outperformed classification models based on single-cell data alone. Our results demonstrate the value of a spatial tissue atlas as a precision medicine tool to guide treatment of patients suffering from autoimmune diseases.

使用例 2:セルタイプの同定によるSpatial Transcriptomeデータのバリデーション

Single-cell resolution is the key!

PhenoCycler image



Other systems

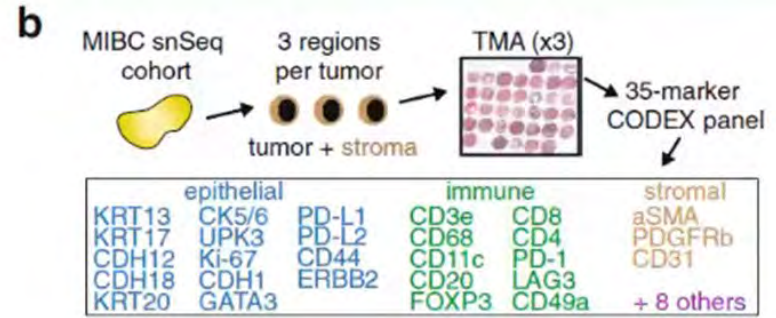


- 組織の連続性がなく、シングルセルデータではない
- データの49%が失われている (Visium)

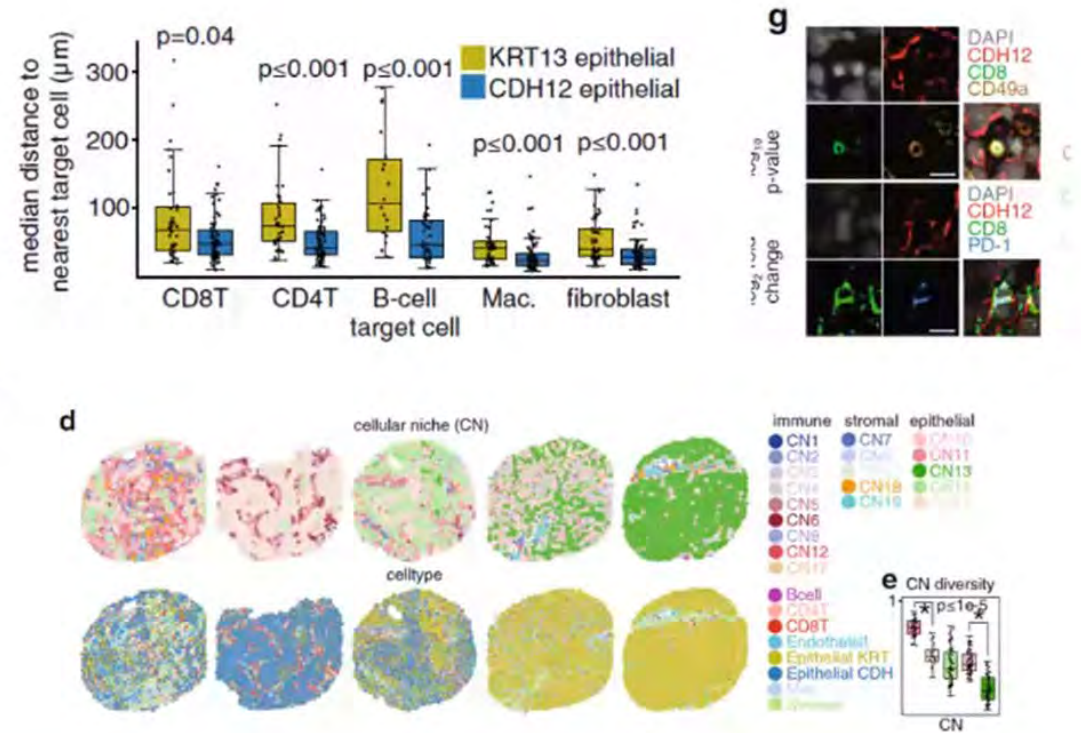
PhenoCyclerによるValidationが有用

CODEX, Together with Sn-Sequencing, Identifies Bio-marker for Cancer

An N-Cadherin 2 expressing epithelial cell subpopulation predicts response to surgery, chemotherapy and immunotherapy in bladder cancer



- 25検体の浸潤性膀胱がんのsnRNAseqで、ネオアジュバント化学療法を伴う/伴わない術後の予後マーカー（予後不良）として、および癌免チェックポイント阻害剤の予後マーカー（予後良好）としてCDH12を同定
- Validationの過程で初めにVisiumを使用し、CDH12が強く発現しているエリアに、疲弊CD8陽性T細胞マーカーであるPDCD1, LAG3, HAVCR2などの発現を確認
- 最終的にPhenCyclerでシングルセルレベルでのCDH12陽性腫瘍細胞と疲弊CD8陽性T細胞の共在（細胞間相互作用）を確認
- CNを利用した相互作用解析



CODEX : True Single Cell Level Protein Expression With Spatial Information

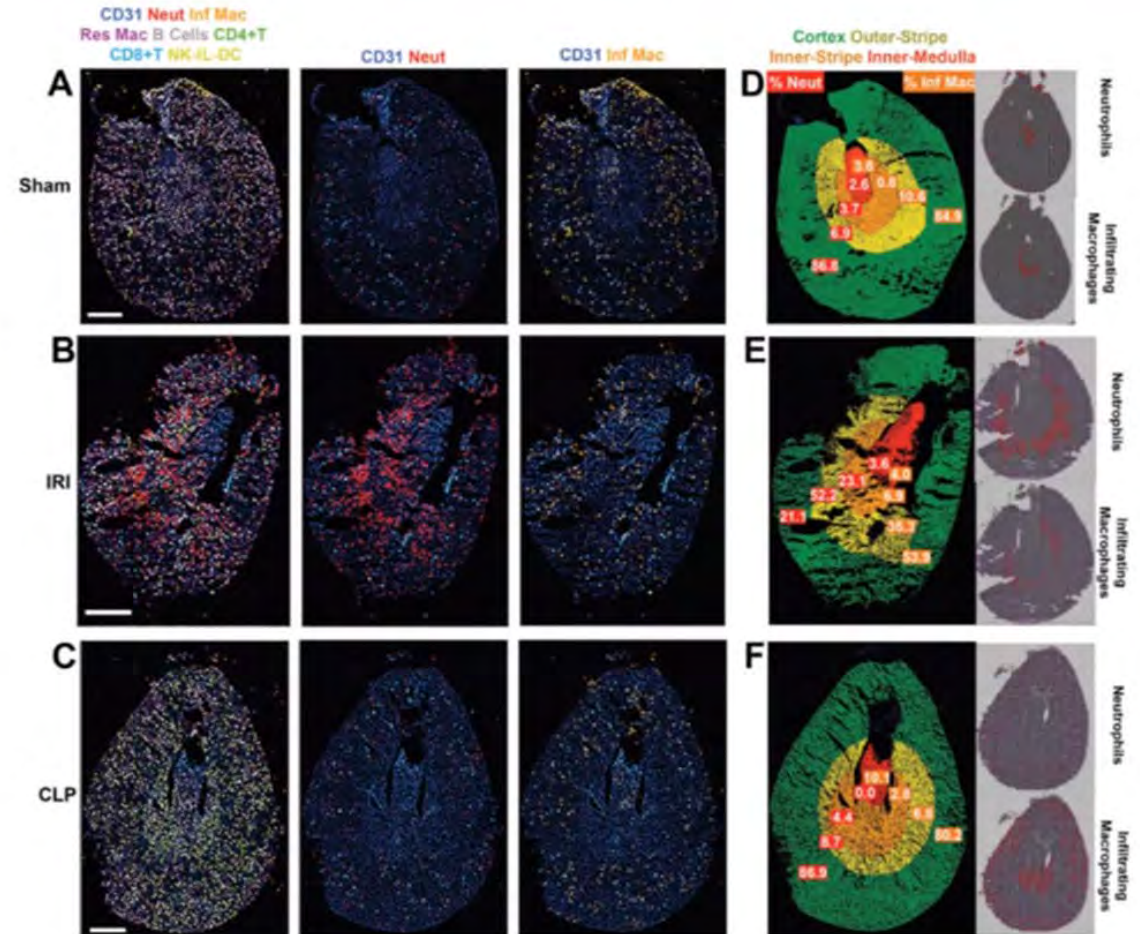
JCI insight

Integration of spatial and single-cell transcriptomics localizes epithelial cell-immune cross-talk in kidney injury

Ricardo Melo Ferreira, ... , Tarek M. El-Achkar, Michael T. Eadon

JCI Insight. 2021;6(12):e147703. <https://doi.org/10.1172/jci.insight.147703>.

- マウスの急性腎臓損傷モデルで空間的トランスクリプトーム解析を実施 (Visium)
- セルタイプ情報をVisiumの各スポットにマップするためにscRNAseq, snRNAseqのデータセットを使用
- Visiumのスポットサイズが55umとシングルセルサイズではなく、メジャーなセルタイプが各スポットにアサインされやすい傾向を観察
- 最終的にCODEXでセルタイプの分布と頻度を検証している



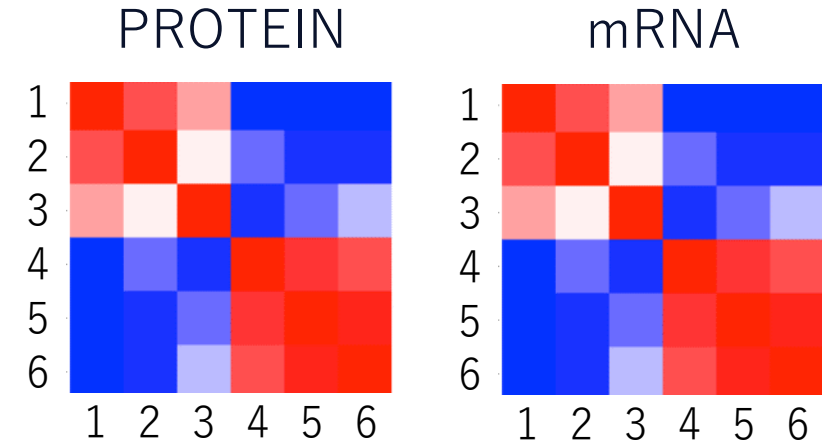
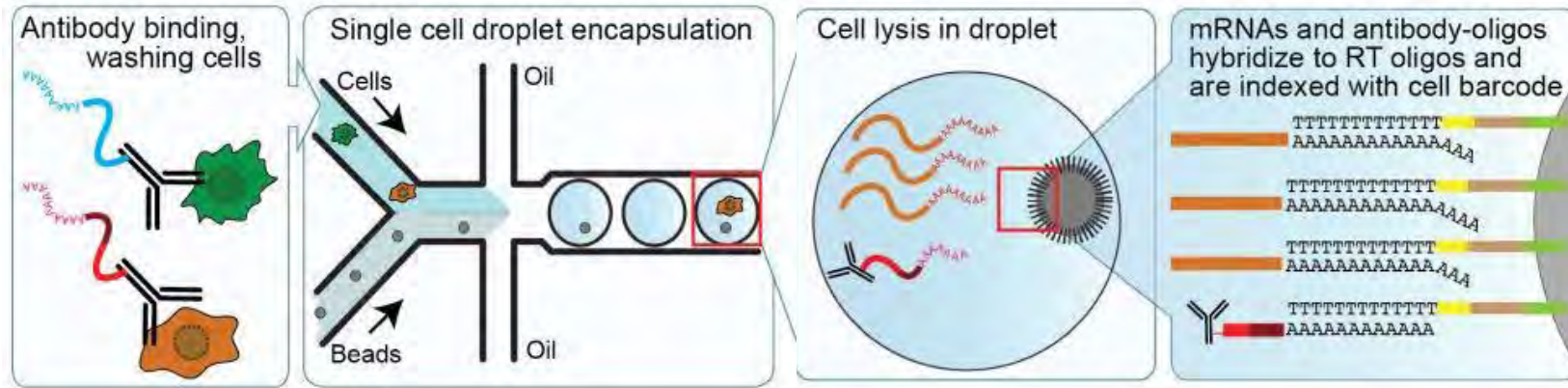
Multiplexed imaging of proteins in toto with **CODEX** validates the localization of immune cell clusters inferred by Spatial Transcriptomics

PhenoCyclerシステムの空間的シングルセルトランスクリプトーム解析への応用

Merger of CITE-Seq and PhenoCycler data

CITE-Seq produces multiplexed PROTEIN and mRNA expression data

CITE-Seq workflow



PhenoCycler (CODEX) results are multiplexed PROTEIN expression data

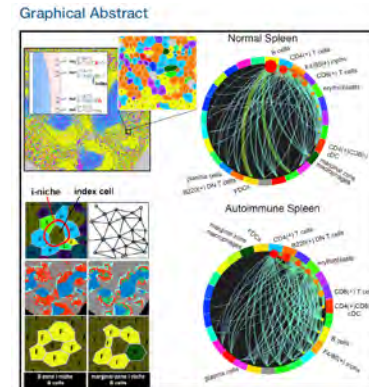
Cell

マウスの脾臓の凍結切片の解析データ

Resource

Deep Profiling of Mouse Splenic Architecture with CODEX Multiplexed Imaging

Cell. 2018 Aug 9;174(4):968-981.e15. doi: 10.1016



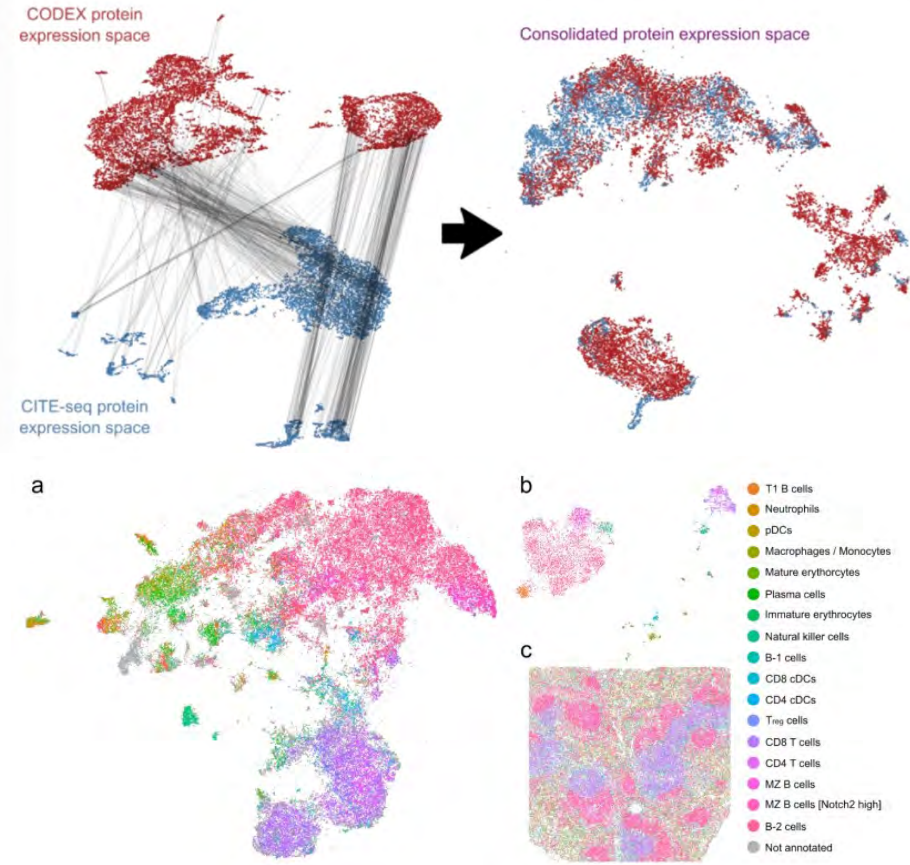
使用例3 : 空間的シングルセルトランスクリプトーム解析への応用 CITE-Seq データと PhenoCycler データの統合

ScienceAdvances

Single-cell transcriptomic analysis of mIHC images via antigen mapping

Science Advances 05 Mar 2021:
Vol. 7, no. 10, eabc5464
DOI: 10.1126/sciadv.abc5464

Kiya W. Govek*, Emma C. Troisi*, Zhen Miao, Rachael G. Aubin, Steven Woodhouse, Pablo G. Camara†



- マウスの脾臓サンプルで33の細胞表面タンパク質をターゲットとしてCITE-seqを実施（7097細胞）。
- 33の表面タンパク質データを相関解析ベースにした独自のアルゴリズム（**STVEA**）でCITE-seqのデータをPhenoCyclerのデータ（Cell. 2018）にマッピング。
- 73%のPhenoCyclerデータ（57819細胞）にマッピングされ、トランスクリプトームをベースにした空間的シングルセル解析が可能に。
- 解析ツール**STVEA**がGitHubに公開されており、利用可能。
- Original Citation: <https://advances.sciencemag.org/content/7/10/eabc5464>

共通するタンパク質のデータで相関解析→
mRNAデータを組織切片データにマッピングして空間的シングルセルトランスクリプトーム解析に応用

Human Biomolecular Atlas Program (HuBMAP) 関連

Highly Multiplexed Immunofluorescence of the Human Kidney using CODEX



Kidney International

Volume 101, Issue 1, January 2022, Pages 137-143

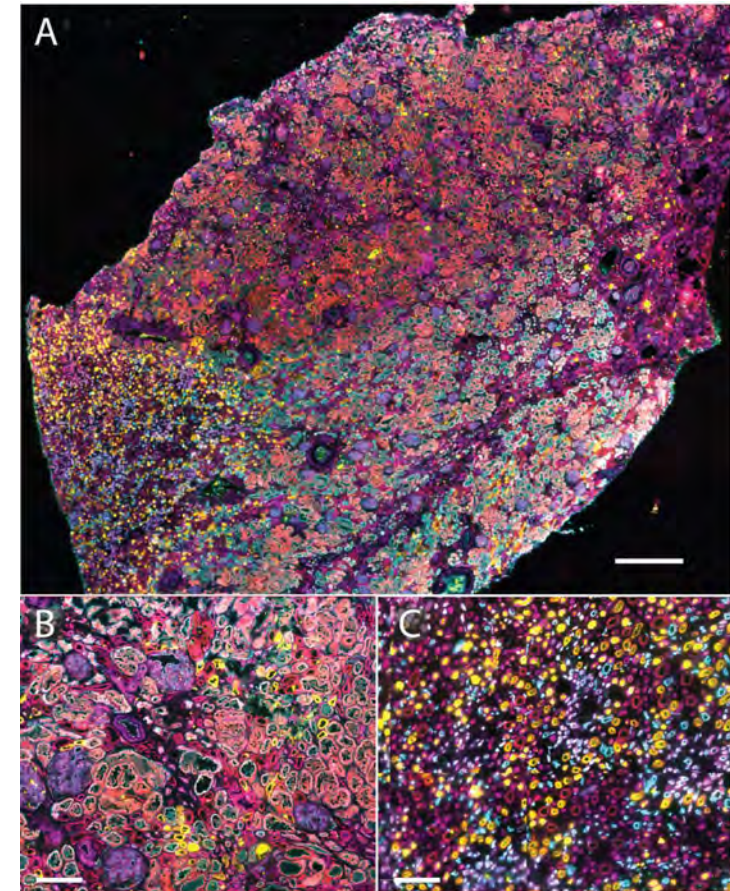


Technical Notes

Highly multiplexed immunofluorescence of the human kidney using co-detection by indexing

Elizabeth K. Neumann^{1,2}, Nathan Heath Patterson^{1,2}, Emilio S. Rivera^{1,2}, Jamie L. Allen^{1,2}, Maya Brewer³, Mark P. deCaestecker³, Richard M. Caprioli^{1,2,4}, Agnes B. Fogo^{3,5,6,7}, Jeffrey M. Spraggins^{2,4,8} ✉

- PhenoCycler (CODEX)で新鮮凍結に使用できる23の腎臓組織マーカーパネルを構築
- カスタムコンジュゲート抗体が機能し、柔軟性を評価
- Imaging Mass cytometryに比べて広い領域 (mm²) のイメージングが可能である点を評価
- 多様な微小環境のシングルセルレベルの解析に利用可能であることが確認された



STAR Protocols

CellPress
OPEN ACCESS

Protocol

Protocol for multimodal analysis of human kidney tissue by imaging mass spectrometry and CODEX multiplexed immunofluorescence

今後の展開① >100Plexのタンパク質検出の実現 (今年中の予定)

Industry's First 103-plex Whole-Slide Dataset

Immune Cell Profiling Core	Advanced Lymphocyte Module	Advanced Myeloid Module	Structural Module	Metabolism	Stress & Death	Advanced Immune Module
CD4	CD107a	CD163	E-cadherin	ASCT2	PARP	HLA-DPB1
CD68	CD21	CD11b	CD31	Na/K ATPase	BAK	LEF1
CD20	Pax5	MPO	Histone H3	HK1	BCL-XL	GAL9
CD11c	FOXP3	CD141	β-actin	LDHA	BAX	CD138
CD8	Gran B	iNOS	Podoplanin	G6PD	BAD	MC Tryptase
HLA-DR	CD38	Immune Activation Module	SMA	IDH2	Cyt. c	OX40
Ki67	CD39		Vimentin	GLUT1	LC3B	ZAP70
CD45RO	CD79D	PD-1	Collagen IV	pNRF2	Beclin-1	CD7
PanCK	TIGIT	PD-L1	CD34	ATPA5	H2AX	CIQA
CD3D	Tissue Specific/ Custom	ICOS	β-catenin	SDHA	Proliferation	CCR6
CD44		TIM3	MMP-9	Cit. Syn		CD15
CD45	TP63	LAG3	ZEB1	CPT1A	pRPS6	AXL
HLA-A	S100A4	IDO1			PCNA	CD227
CD14	TFAM	CD40			Cyc D1	
CD56	GP100	HLA-E				
CD57	LaminB1	IFNG				
CD19	CK17	CD69				
CD2	CK19					
CD1a	GATA3					
	SOX2					
	EpCAM					
	Caveolin					

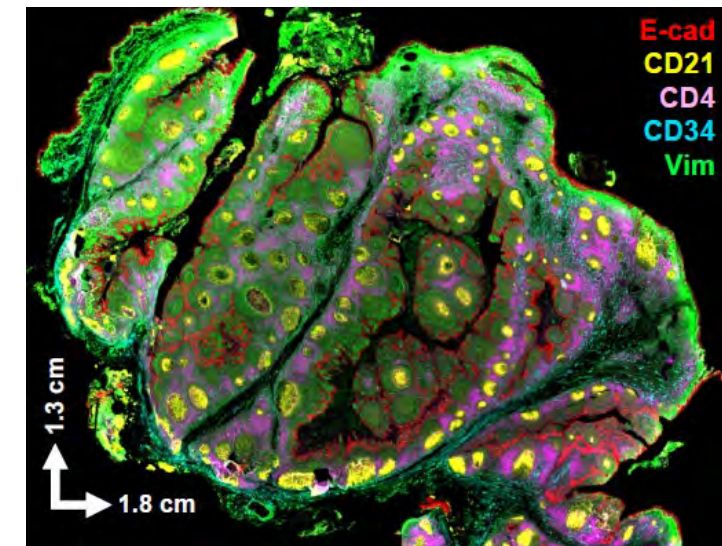
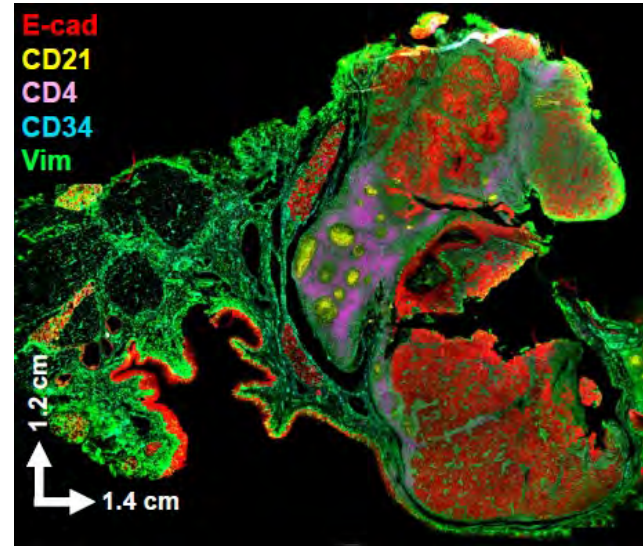
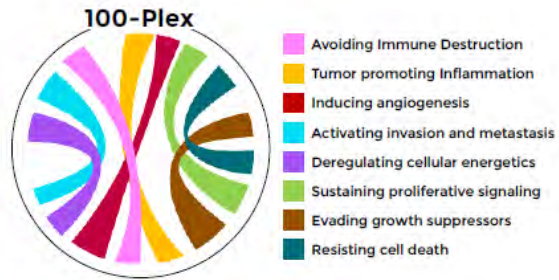


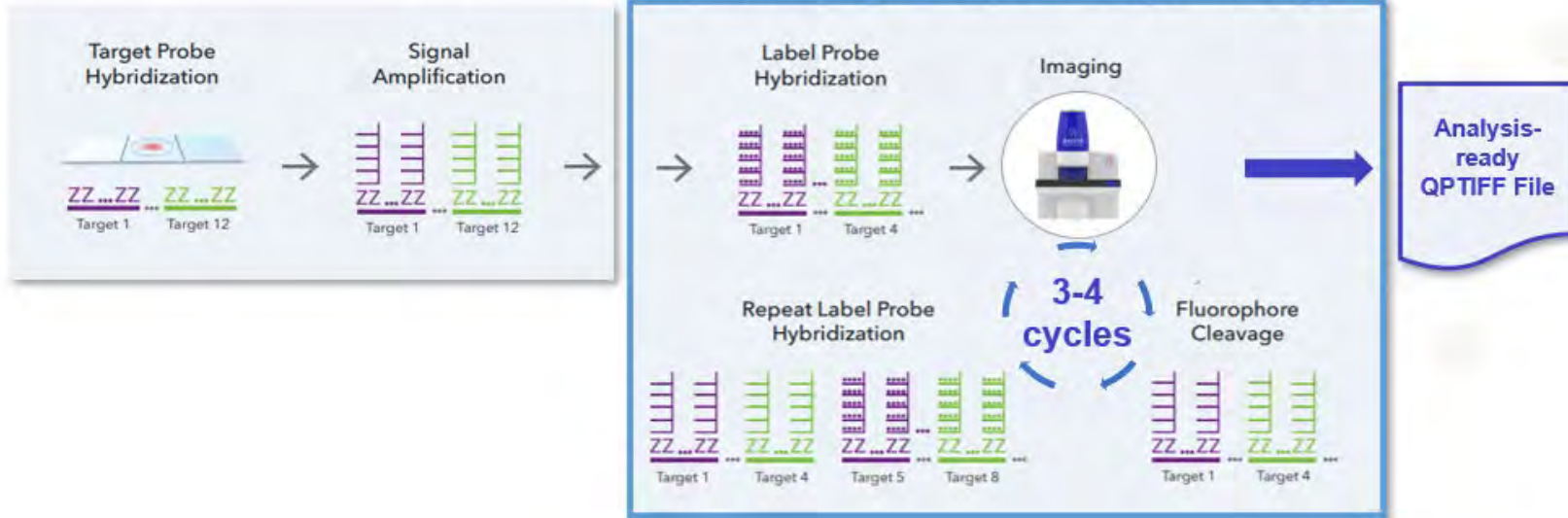
TABLE 1	SPECIMEN	
Tissue Type	FFPE Oropharyngeal Squamous Cell Carcinoma	FFPE Human Tonsil
Markers	103 Antibodies	103 Antibodies
Tissue imaging area	1.2 cm x 1.4 cm	1.3 cm x 1.8 cm
Fluidics time	38 cycles 44 hours	38 cycles 50 hours
Data size	24 gigabytes	46 gigabytes
Number of cells	832,823 cells	2,253,539 cells

今後の展開② 12plex RNAScopeの実現 (今年中の予定)

RNAScope HiPlex v2



Automated HiPlex v2 Workflow on PhenoCycler-Fusion System



今後の展開③ 独自開発の100plex RNA検出、タンパク質とRNAの同時検出の実現 (来年前半の予定)

3. Spatial Transcriptomics Workflow is Compatible with a Broad Set of Formalin-fixed, Paraffin-embedded (FFPE) Tissues

3.1 104-Plex RNA Workflow on Human Tissue Microarray

RNA detection across a human tissue microarray on the PhenoCycler-Fusion with a 104-plex RNA panel. Detection of RNA targets produces distinct labeling patterns in different tissue types. All cores are 2mm in diameter and all samples are human FFPE.

3.2 102-Plex RNA Workflow on Human HNC Squamous Cell Carcinoma Sample

RNA spatial phenotyping of a human head and neck cancer tissue on the PhenoCycler-Fusion with a 102-plex RNA panel. The images show a whole slide view (left) and zoomed-in ROIs to illustrate different marker combinations and tissue features.

5. Assay Compatibility Enables Same-Section Multiomic Spatial Phenotyping

Same section, spatial multiomic data

ACKR1 CD21-P CD45-P CD68 ICHAM1 MPO-P PanCK-P

B

- B cells
- Connective Tissue
- Endothelial cells
- Epithelial cells
- Glandular cells
- Macrophages
- Necrotic Tissue
- Other
- Plasma cells
- T cells
- Tumor cells

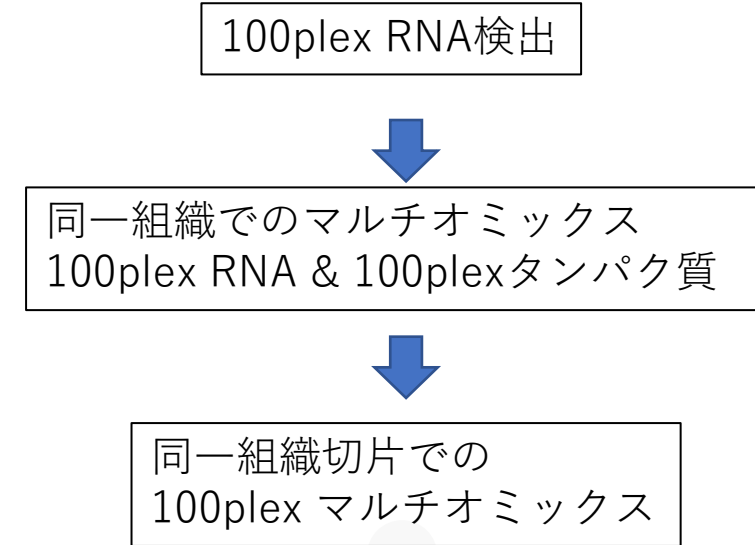
C

CD68-P ANXA1 IML KRT10 DAPI TP53 Keratin 5-P

D

CD68-P ANXA1 IML KRT10 DAPI TP53 Keratin 5-P

(A) Same-section, multiomic spatial phenotyping of head and neck cancer tissue. The compatibility of our high-plex protein and RNA detection assays enables multiomic analyses on the same tissue section. (B) Spatial projection of key cell types identified through clustering of the multiomic dataset. (C) The same ROI as shown in the RNA-only panel in Section 4.1, but with protein targets highlighted for emphasis. (D) The same ROI as shown in the RNA-only panel, but with additional protein markers, including additional Keratins and CD68 to denote macrophages. This structure shows the attack of a necrotic region of tissue and potential destruction of the Keratin border.



Spatial Multiomics Grant Program
Rapid, Unbiased *In Situ* RNA & Protein Detection
 Application Deadline: September 30, 2022

CODEX技術を用いた空間的シングルセル解析 まとめ

- CODEX技術を利用して～60種類のタンパク質を標的にシングルセルの解像度での多重蛍光免疫染色が可能で、以下のアプリケーションで主に使用されている
 - ①微小環境におけるシングルセルレベルでの細胞間相互作用、領域間相互作用の解析
 - ②セルタイプの存在を証明する目的でSpatial TranscriptomeデータのValidationに使用
 - ③scRNAseq (CITE-seq)とのデータ統合による空間的シングルセルトランスクリプトーム解析
- セルセグメンテーションおよびセルタイピングの精度向上が研究テーマの一つになっており、核をベースにした手法から、細胞質、細胞膜のマーカースも含め、かつAIを利用して細胞境界の予測、セルタイピングを行う手法の開発がすすめられている。
- 技術的にはスライドガラスの全面スキャンが可能になっており、5スライド/週で解析が可能
- 100plexのタンパク質またはRNAの検出が可能になるとともに（今年度）、RNAとタンパク質の同時検出も可能になる予定（来年度）
- レセプターリガンド解析や細胞内局在の解析に耐える解像度の実現が今後の課題

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