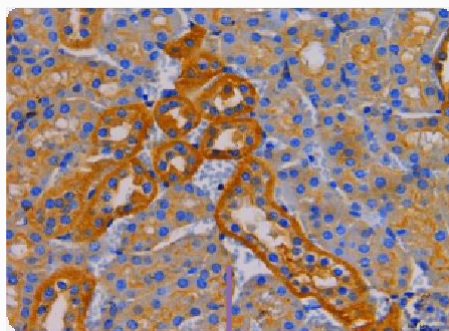


基于特殊染色方式的原位空间DSP分析策略

李振亚 博士

空间生物学的革命正在发生

探究微环境中组织结构、细胞亚型分布、特殊基因表达的重要性

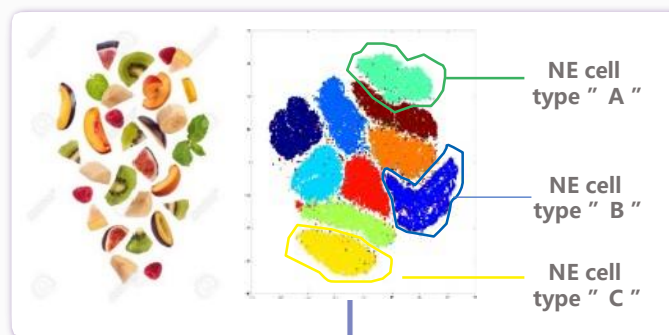


TCGA 和 bulk 组织
NGS 分析
2006 年至今

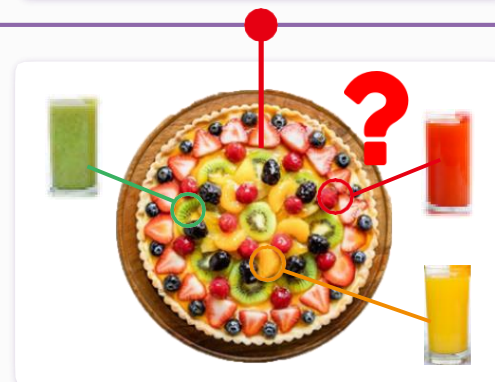
组织形态及功能
传统方式



单细胞测序，细胞分群
2012 年至今



组学结果如何与组织原位空间
信息对应起来进行验证?



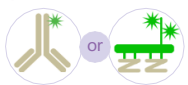
DSP技术原理及流程

- 石蜡样本/冰冻切片/穿刺样本等多样本、珍贵样本策略
- 前处理简单, 无需优化, 视野区域广阔, 无需盲选

- 靶向检测, 对中低表达靶点友好
- 自动化程度高, 结果可重复性强

特定标记荧光抗体(或探针) 与所选待测指标共孵育

形态学标记荧光抗体或RNA探针



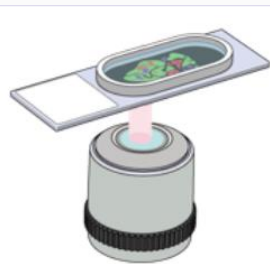
蛋白检测 待测靶点抗体+特定标记荧光抗体(或 探针) 共孵育



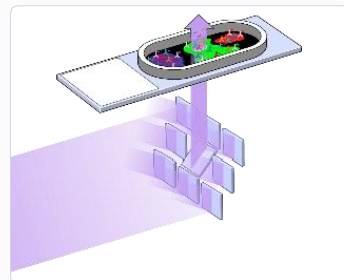
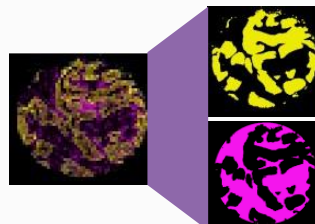
RNA 检测 探针孵育+特定标记荧光抗体(或探针) 共孵育



形态学标记荧光成像 ROI (感兴趣区域) 圈选

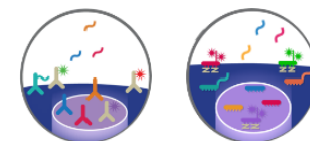


区域划分 (Segment, 可选) 紫外光解 DSP barcode

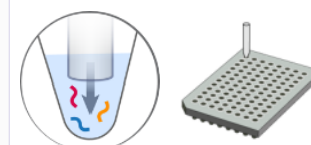


逐个 ROI 收集 DSP barcode 到 96 孔收集板

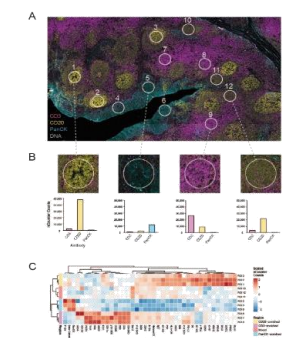
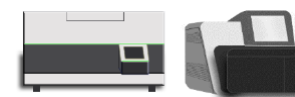
紫外光照射, linker断裂, DSP barcode解离至上层缓冲液



毛细管将解离的DSP barcode收 集至96孔板



计数、测序和数据分析



DSP空间组学的兴趣区域(ROI)选择

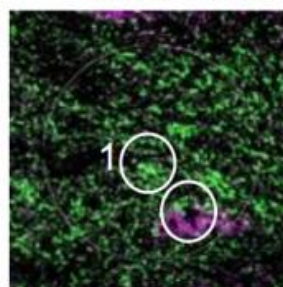
ROI的选择是DSP技术最大化应用的核心环节

Five Unique Fluorescent Profiling Modalities

Geometric



CD3 PanCK DNA

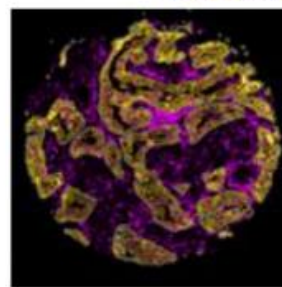


Assess heterogeneity
(e.g. tumor margin vs. core, cortical layers)

Segmentation

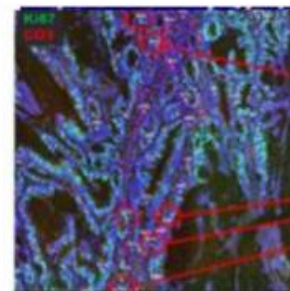


PanCK DNA



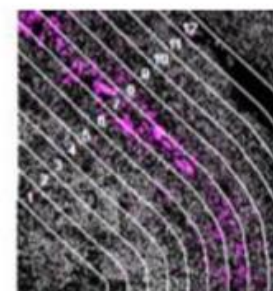
Evaluate biological compartments
(e.g. Tumor-TME)?

Cell Type/Rare cell



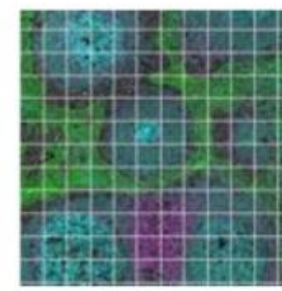
Profile cell populations
(e.g. CD3+ cells, GFAP+ cells)

Contour



Determine proximal response
(e.g. CART, AD plaque)

Gridded

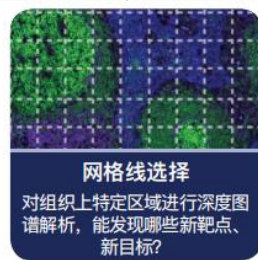
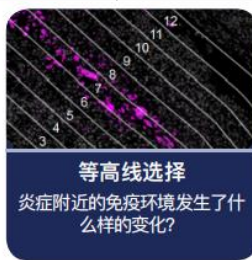
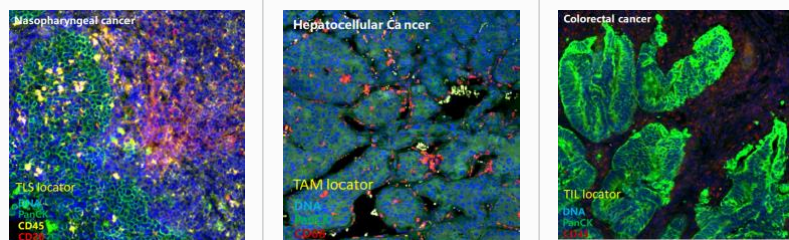


Perform deep mapping
(e.g. 96 ROI)

DSP空间组学的兴趣区域(ROI)选择

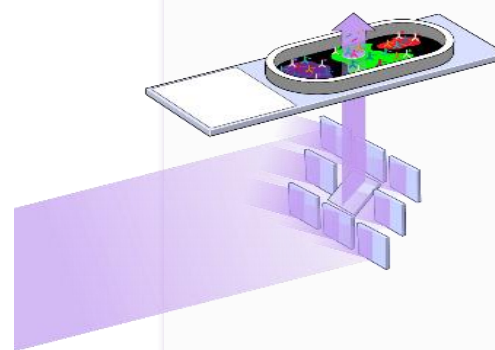
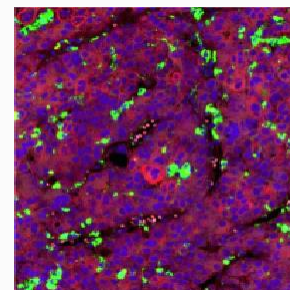
ROI的选择是DSP技术最大化应用的核心环节

特定组织结构区域选取策略

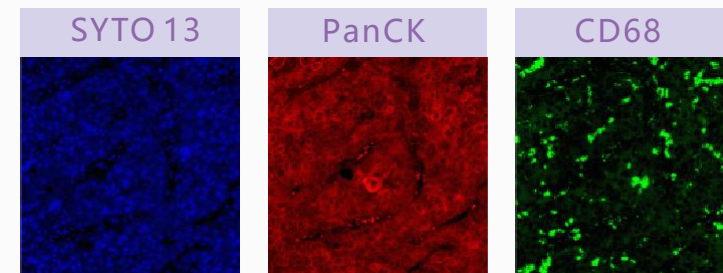


特定细胞群选取策略

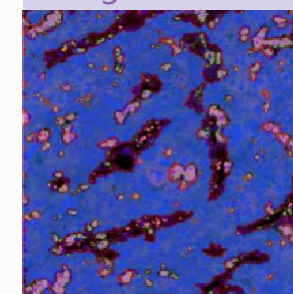
Full Channel Overlay



Single Channel (Mask)



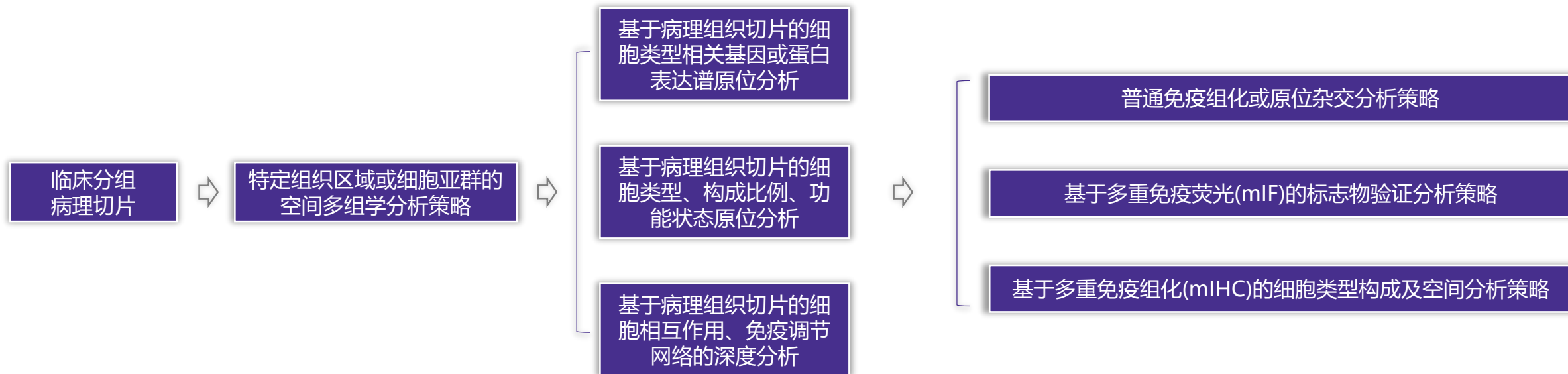
Segmentation



基于临床病理切片的常见空间分析策略组合

基于数字化空间分析技术(DSP)的深度分析策略

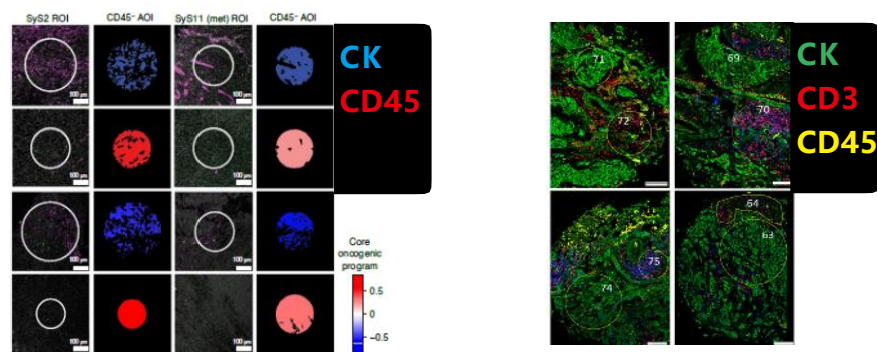
其他验证性空间分析策略



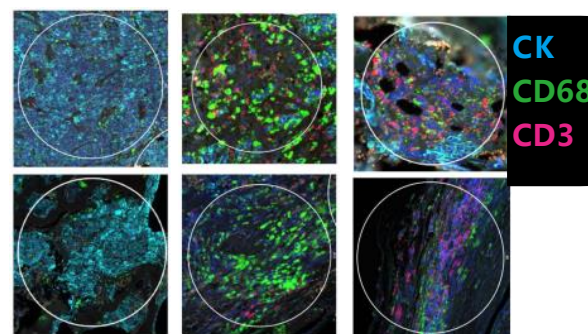
DSP空间组学分析相关细胞标志物选择

合适的组织结构或细胞群标志物，有助于ROI的选择和后续分析。

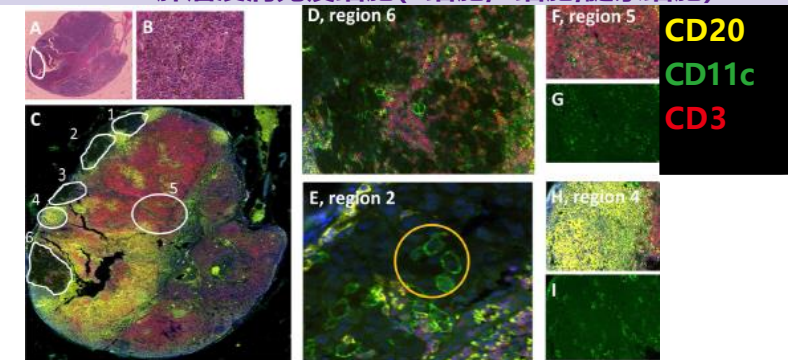
肿瘤及肿瘤浸润免疫细胞(白细胞, T细胞)



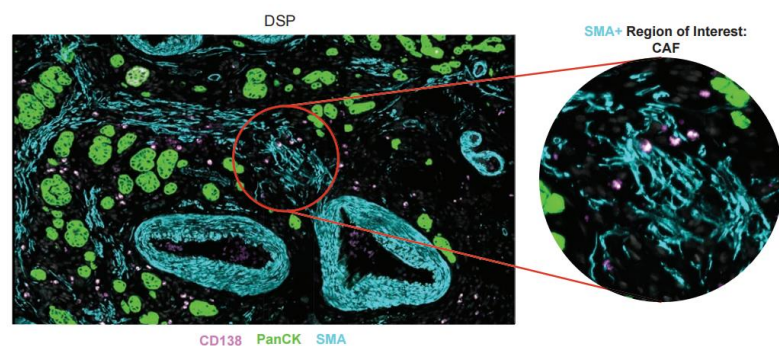
肿瘤及肿瘤浸润免疫细胞(T细胞,巨噬细胞)



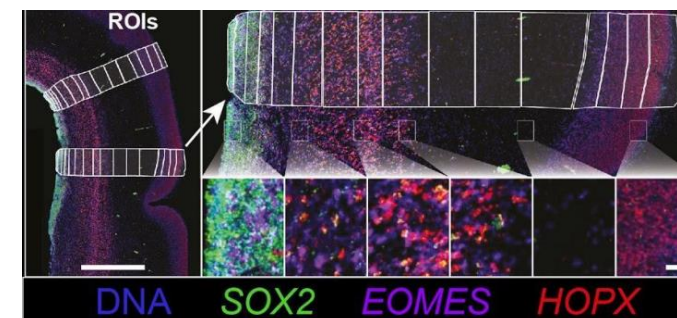
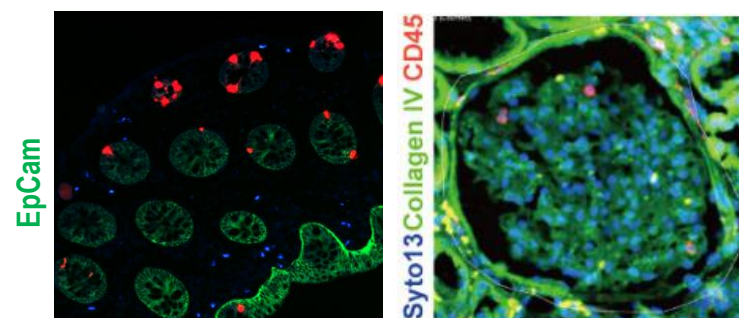
肿瘤浸润免疫细胞(T细胞,B细胞,髓系细胞)



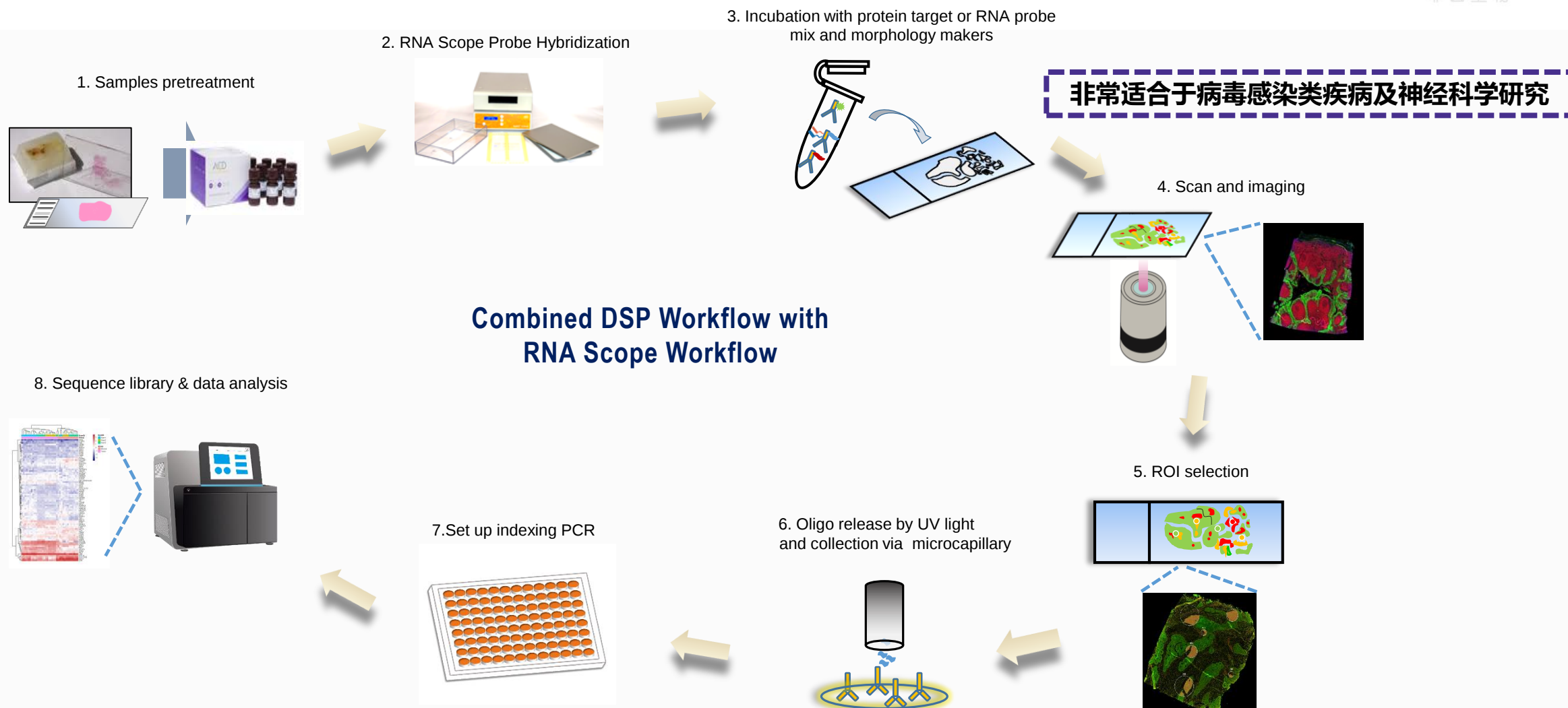
肿瘤相关基质细胞(成纤维类)



其他特殊标志物染色

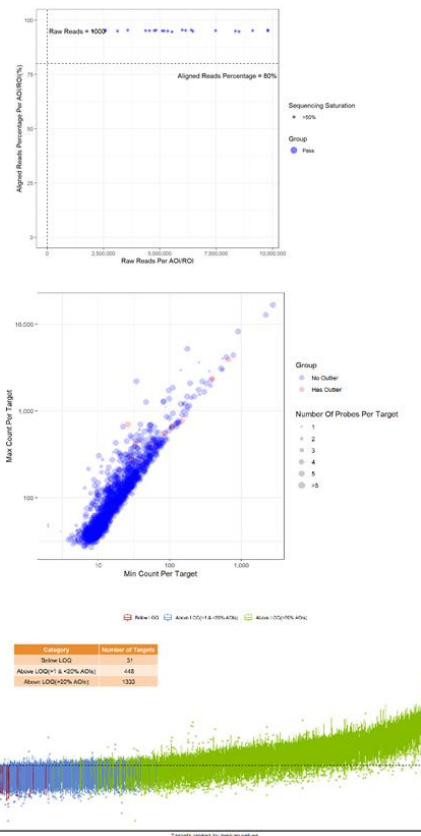
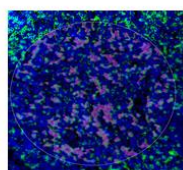
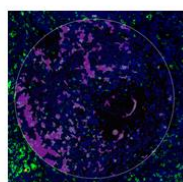
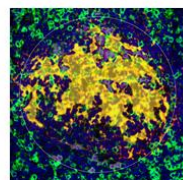
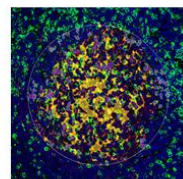
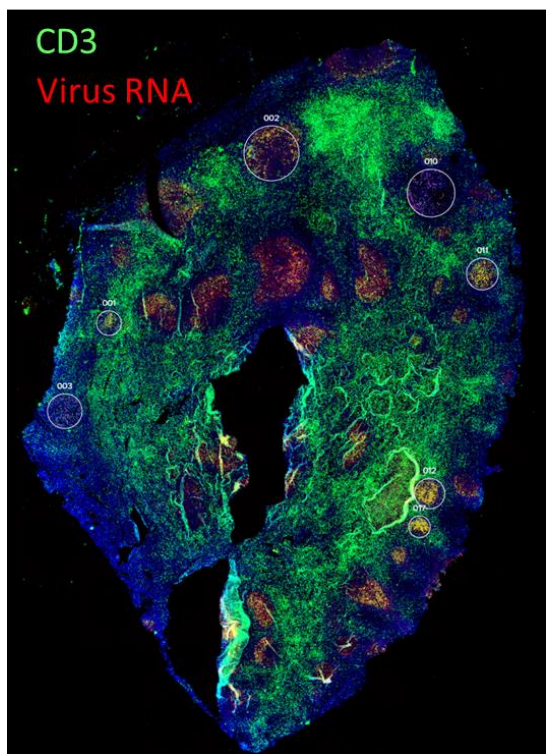


结合RNAScope技术的DSP空间分析策略



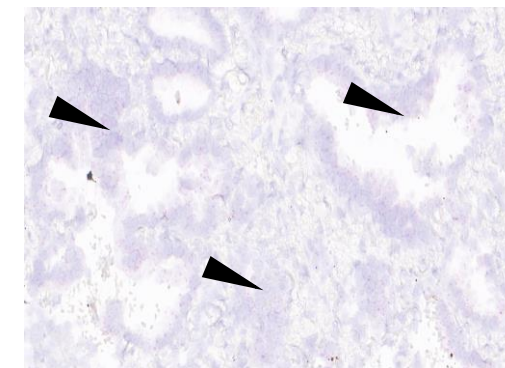
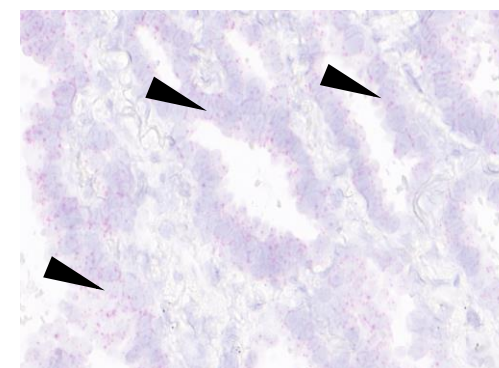
结合RNA原位杂交的DSP空间分析策略

RNAScope

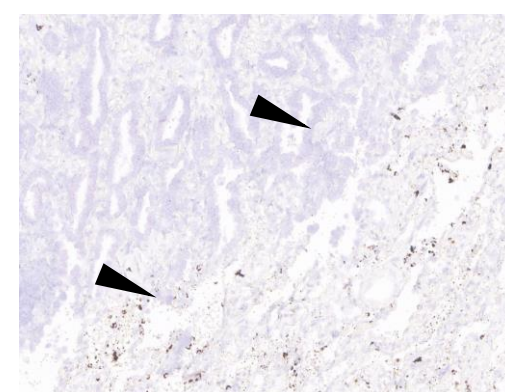
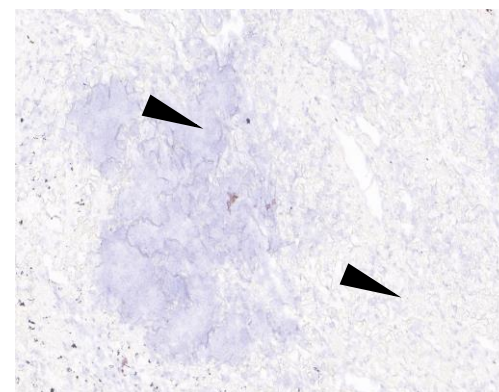


Base Scope

Amplified HER2

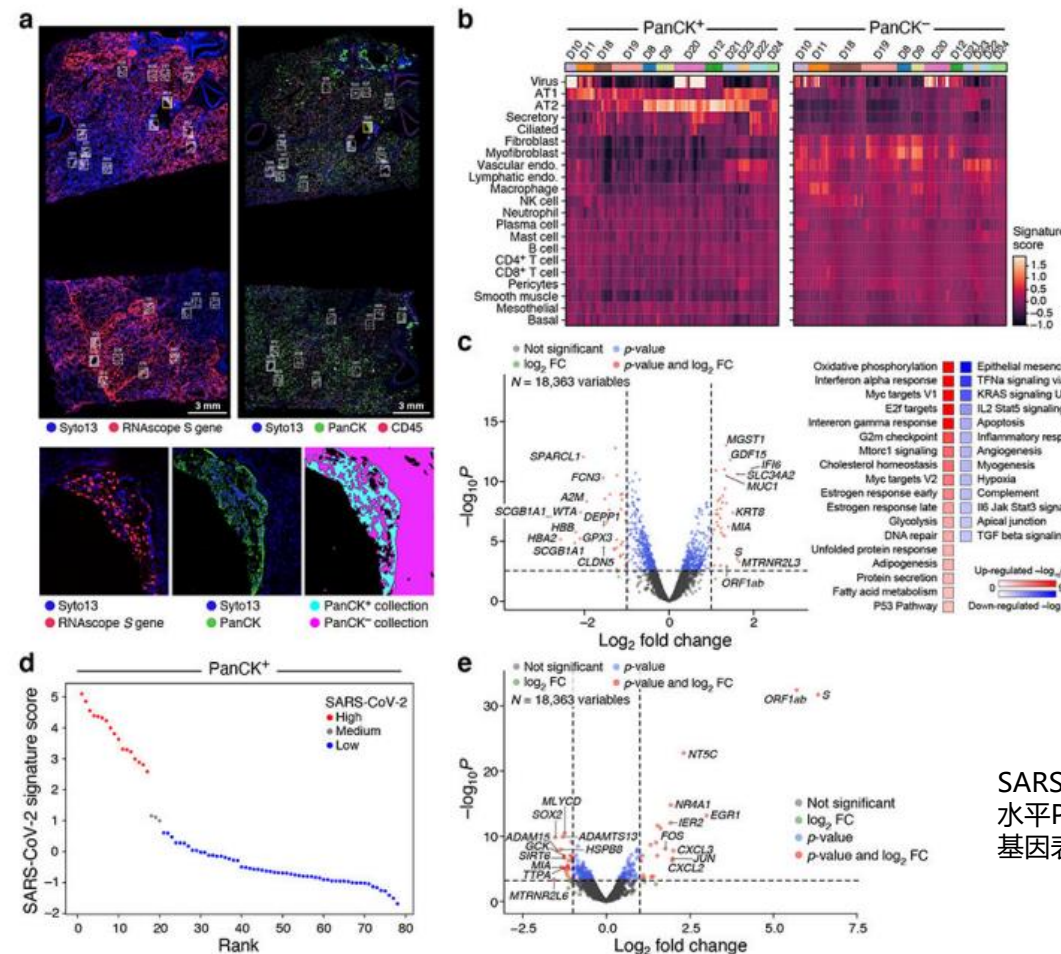
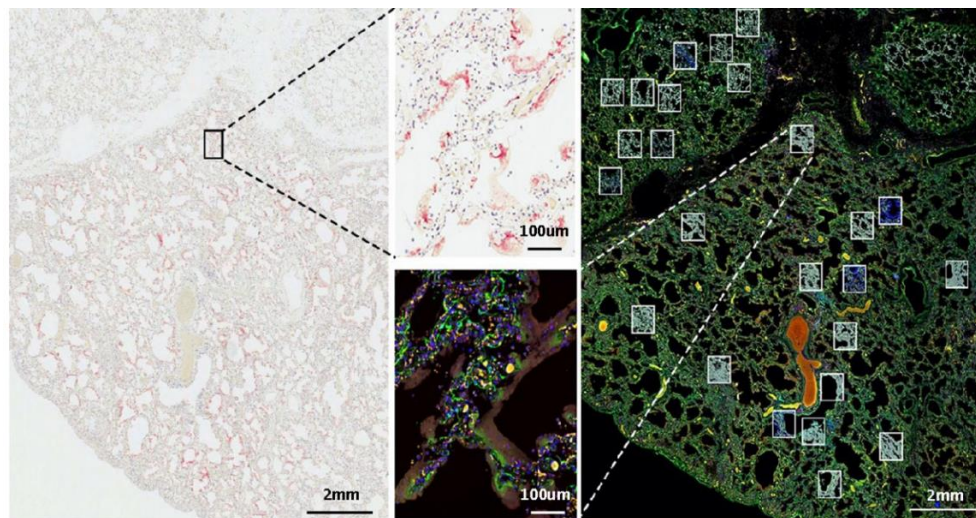


Non-Amplified regions



RNA原位杂交分析+DSP分析的研究的策略

利用RNAScope去确定病毒分布，根据病毒分布确定兴趣区域，利用DSP-WTA技术，获得数据后分析病毒造成肺组织结构转变及免疫应答的变化

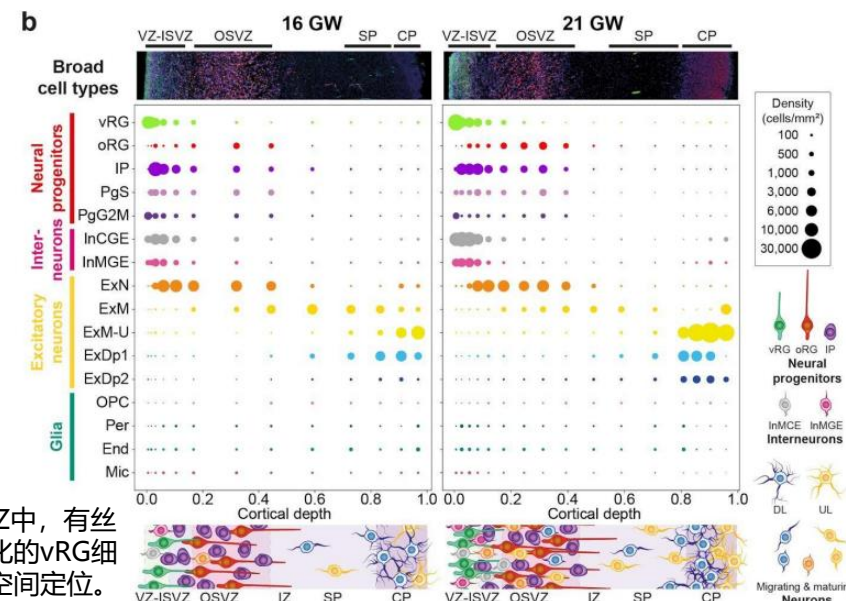
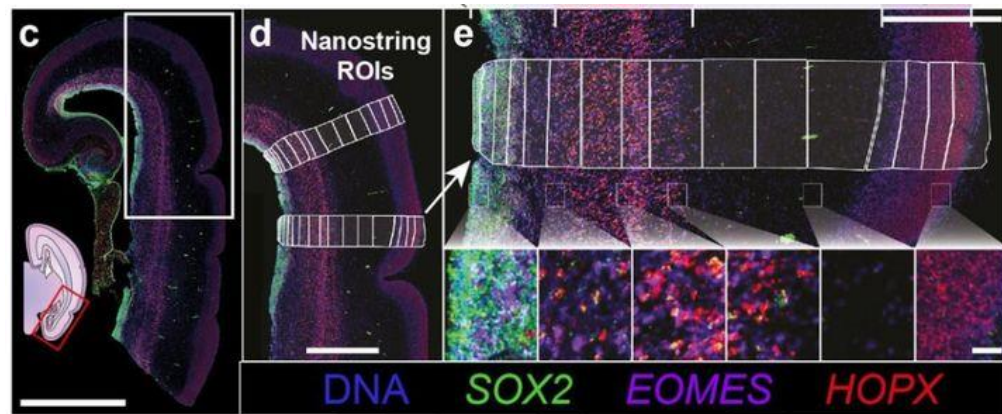


PanCK+和PanCK-的肺泡AOIs之间，以及健康人及COVID感染者AOI之间的细胞组成差异

健康人及COVID感染者PanCK+ AOI之间的基因表达差异及功能富集特征

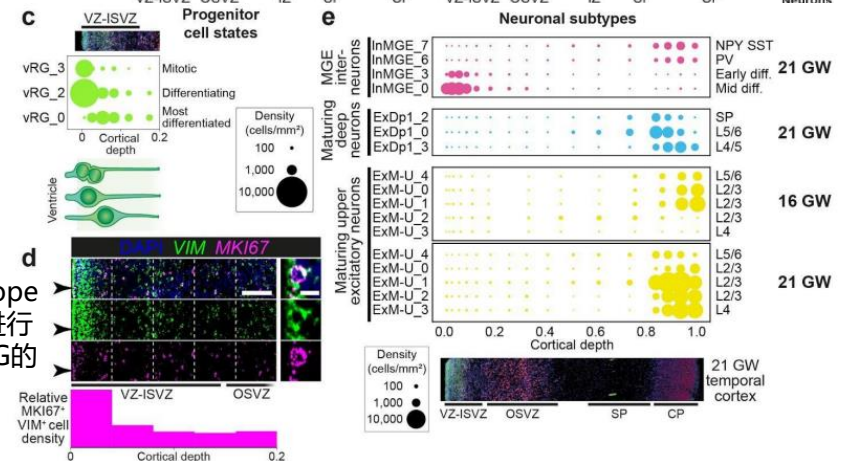
SARS-CoV-2高水平和低水平PanCK+ AOI之间的基因表达差异

DSP空间组分析绘制发育中人类新皮质的细胞结构



在16G和21GW时，横跨
颞叶皮层深度的多细胞类型
的空间图谱，利用
WTA-Cell2Location进行
细胞密度分析

在VZ-ISVZ中，有丝
分裂和分化的vRG细胞
状态的空间定位。



神经元亚型的映射揭示
了不同的空间定位，反
映了皮层发育过程的
构成和分化时序

利用
RNAscope
和mIF进行
分裂vRG的
定位

(1) Cell type reference signatures
(Polioudakis et al.)

(3) Spatial locations
of cell types

(2) Spatially resolved
gene expression profiles

Human fetal
cortex

scRNA-seq

NanoString
WTA

Gene 1
Gene 2
Gene 3
Gene n

Cortical depth

cell2location
WTA model

Density (cells/mm²)

oRG density

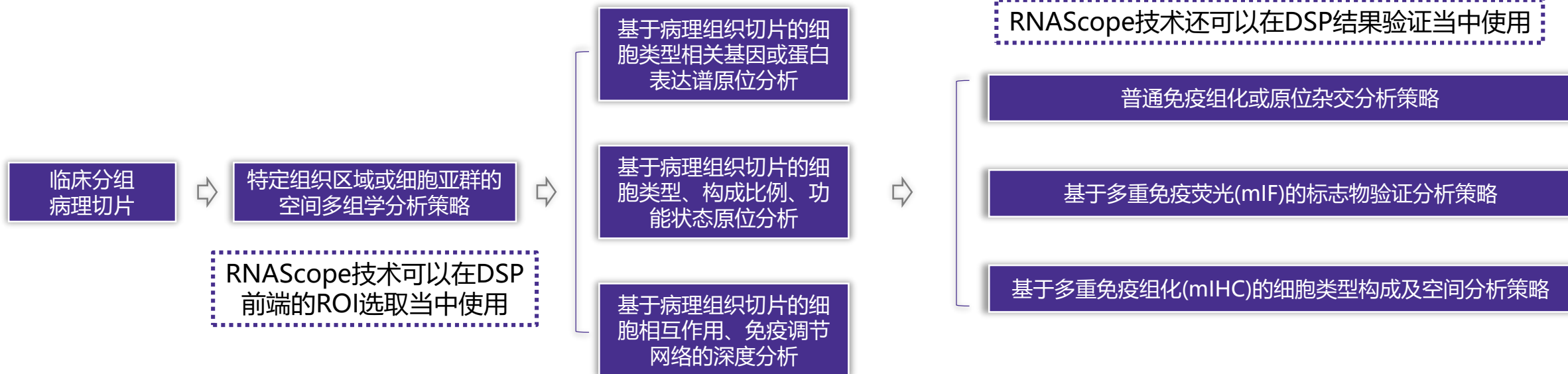
Cortical depth

基于scRNA-seq和DSP-WTA数据，利用Cell2location算法对细胞类型进行空间映射的工作流程

结合RNAScope技术的空间分析策略组合

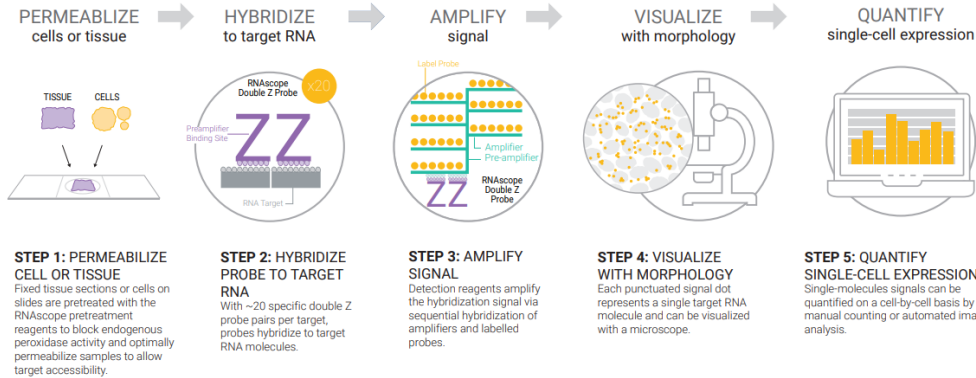
基于数字化空间分析技术(DSP)的深度分析策略

其他验证性空间分析策略



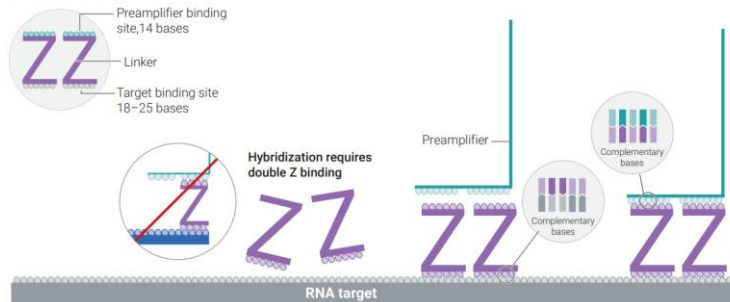
RNAScope技术的原理

RNAscope Assay Workflow

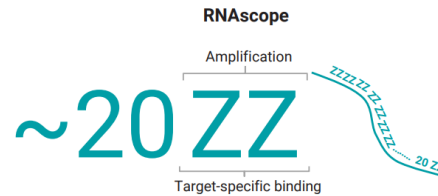
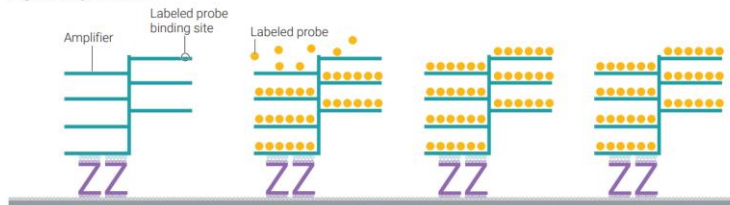


Probe design

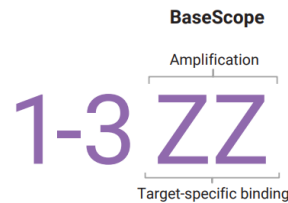
Z target probe design



Signal amplification



- 20 ZZ pairs per each mRNA or lncRNA target
- Single RNA molecule detection
- mRNA or lncRNA target (>300nt)



- 1-3 ZZ pair per target
- Single RNA molecule detection
- Exon junction/splice variants, short target/highly homologous sequences (50-300 nt) and point mutations

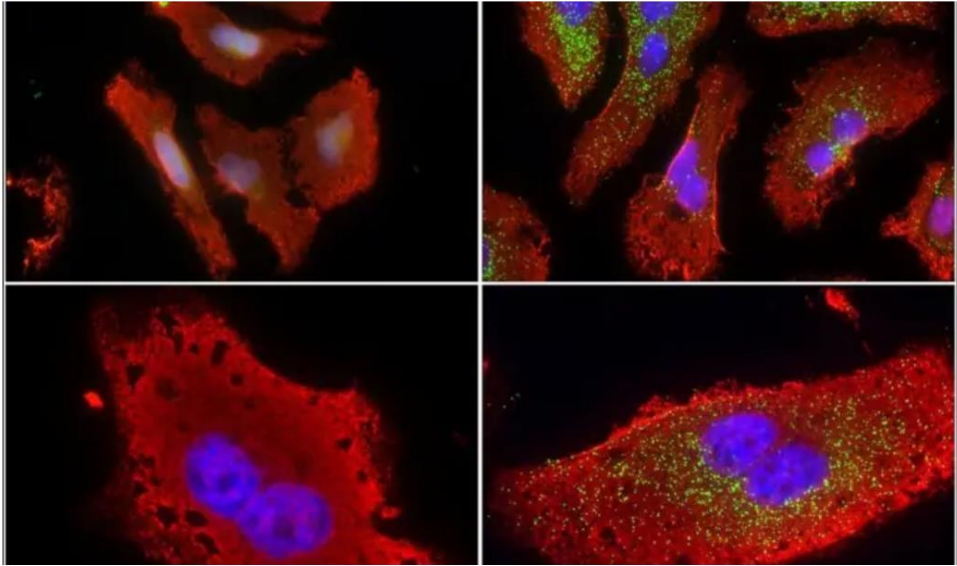
	RNAscope Assay	RNAscope HiPlex Assay	BaseScope Assay
Number of ZZ Pair per target	• Standard probe design is 20 ZZ probes (minimum of 6 ZZ probes)	• Standard probe design is 20 ZZ probes (minimum of 6 ZZ probes)	• 1 to 3 ZZ probes based on the application
Target	• mRNA >300 bases • lncRNA >300 bases	• mRNA >300 bases • lncRNA >300 bases	• Exon junctions/ splice variants • RNA targets between 50 to 300 nucleotides • Validated point mutations
Application	• lncRNA and mRNA >300 bases	• lncRNA and mRNA >300 bases	• Exon junctions/splice variants, circular RNA, gene fusion, gene knockout • Short/highly homologous sequences, TCRs and CDR sequence for T cell clones, pre-miRNA, gene editing/CRISPR, CAR-T cell validation and detection • Point mutation, short InDel, homologues
Multiplex capability	Single to up to 4-plex	8 to 12-plex	Single to duplex
Detection	Chromogenic or fluorescent	Fluorescent	Chromogenic
Sample type	FFPE cells and tissues Fresh frozen tissues Fixed frozen tissues Cultured cells Whole mount	Fresh frozen tissues Fixed frozen tissues	FFPE cells and tissues Fresh frozen tissues Fixed frozen tissues Cultured cells



RNAScope探针的广泛应用

RNAscope Catalog Probes are available for over 100 different viral species and ever growing. Click on species name to see corresponding probes.

Adeno-associated virus	Alpaca Polyomavirus	Andes virus
Avian infectious bronchitis virus	Bat hepadnavirus	Bat hepatovirus
Bovine astrovirus	Bovine papillomavirus	Bovine parvovirus
Canine bocavirus	Canine herpesvirus	Canine papillomavirus
Canine parvovirus	Cercopithecine herpesvirus	Chikungunya virus
Circovirus	Citrus tristeza virus	COVID-19 coronavirus
Cynomolgus macaque cytomegalovirus	Dengue virus	Eastern equine encephalitis virus
Enterovirus	Equid herpesvirus	Equine arteritis virus

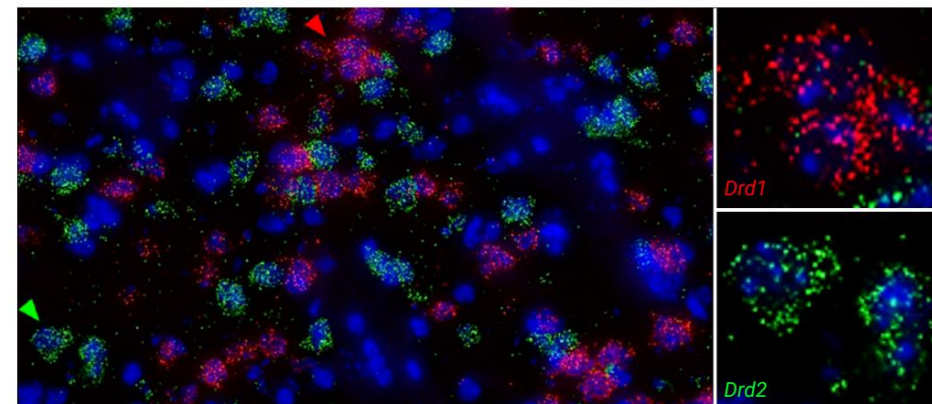
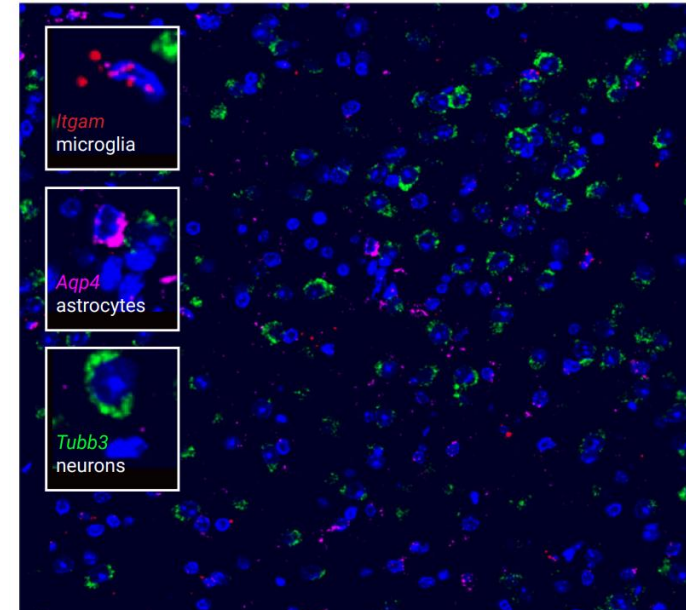
Feline herpesvirus	Feline immunodeficiency virus	Feline infectious peritonitis virus
Feline papillomavirus	Frog virus	Hedgehog hepatovirus
Hendra virus		
Hepatitis C virus		
Hepatitis GB Virus		
Human coronavirus		
Human herpesvirus	HCV-uninfected (left) or HCV-infected (right) HuH-7 cells were hybridized with probes targeting HCV mRNA (green). Cells were costained with 18S rRNA target probes (red) as an internal control for assay success. Nuclei were counterstained with DAPI (blue).	
Human parechovirus		
Human T-lymphotropic virus 1		



RNA Scope 探针的广泛应用

Cell Type	Markers*
Neuroepithelial cells	<i>Nestin (Nes)</i> , <i>Sox2</i> , <i>Notch1</i> , <i>Hes1</i> , <i>Hes3</i> , <i>E-cadherin (Cdh1)</i> , <i>Occludin (Ocl)</i>
Radial glia	<i>Vimentin (Vim)</i> , <i>Nestin (Nes)</i> , <i>Pax6</i> , <i>Hes1</i> , <i>Hes5</i> , <i>Gfap</i> , <i>Glast (Slc1a3)</i> , <i>Blbp (Fabp7)</i> , <i>Tnc</i> , <i>N-cadherin (Cdh2)</i> , <i>Sox2</i>
Intermediate progenitors	<i>Tbr2 (Eoms)</i> , <i>Mash1 (Ascl1)</i>
Immature neurons	<i>Doublecortin (Dcx)</i> , β III tubulin (<i>Tubb3</i>), <i>NeuroD1</i> , <i>Tbr1</i> , <i>Stathmin 1 (Stmn)</i>
Oligodendrocyte precursor cells	<i>Pdgfr alpha (Pdgfra)</i> , <i>Ng2 (Cspg4)</i>
Mature oligodendrocytes	<i>Olig1</i> , <i>Olig2</i> , <i>Olig3</i> , <i>Mbp</i> , <i>Osp (Spp1)</i> , <i>Mog</i> , <i>Sox10</i>
Schwann cells	<i>Mpz</i> , <i>Ncam</i> , <i>Gap43</i> , <i>S100</i>
Astrocytes	<i>Gfap</i> , <i>Eaat1 (Slc1a3)</i> , <i>Eaat2 (Slc1a2)</i> , <i>Glutamine synthetase (Glul)</i> , <i>S100-beta (S100b)</i> , <i>Aldh1l1</i>
Microglia	<i>Cd11b</i> , <i>Cd45</i> , <i>Iba1 (Aif1)</i> , <i>F4/80 (Emr1)</i> , <i>Cd68</i> , <i>Cd40</i>
Mature neurons	<i>NeuN (RbFox3)</i> , <i>Map2</i> , <i>160kDa neurofilament medium (Nefm)</i> , <i>200kDa neurofilament heavy (Nefh)</i> , <i>Synaptophysin (Syn)</i> , <i>Psd95</i>
Glutamatergic neurons	<i>Vglut1 (Slc17a7)</i> , <i>Vglut2 (Slc17a6)</i> , <i>Nmdar1 (Grin1)</i> , <i>Nmdar2b (Grin2b)</i> , <i>Glutaminase (Gls)</i> , <i>Glutamine synthetase (Glul)</i>
GABAergic neurons	<i>Gaba Transporter 1 (Slc6a1)</i> , <i>Gaba Receptor 1 (Gabra1)</i> , <i>Gaba Receptor 2 (Gabra2)</i> , <i>Gad 65 (Gad2)</i> , <i>Gas67 (Gad1)</i>
Dopaminergic neurons	<i>Tyrosine Hydroxylase (Th)</i> , <i>Dopamine Transporter (Chat)</i> , <i>FoxA2</i> , <i>Girk2 (Kcnj6)</i> , <i>Nurr1 (Nr4a2)</i> , <i>Lmx1b</i>
Serotonergic neurons	<i>Tryptophan Hydroxylase (Tph)</i> , <i>Serotonin Transporter (Slc6a4)</i> , <i>Pet1</i>
Cholinergic neurons	<i>Choline Acetyltransferase (Chat)</i> , <i>Vesicular Acetylcholine Transporter (Slc18a3)</i> , <i>Acetylcholinesterase (Ache)</i>

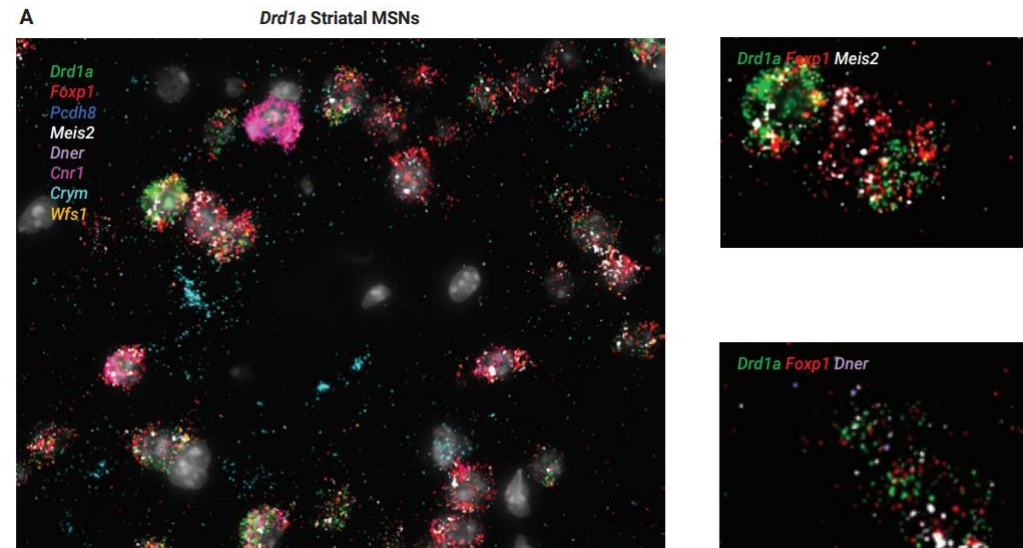
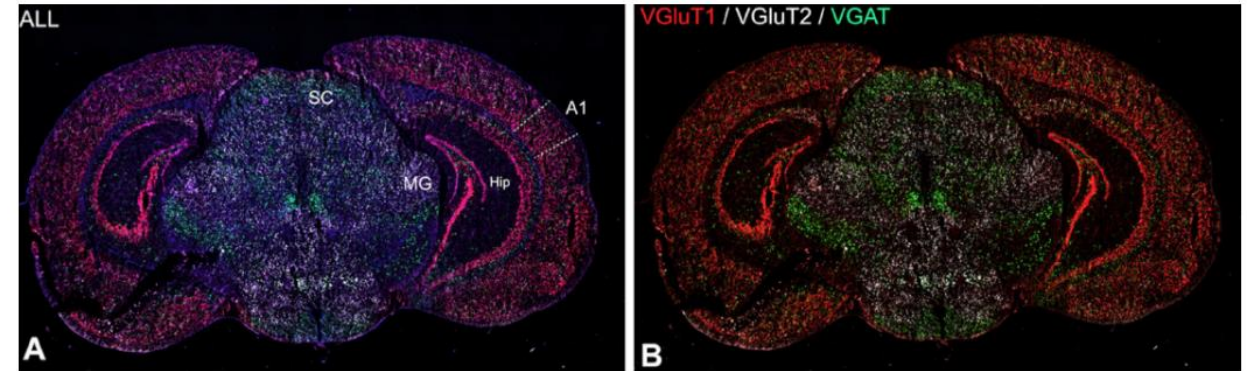
* Cell markers may not have been tested and confirmed internally by ACD; recommendations may be based on literature searches and protein detection by IHC therefore RNA levels may vary from protein levels



RNAScope探针的广泛应用

Cell Type	Markers*
Neuroepithelial cells	<i>Nestin (Nes), Sox2, Notch1, Hes1, Hes3, E-cadherin (Cdh1), Occludin (Ocl)</i>
Radial glia	<i>Vimentin (Vim), Nestin (Nes), Pax6, Hes1, Hes5, Gfap, Glast (Slc1a3), Blbp (Fabp7), Tnc, N-cadherin (Cdh2), Sox2</i>
Intermediate progenitors	<i>Tbr2 (Eoms), Mash1 (Ascl1)</i>
Immature neurons	<i>Doublecortin (Dcx), β III tubulin (Tubb3), NeuroD1, Tbr1, Stathmin 1 (Stmn)</i>
Oligodendrocyte precursor cells	<i>Pdgfr alpha (Pdgfra), Ng2 (Cspg4)</i>
Mature oligodendrocytes	<i>Olig1, Olig2, Olig3, Mbp, Osp (Spp1), Mog, Sox10</i>
Schwann cells	<i>Mpz, Ncam, Gap43, S100</i>
Astrocytes	<i>Gfap, Eaat1 (Slc1a3), Eaat2 (Slc1a2), Glutamine synthetase (Glul), S100-beta (S100b), Aldh1l1</i>
Microglia	<i>Cd11b, Cd45, Iba1 (Aif1), F4/80 (Emr1), Cd68, Cd40</i>
Mature neurons	<i>NeuN (RbFox3), Map2, 160kDa neurofilament medium (Nefm), 200kDa neurofilament heavy (Nefh), Synaptophysin (Syn), Psd95</i>
Glutamatergic neurons	<i>Vglut1 (Slc17a7), Vglut2 (Slc17a6), Nmdar1 (Grin1), Nmdar2b (Grin2b), Glutaminase (Gls), Glutamine synthetase (Glul)</i>
GABAergic neurons	<i>Gaba Transporter 1 (Slc6a1), Gaba Receptor 1 (Gabra1), Gaba Receptor 2 (Gabra2), Gad 65 (Gad2), Gas67 (Gad1)</i>
Dopaminergic neurons	<i>Tyrosine Hydroxylase (Th), Dopamine Transporter (Chat), FoxA2, Girk2 (Kcnj6), Nurr1 (Nr4a2), Lmx1b</i>
Serotonergic neurons	<i>Tryptophan Hydroxylase (Tph), Serotonin Transporter (Slc6a4), Pet1</i>
Cholinergic neurons	<i>Choline Acetyltransferase (Chat), Vesicular Acetylcholine Transporter (Slc18a3), Acetylcholinesterase (Ache)</i>

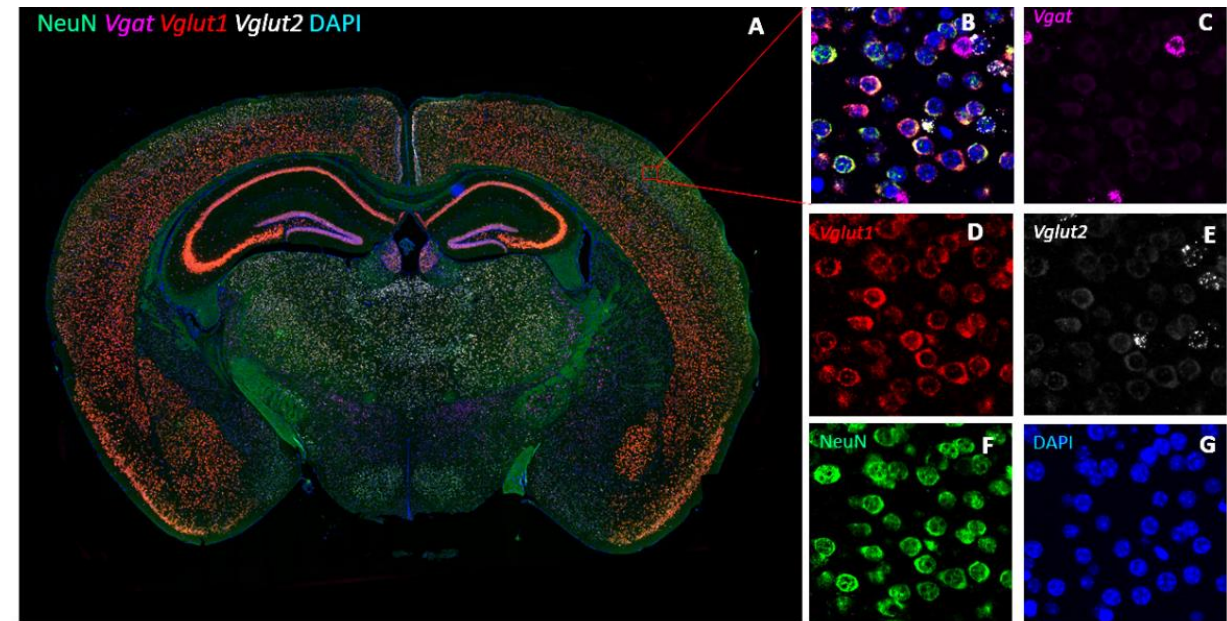
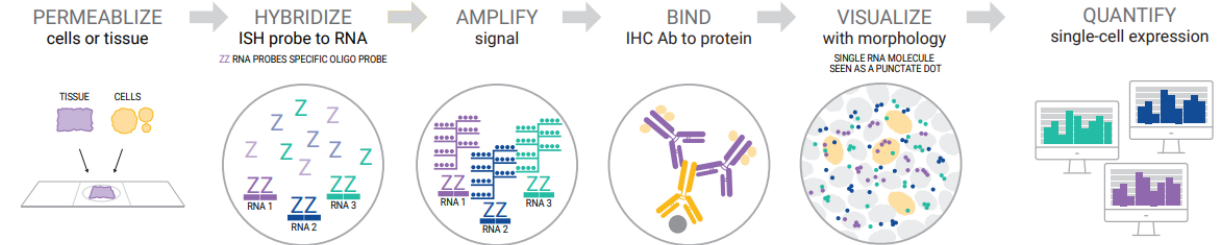
* Cell markers may not have been tested and confirmed internally by ACD; recommendations may be based on literature searches and protein detection by IHC therefore RNA levels may vary from protein levels



RNA Scope 探针的广泛应用

Cell Type	Markers*
Neuroepithelial cells	<i>Nestin (Nes)</i> , <i>Sox2</i> , <i>Notch1</i> , <i>Hes1</i> , <i>Hes3</i> , <i>E-cadherin (Cdh1)</i> , <i>Occludin (Ocl)</i>
Radial glia	<i>Vimentin (Vim)</i> , <i>Nestin (Nes)</i> , <i>Pax6</i> , <i>Hes1</i> , <i>Hes5</i> , <i>Gfap</i> , <i>Glast (Slc1a3)</i> , <i>Blbp (Fabp7)</i> , <i>Tnc</i> , <i>N-cadherin (Cdh2)</i> , <i>Sox2</i>
Intermediate progenitors	<i>Tbr2 (Eoms)</i> , <i>Mash1 (Ascl1)</i>
Immature neurons	<i>Doublecortin (Dcx)</i> , β III tubulin (<i>Tubb3</i>), <i>NeuroD1</i> , <i>Tbr1</i> , <i>Stathmin 1 (Stmn)</i>
Oligodendrocyte precursor cells	<i>Pdgfr alpha (Pdgfra)</i> , <i>Ng2 (Cspg4)</i>
Mature oligodendrocytes	<i>Olig1</i> , <i>Olig2</i> , <i>Olig3</i> , <i>Mbp</i> , <i>Osp (Spp1)</i> , <i>Mog</i> , <i>Sox10</i>
Schwann cells	<i>Mpz</i> , <i>Ncam</i> , <i>Gap43</i> , <i>S100</i>
Astrocytes	<i>Gfap</i> , <i>Eaat1 (Slc1a3)</i> , <i>Eaat2 (Slc1a2)</i> , <i>Glutamine synthetase (Glul)</i> , <i>S100-beta (S100b)</i> , <i>Aldh1l1</i>
Microglia	<i>Cd11b</i> , <i>Cd45</i> , <i>Iba1 (Aif1)</i> , <i>F4/80 (Emr1)</i> , <i>Cd68</i> , <i>Cd40</i>
Mature neurons	<i>NeuN (RbFox3)</i> , <i>Map2</i> , <i>160kDa neurofilament medium (Nefm)</i> , <i>200kDa neurofilament heavy (Nefh)</i> , <i>Synaptophysin (Syn)</i> , <i>Psd95</i>
Glutamatergic neurons	<i>Vglut1 (Slc17a7)</i> , <i>Vglut2 (Slc17a6)</i> , <i>Nmdar1 (Grin1)</i> , <i>Nmdar2b (Grin2b)</i> , <i>Glutaminase (Gls)</i> , <i>Glutamine synthetase (Glul)</i>
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Dopaminergic neurons	<i>Tyrosine Hydroxylase (Th)</i> , <i>Dopamine Transporter (Chat)</i> , <i>FoxA2</i> , <i>Girk2 (Kcnj6)</i> , <i>Nurr1 (Nr4a2)</i> , <i>Lmx1b</i>
Serotonergic neurons	<i>Tryptophan Hydroxylase (Tph)</i> , <i>Serotonin Transporter (Slc6a4)</i> , <i>Pet1</i>
Cholinergic neurons	<i>Choline Acetyltransferase (Chat)</i> , <i>Vesicular Acetylcholine Transporter (Slc18a3)</i> , <i>Acetylcholinesterase (Ache)</i>

* Cell markers may not have been tested and confirmed internally by ACD; recommendations may be based on literature searches and protein detection by IHC therefore RNA levels may vary from protein levels



nCounter技术广泛应用于多靶标表达验证



COVID-19 **Research Solutions** >



nCounter® **Host Response Panel** >



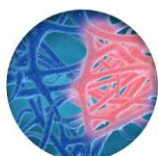
nCounter® **Immune Exhaustion Panel** >



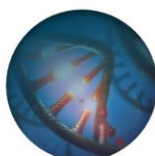
nCounter® **Alzheimer's Disease Panel** >



nCounter® **Glial Profiling Panel** >



nCounter® **Inflammation Panel** >



nCounter® **miRGE Assay** >



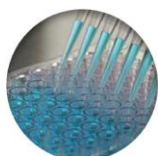
nCounter® **miRNA Expression Panels** >



nCounter® **Neuroinflammation Panel** >



nCounter® **Neuropathology Panel** >



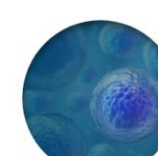
nCounter® **PlexSet™ Reagents** >



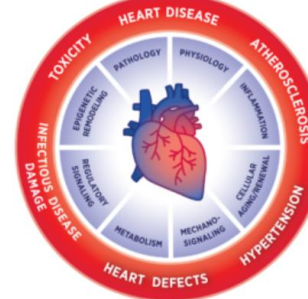
nCounter® **TCR Diversity Panel** >



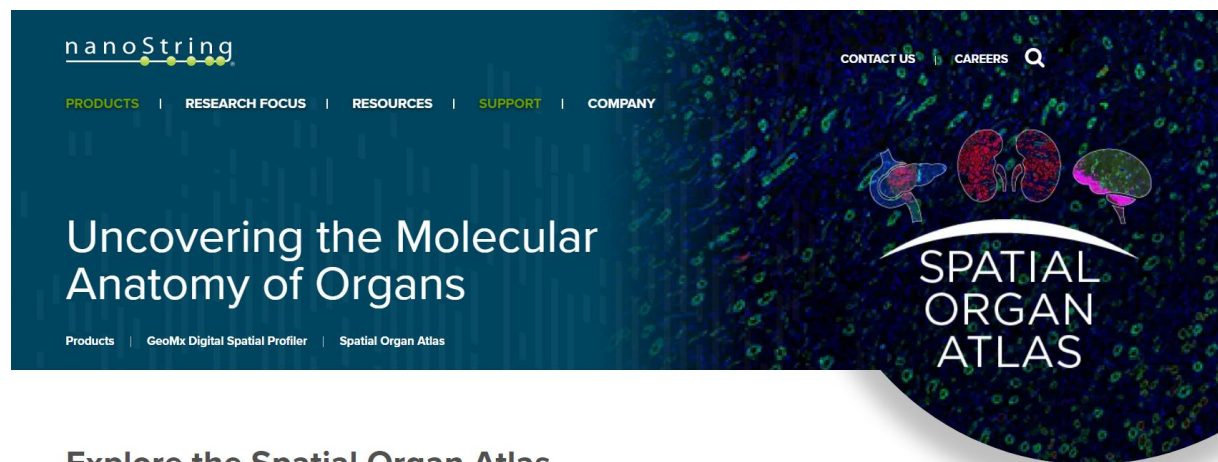
nCounter® **Viral Panel Plus Menu** >



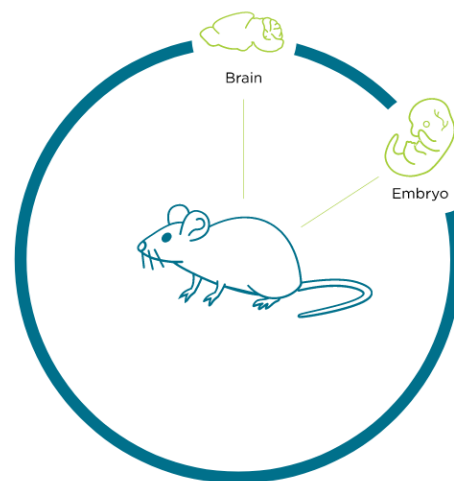
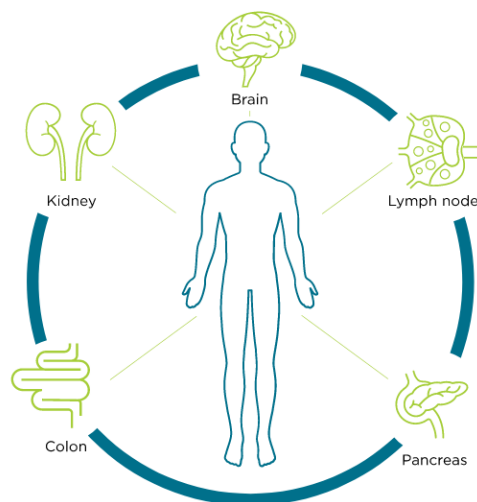
nCounter® **Stem Cell Characterization Panel** >



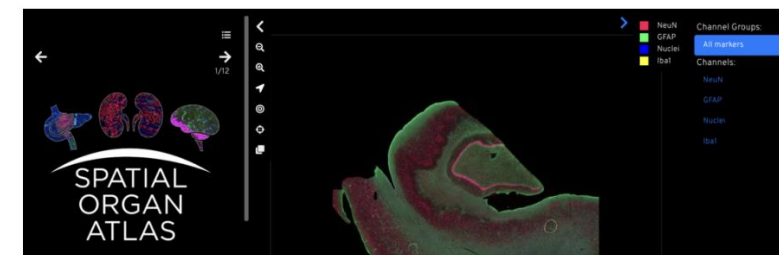
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HUMAN BRAIN METADATA

DATA DOWNLOADS

This cohort contains 5 samples:

- Sex: Male
- Race: White
- Age Range: 70 years to 90 years
- BMI: 22 to 29

Whole Transcriptome Atlas Profiling:

- >18,000 genes
- 5µm FFPE tissue section

Area of Illumination (AOI) Profiling Strategy:

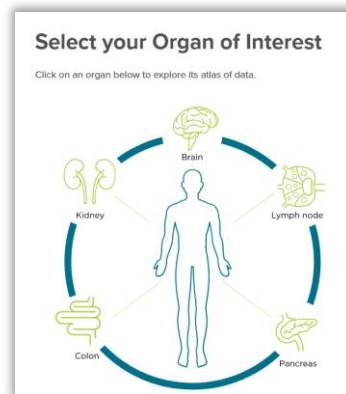
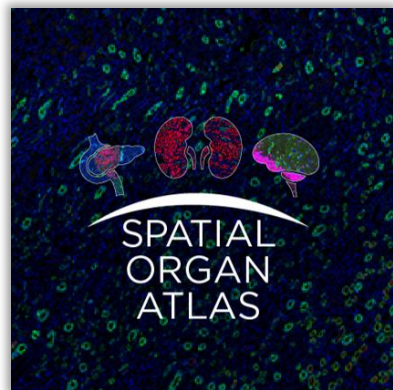
- 252 AOIs total
- Subregions of cortex and hippocampus
- Cell type enriched and geometric areas of illumination

Morphology Markers:

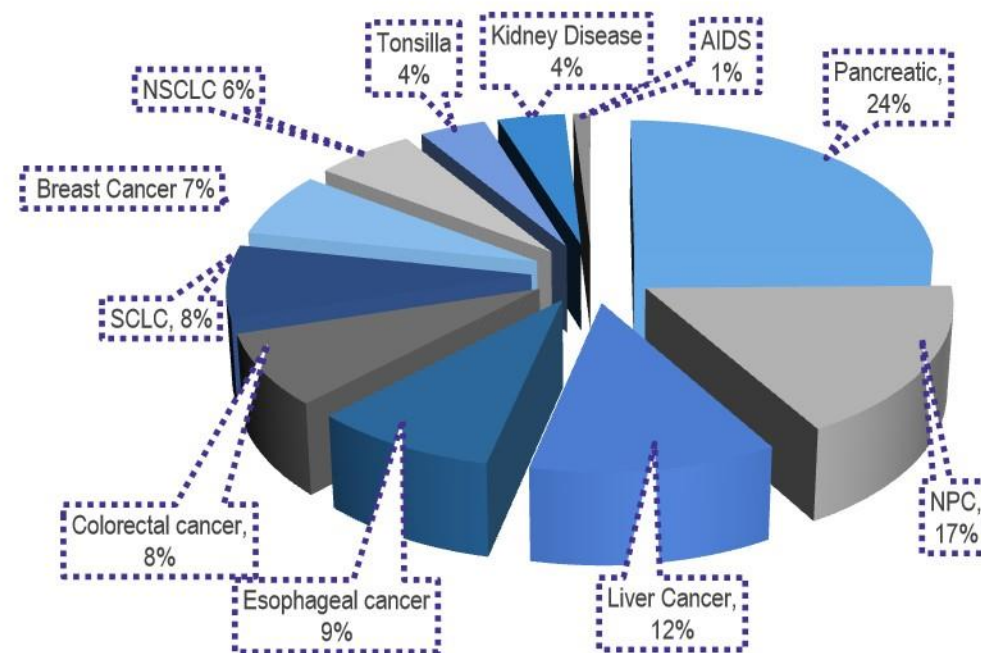
- NeuN: Neurons
- Iba1: Microglia
- GFAP: Astrocytes
- DNA: Nuclei

国内最大的空间组学分析平台

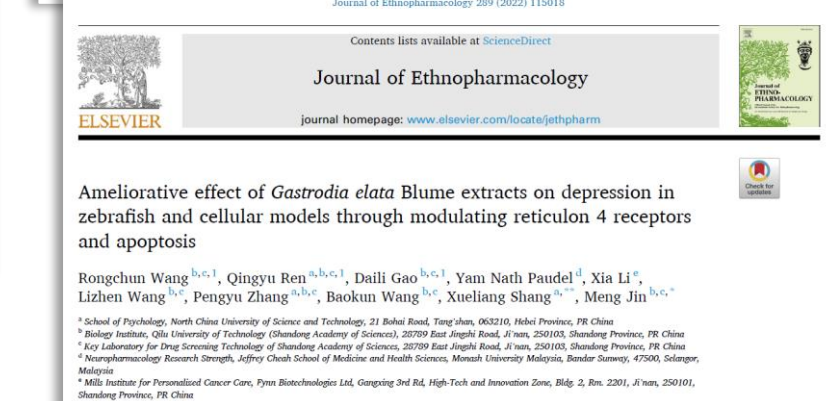
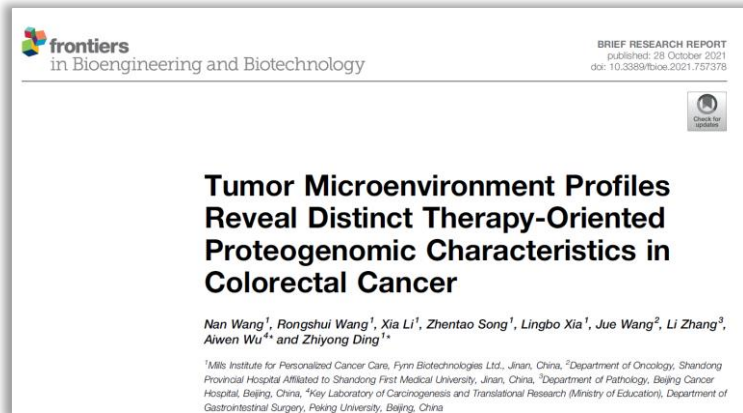
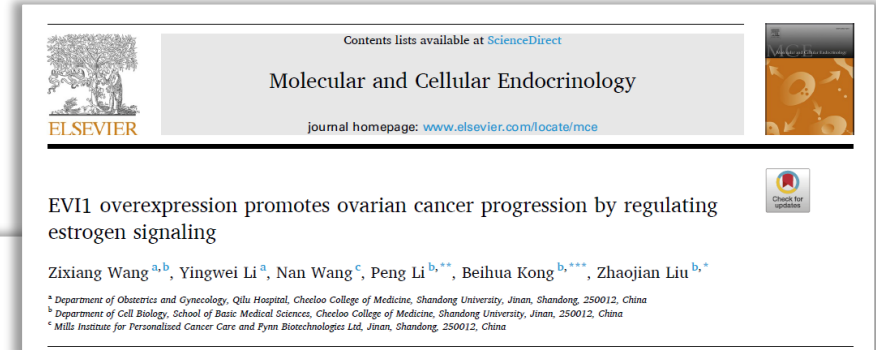
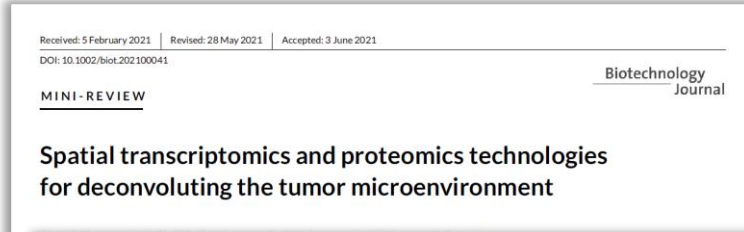
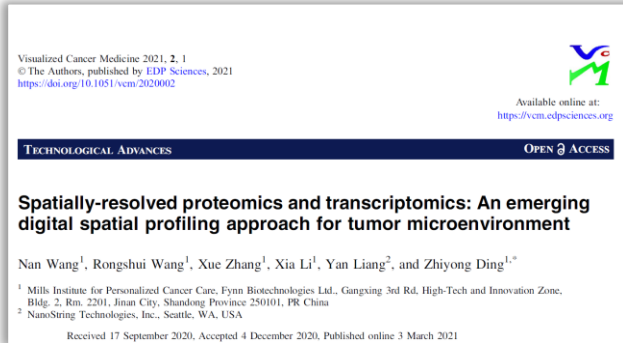
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150+ 基于DSP空间分析技术的临床转化医学研究项目



非因生物助力创新科学研究



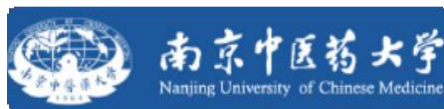
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