

Tissue Cytometry : FACS Language, Tissue Story

用流式細胞儀的語言，訴說組織切片的故事

TissueFAXS

類流式組織細胞定量分析儀



Headquarter

TissueGnostics , Vienna (2003)

International Offices

TissueGnostics Romania SRL, Iasi, Romania (2005)

TissueGnostics USA Inc., Los Angeles (2007)

TG Representation New York, US East coast (2013)

TissueGnostics China Division, Beijing (2014)

Distribution partners for 30+ countries.

2025: TG celebrates 22nd Anniversary!



It's official: **TG wins** at the
3rd International **Scanner Contest!**
First Prize in "Image Analysis"



Principles of TissueFAXS

Image

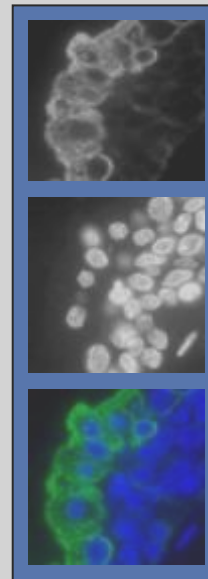


樣本種類:
各類組織切片
組織微陣列TMA
細胞抹片

全自動高速拍攝:
可見光/螢光樣本
自動對焦拍攝
全景無縫拼接

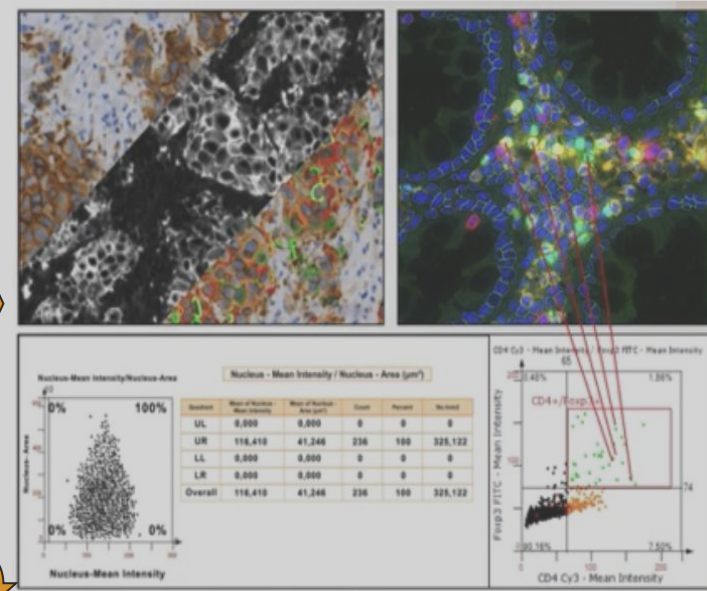
Analysis

Result I



影像分析:
全彩IHC自動分色
專利組織細胞辨識
18種強度及型態量化參數

Result II



流式數據報告產出:
功能性二維散點圖
數據 ↔ 細胞聯動
ROI分析自動報告輸出

The TissueFAXS™ product family

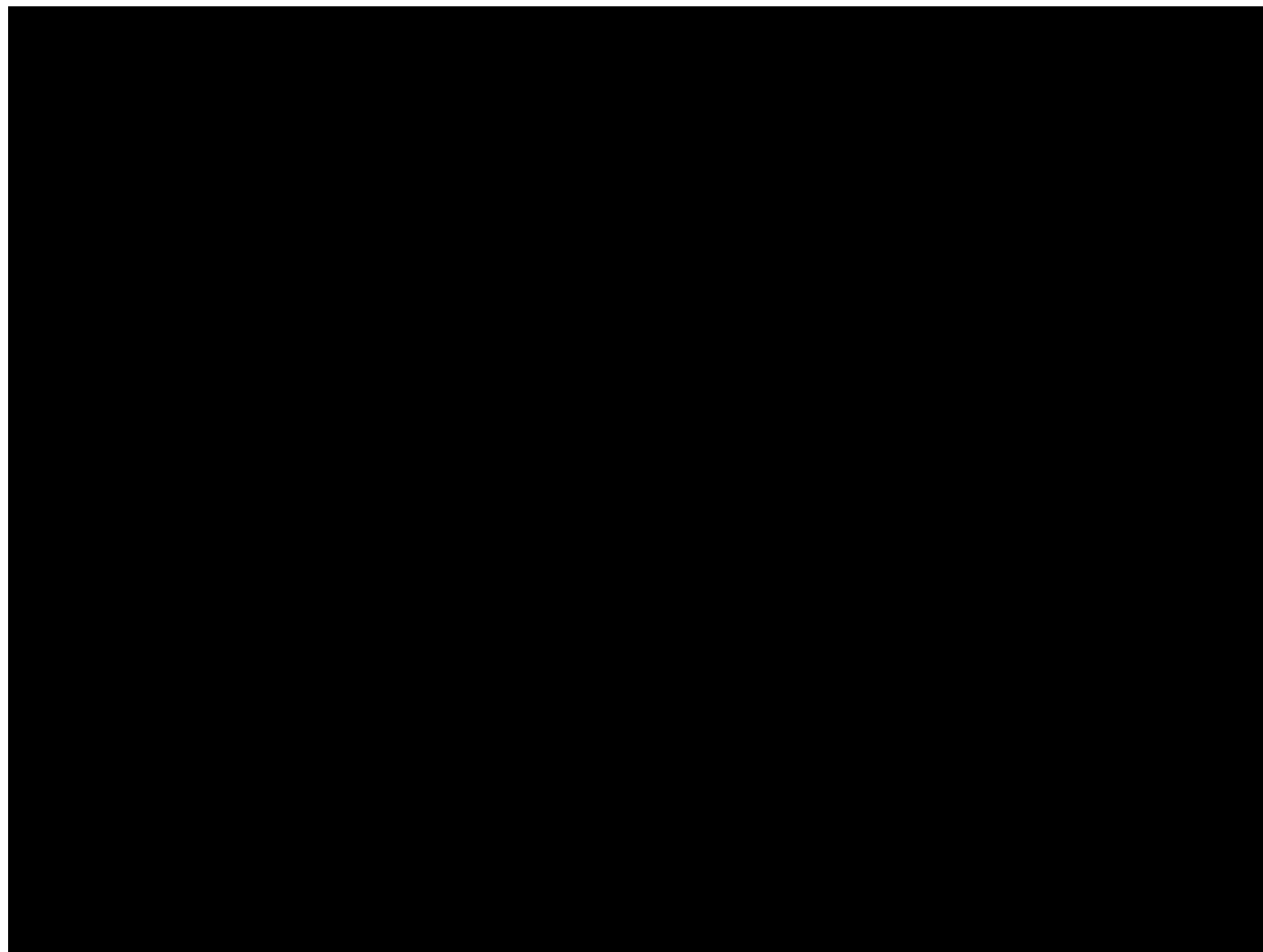
TISSUEGNOSTICS
PRECISION THAT INSPIRES

TissueFAXS is a microscope based slide scanner with ultimate flexibility and image quality



Automatic scan IHC and/or IF sample with different configuration by user demand.

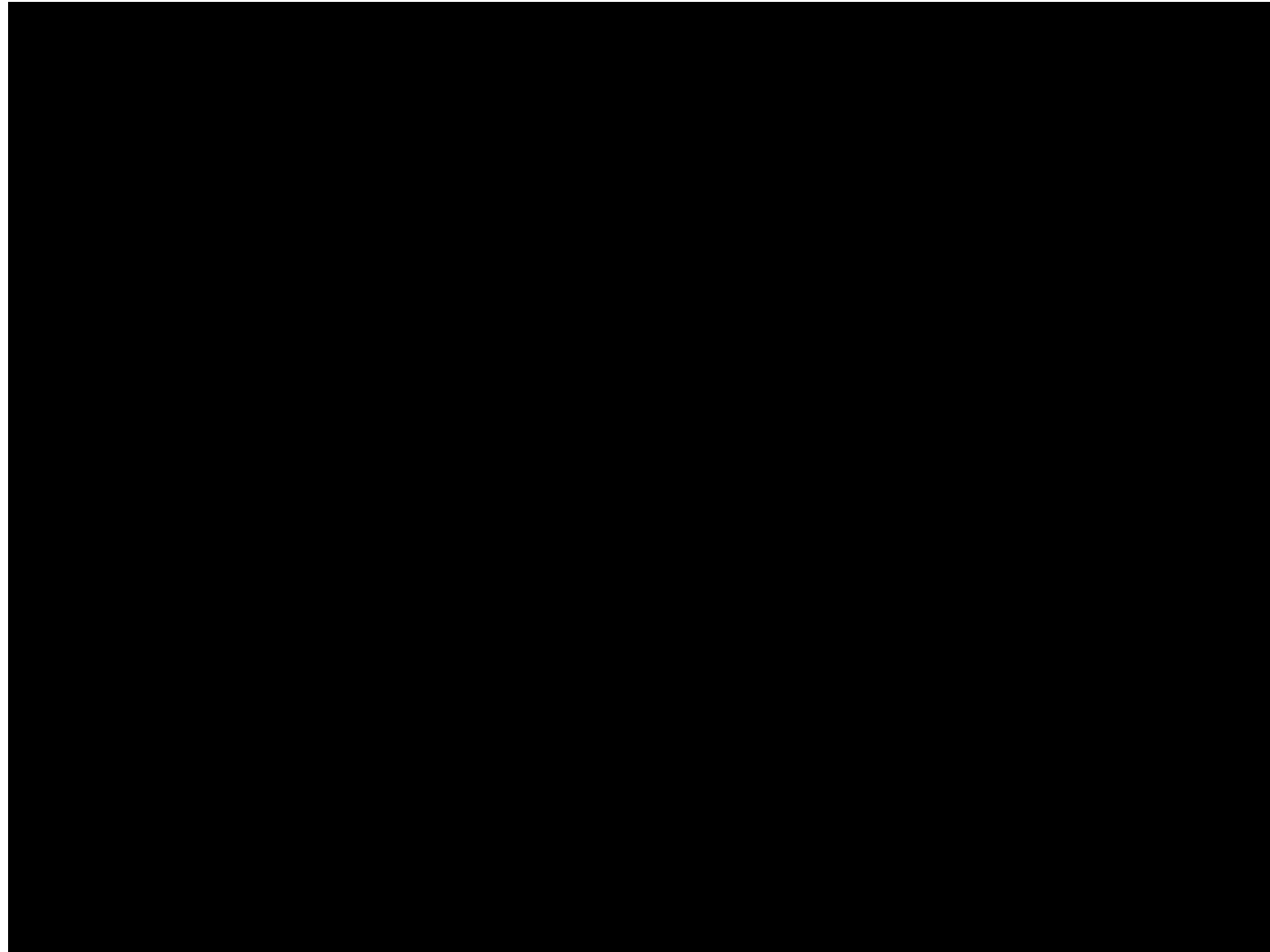
Tissuegnostics High Throughput Scanning Solution for Immunohistochemistry sample



1cm² tissue only 47s !

- Focus strategy = 7X7
- Exposure time 2.5ms

Tissuegnostics High Throughput Scanning Solution for Immunofluorescence sample



1cm² tissue with 4 FL
less than 12 mins!

- Focus strategy = 7X7
- Exposure time < 50 ms

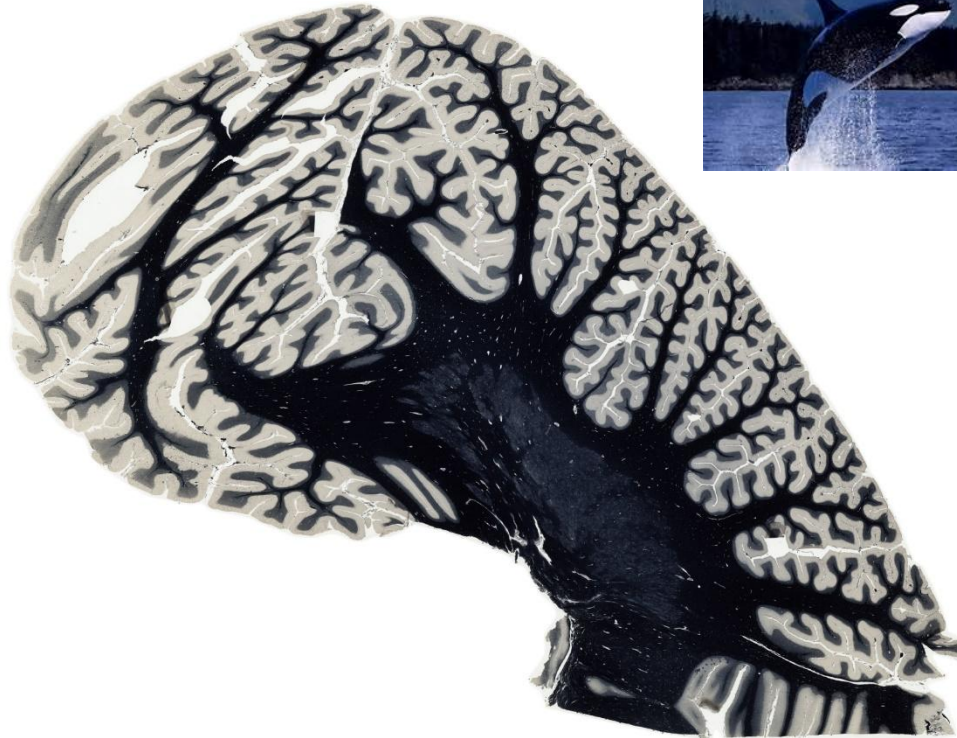
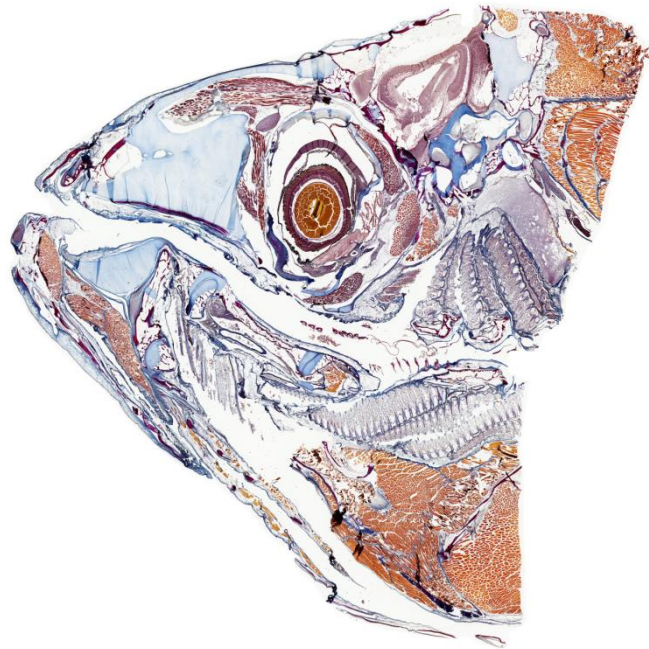
The Virtual Slide in IHC



The *digital sample / virtual slide* might consist of thousands of individual fields of view!

The Virtual Slide in IHC

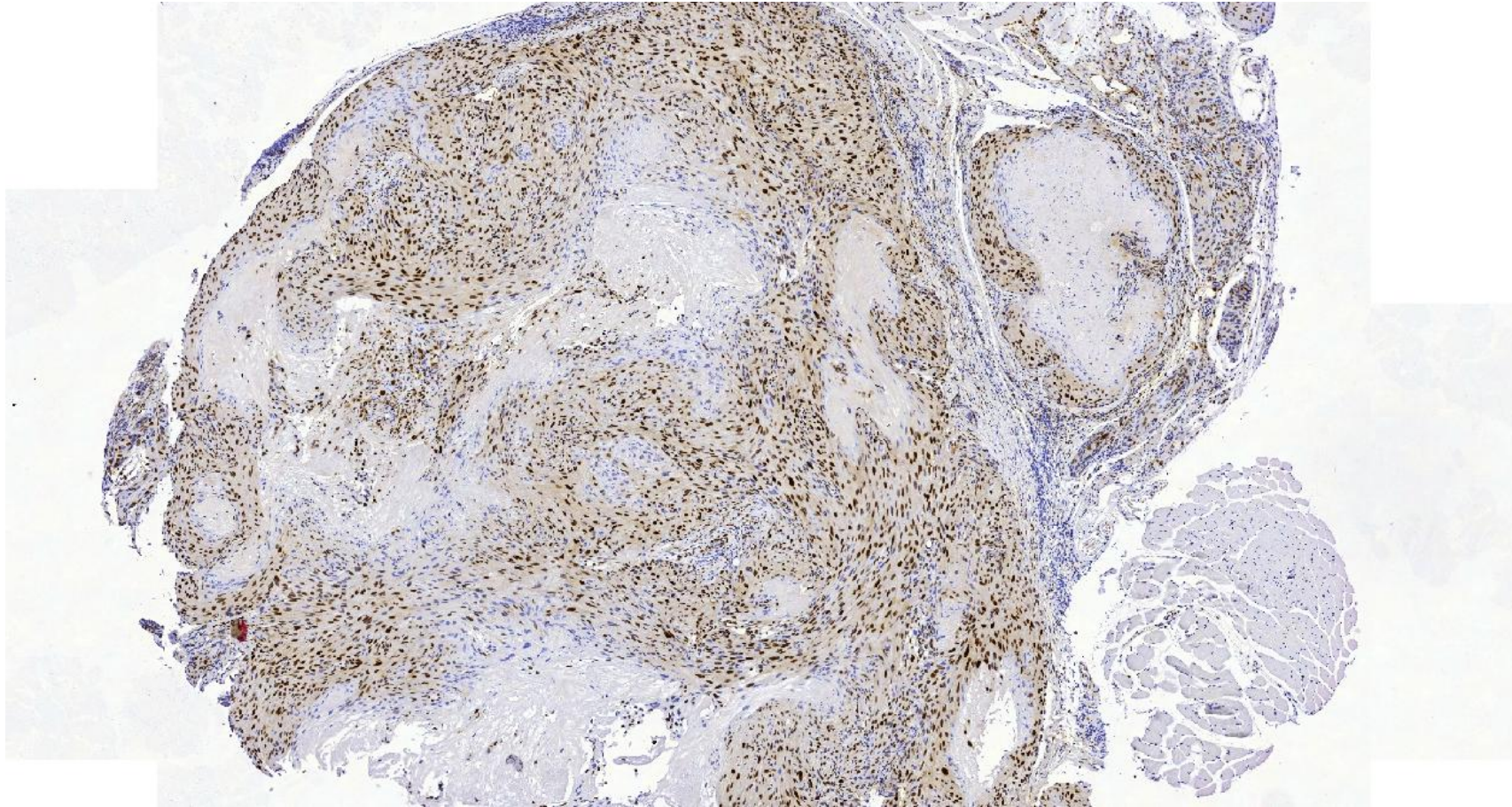
Over-sized slide



The *digital sample / virtual slide* might consist of thousands of individual fields of view!

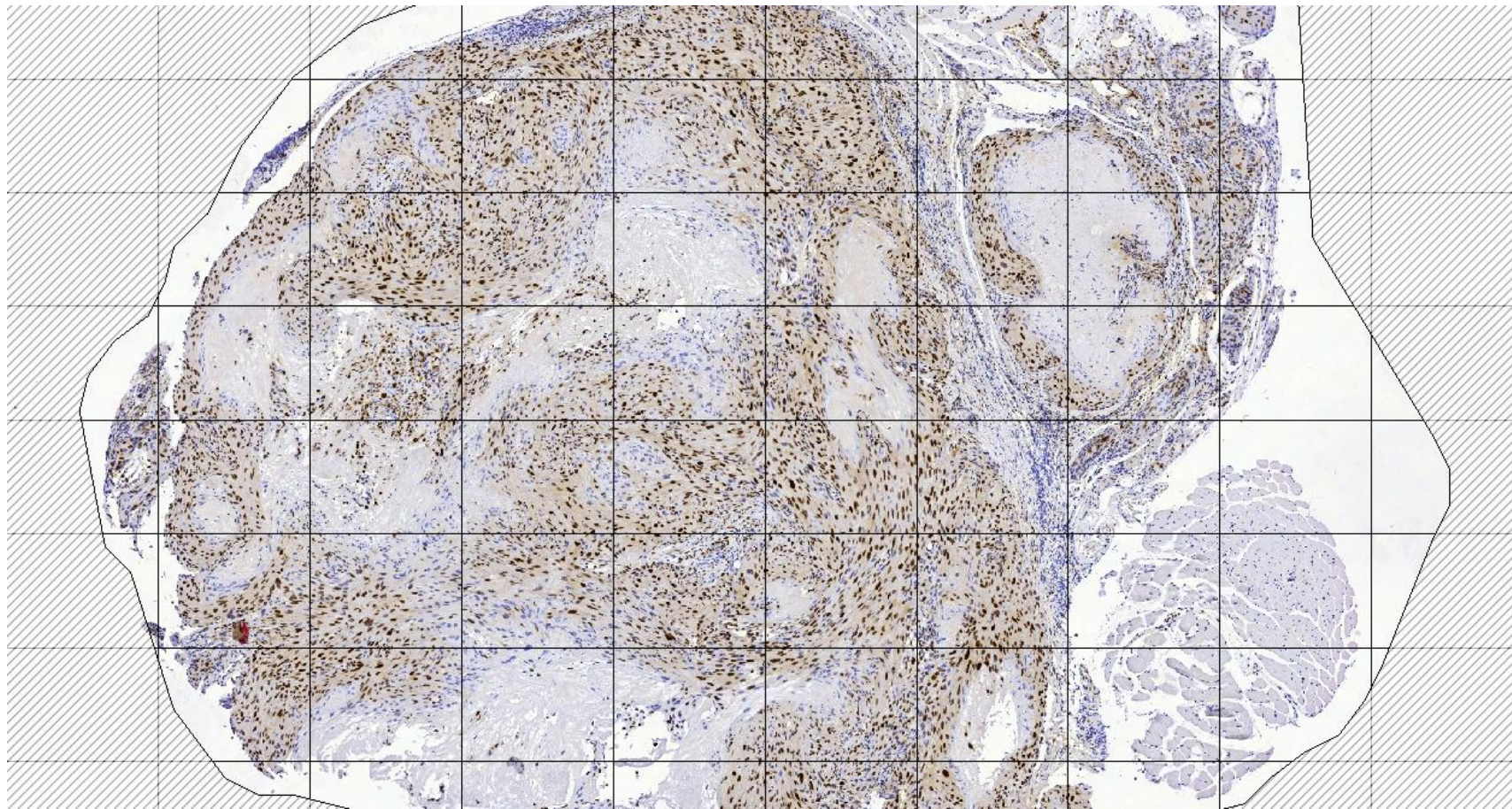
The Virtual Slide in IHC

Slide overview



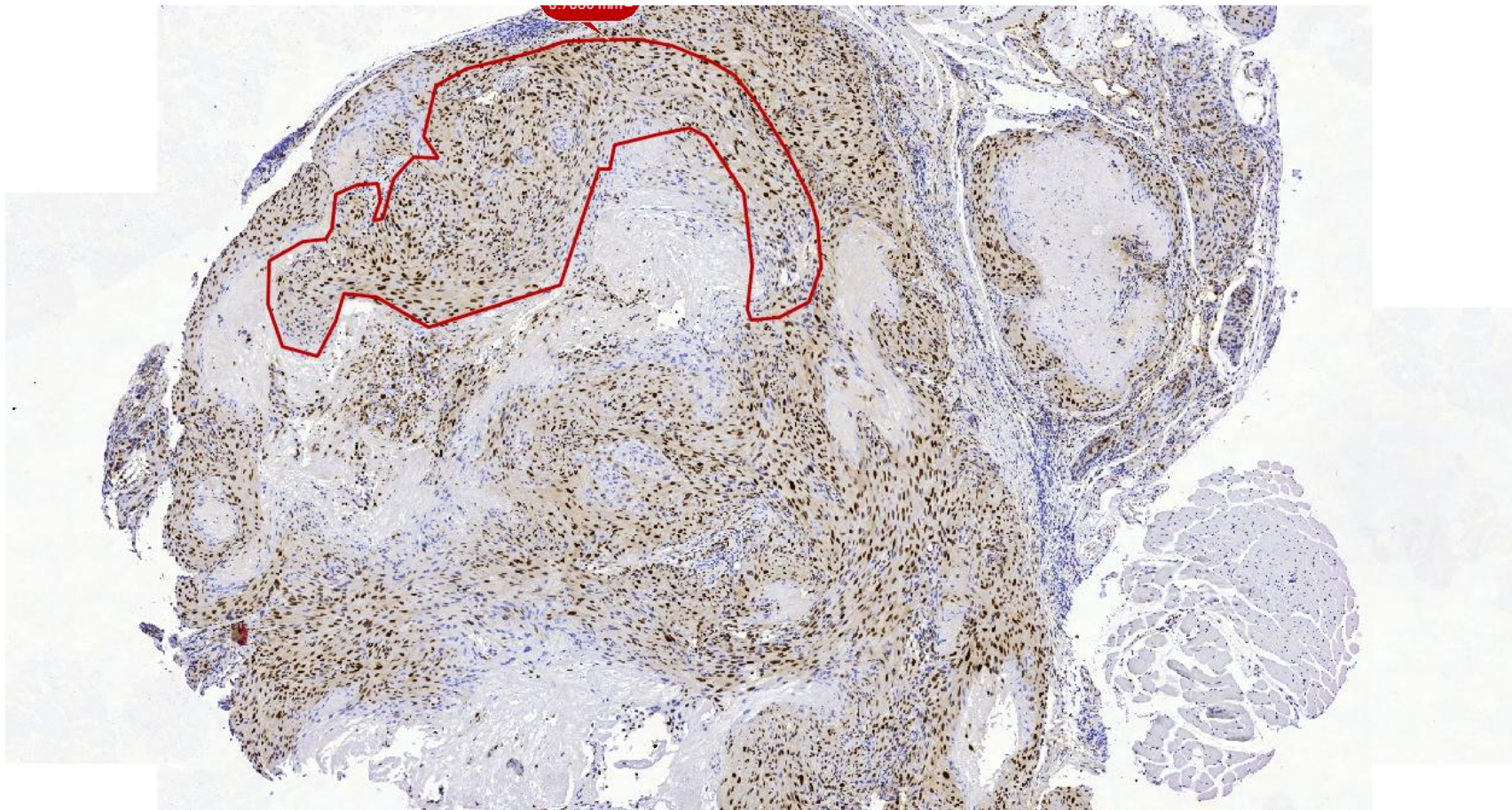
The Virtual Slide in IHC

Corp and FOV line



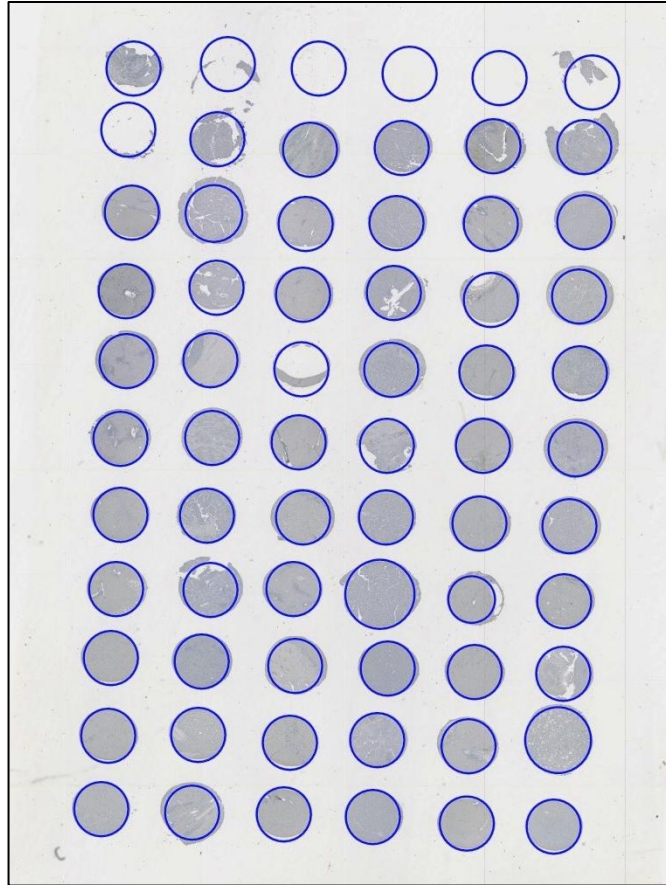
The Virtual Slide in IHC

Category in virtual slide



The Virtual Slide in IHC

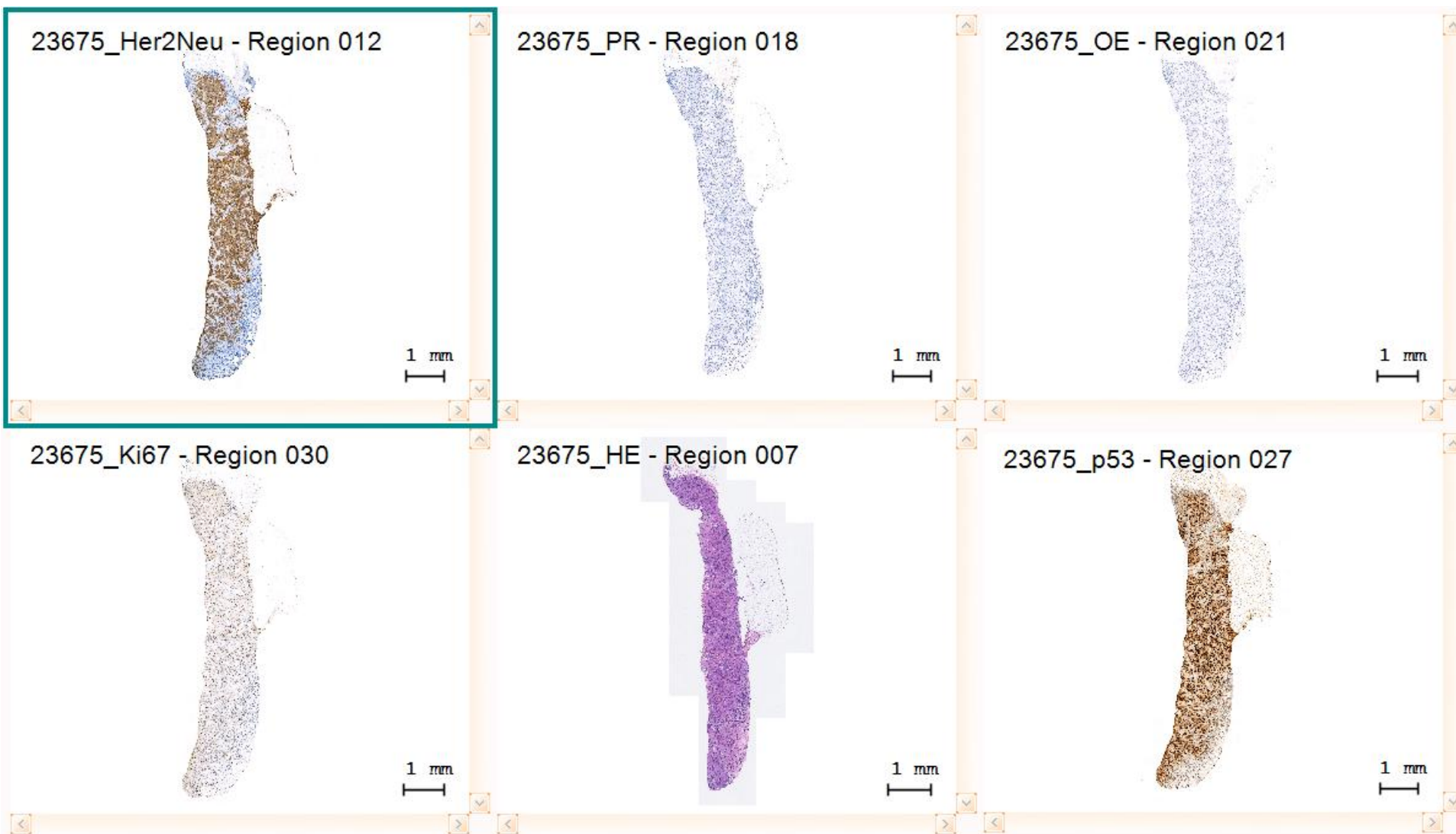
TMA acquisition and manage



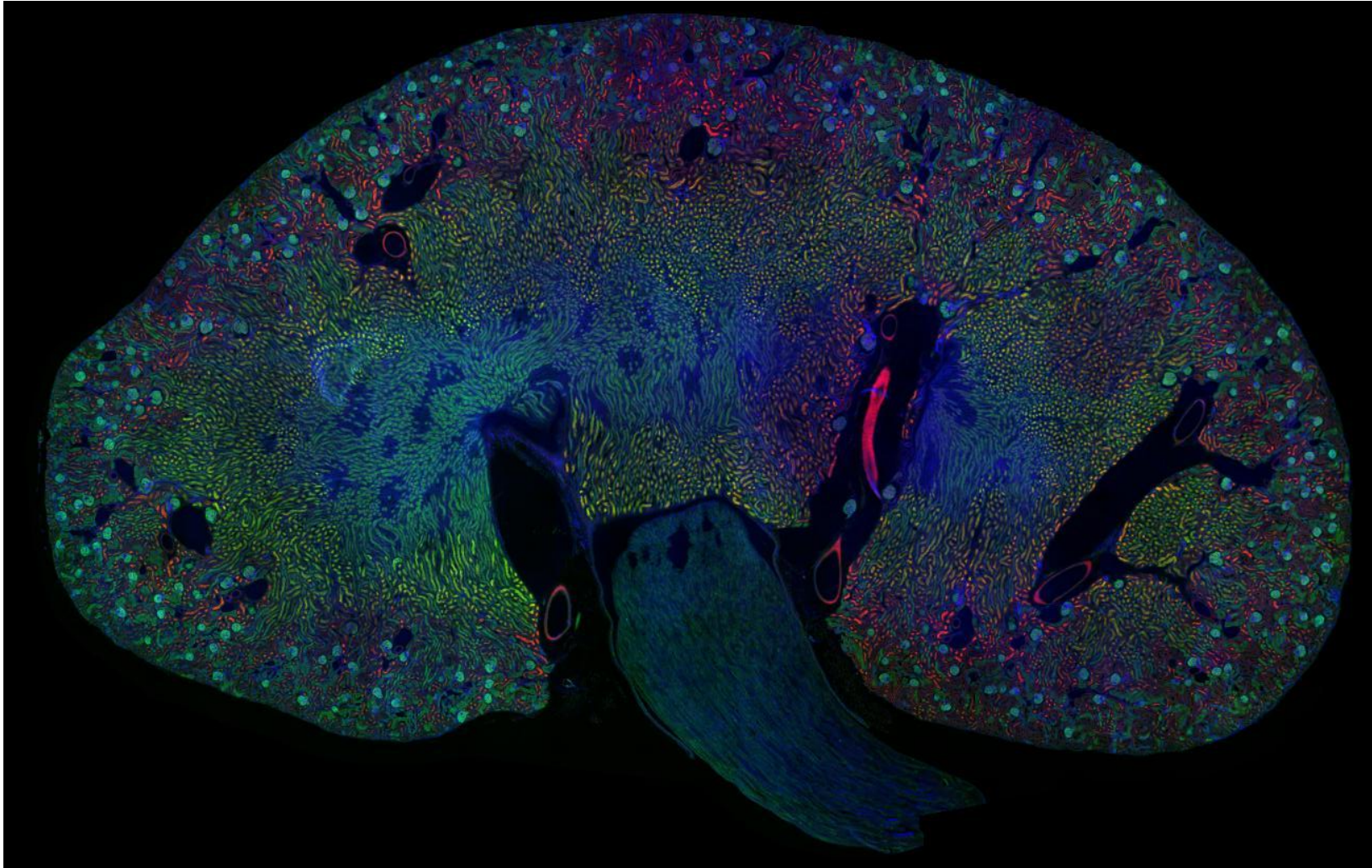
- Auto detect each core
- Missing core identify
- Block grouping
- Each core size and shape can be manually correct
- Block angle and cores distance can be manually correct
- Only scan identified core
- Focus once on each core

The Virtual Slide in IHC

Consecutive sections



The Virtual Slide in IF



The *digital sample / virtual slide* might consist of thousands of individual fields of view (FOV)!

The Virtual Slide in IF

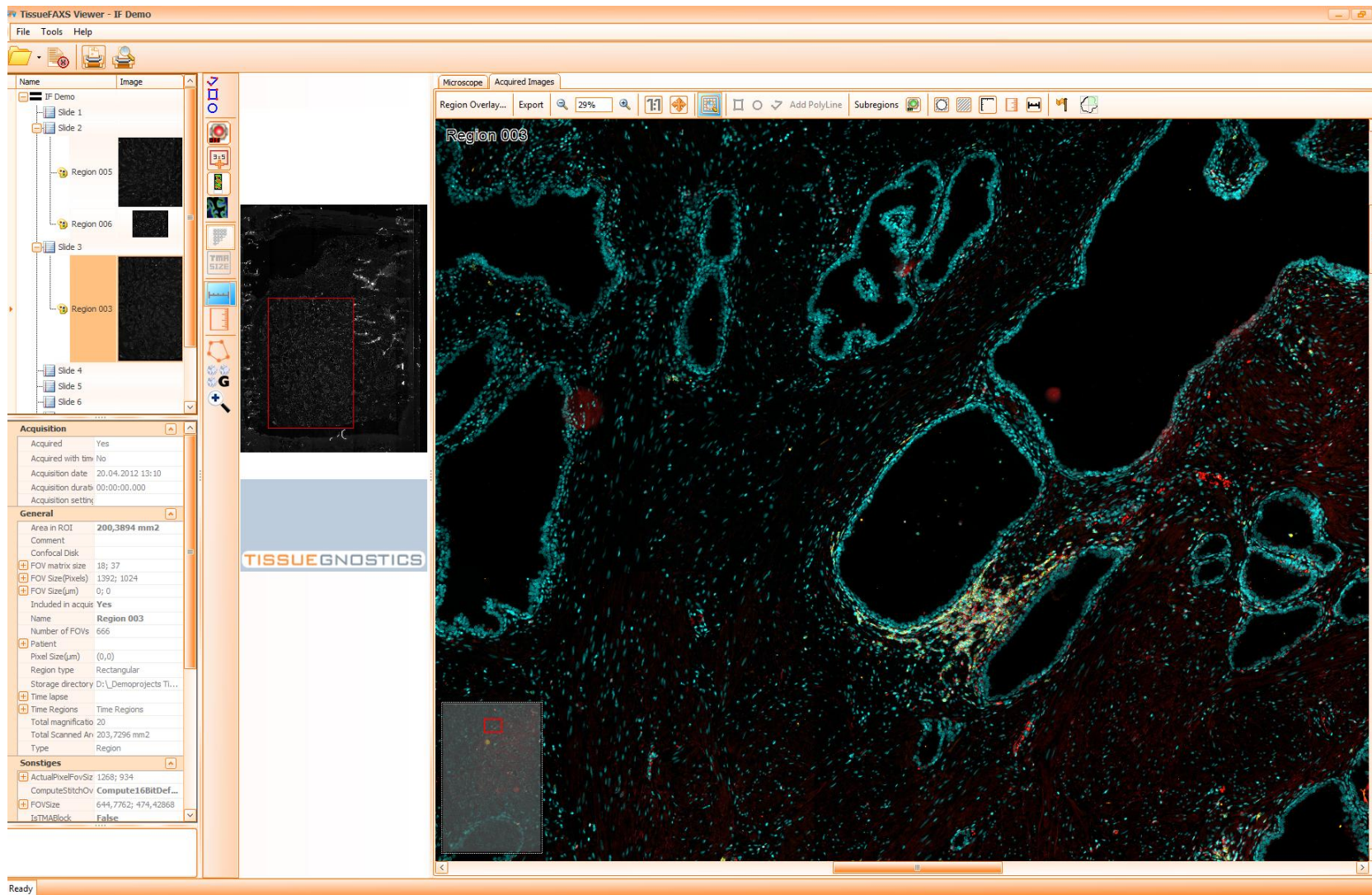


Scanning of 50 μm thick retina

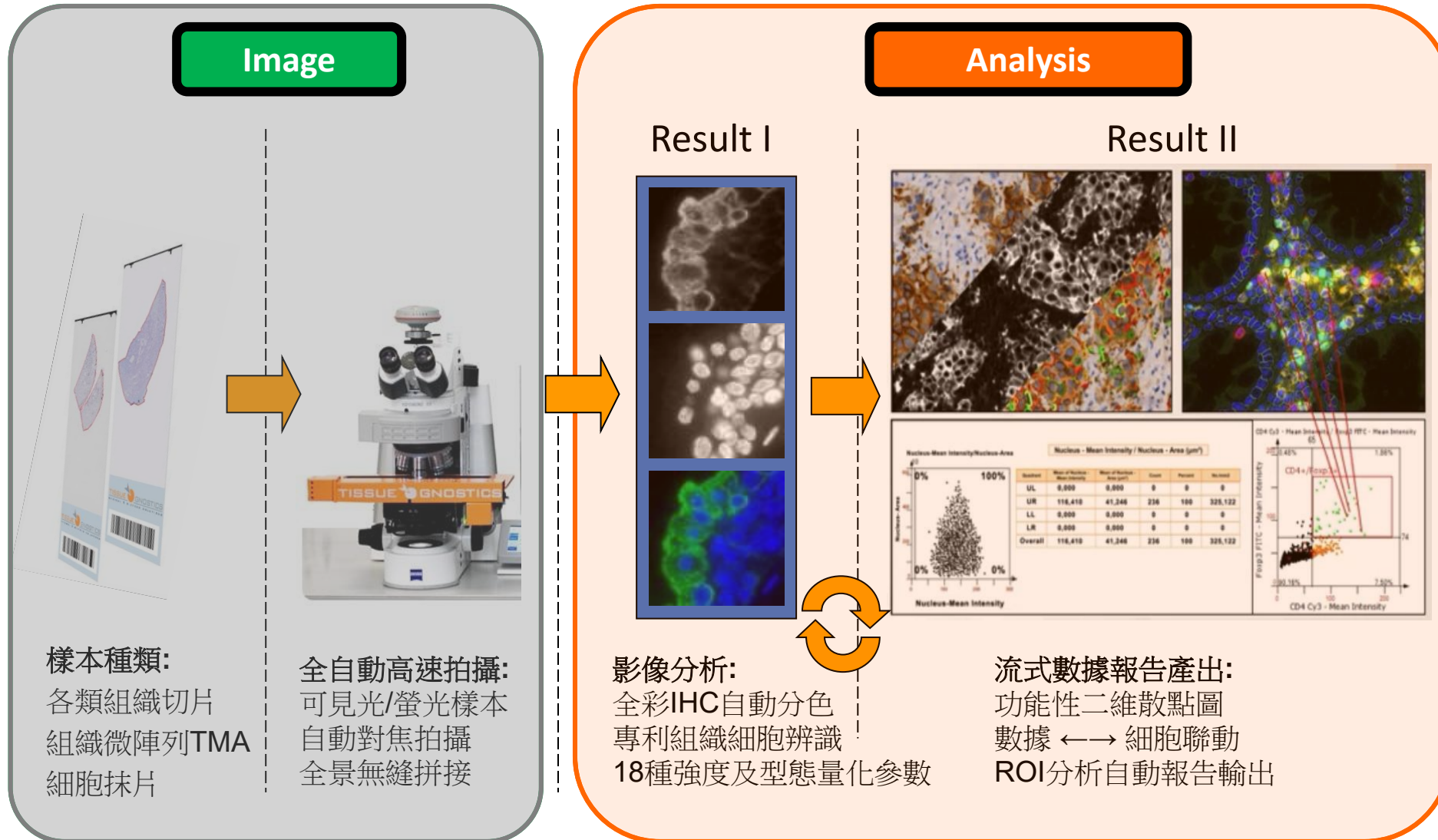
TissueFAXS provide specific image stacking algorithm for thick sample without blur effect

TissueFAXS™ VIEWER – Freeware!

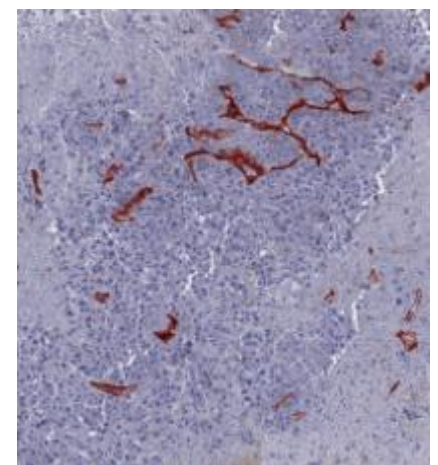
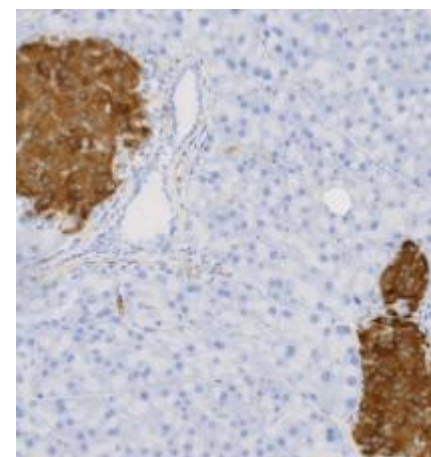
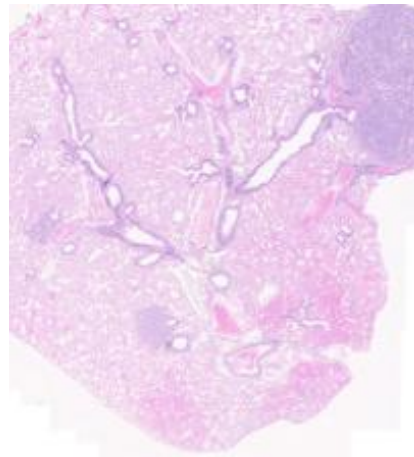
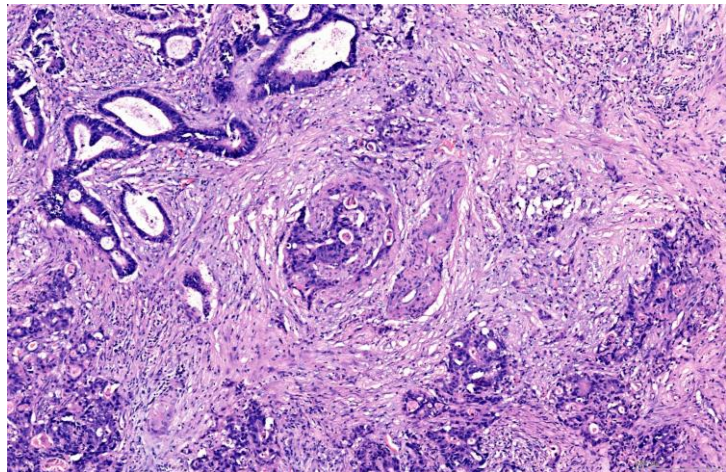
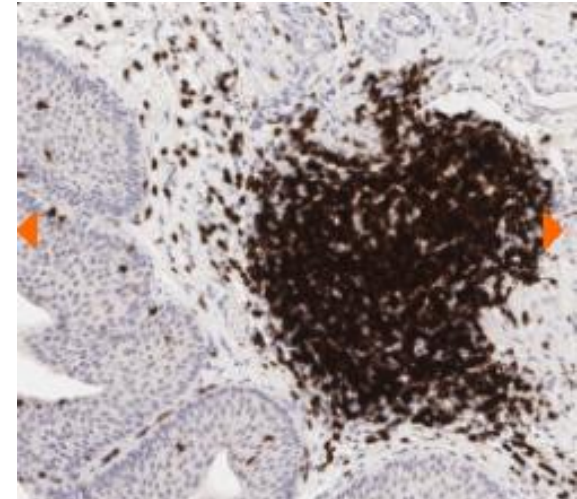
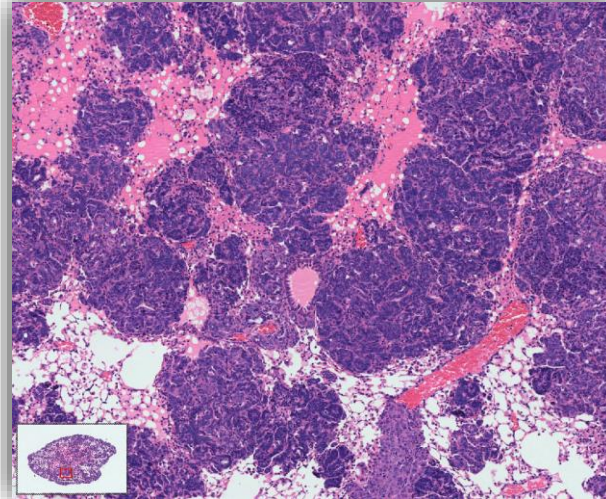
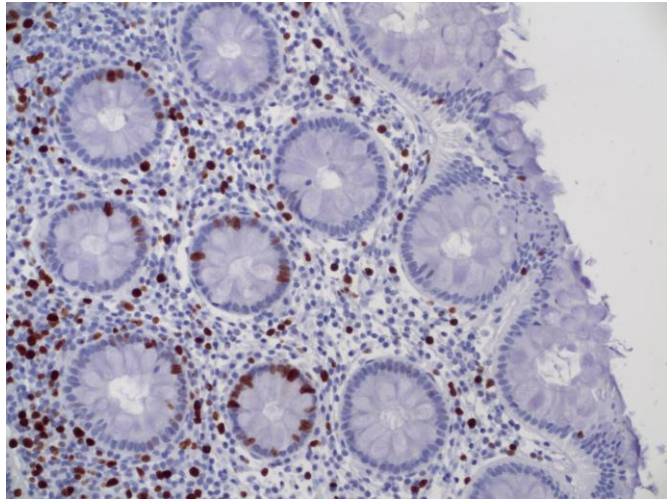
TISSUEGNOSTICS
PRECISION THAT INSPIRES



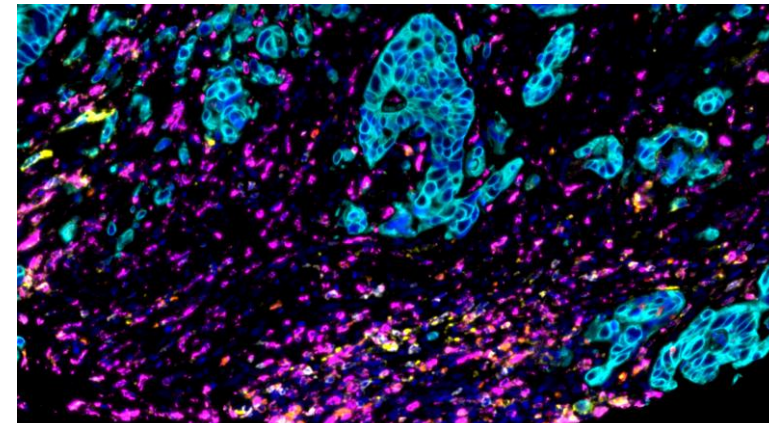
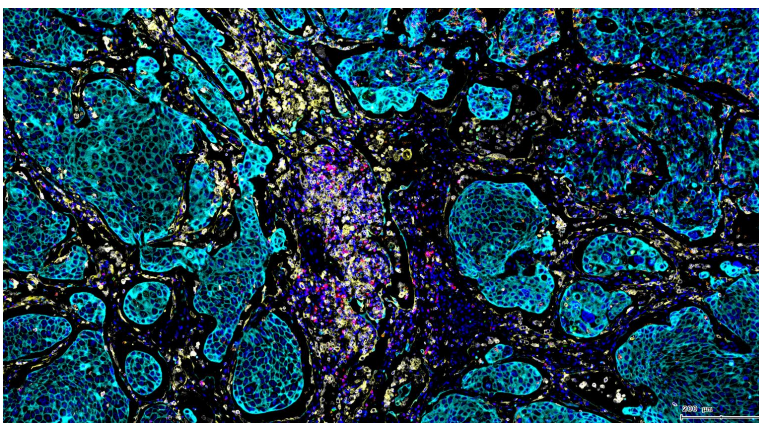
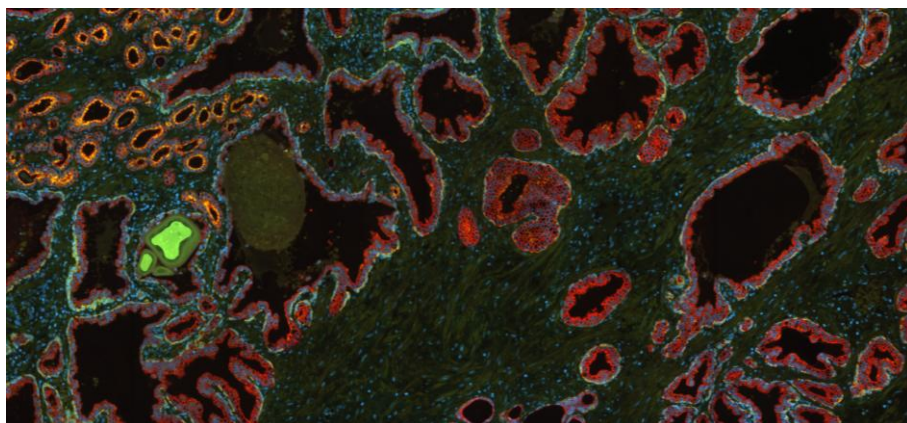
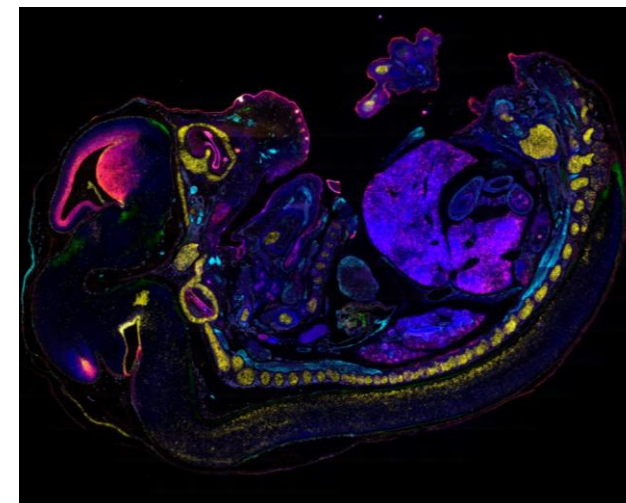
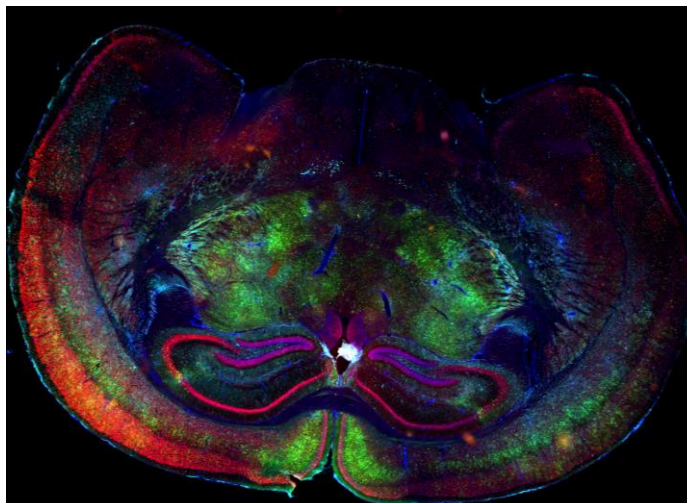
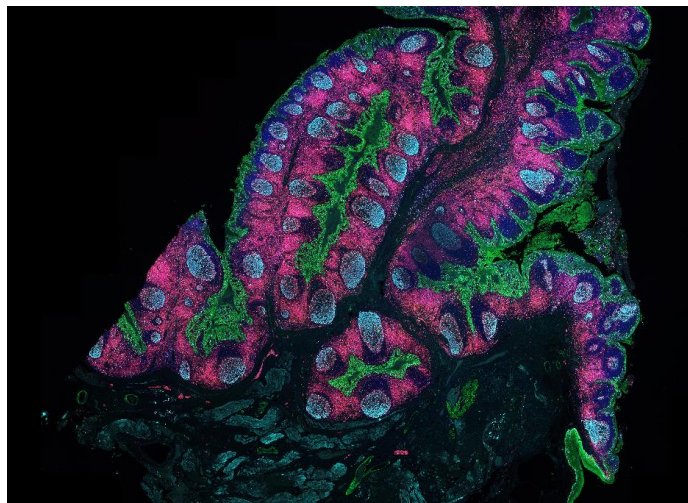
Principles of TissueFAXS



From Image...



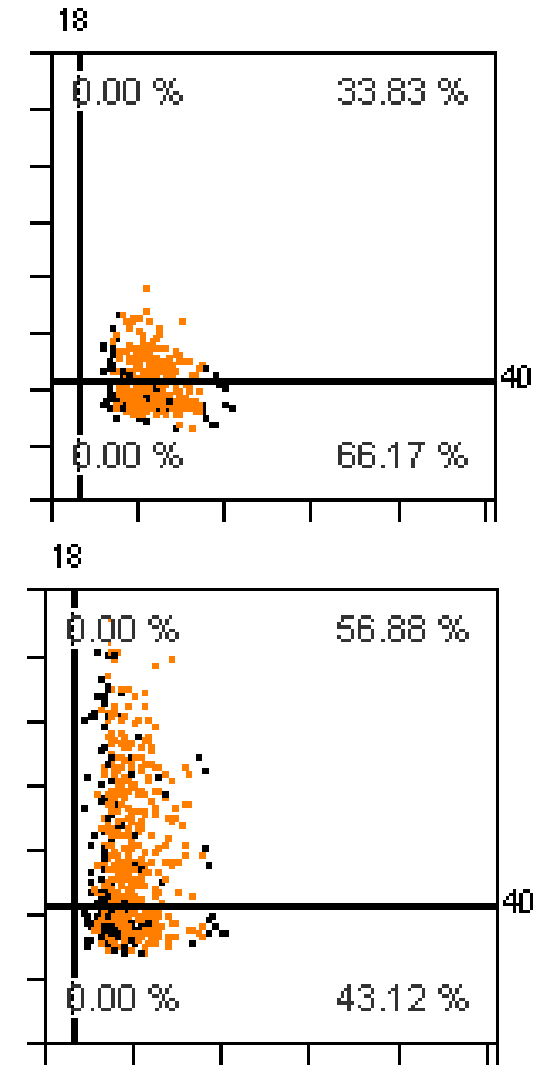
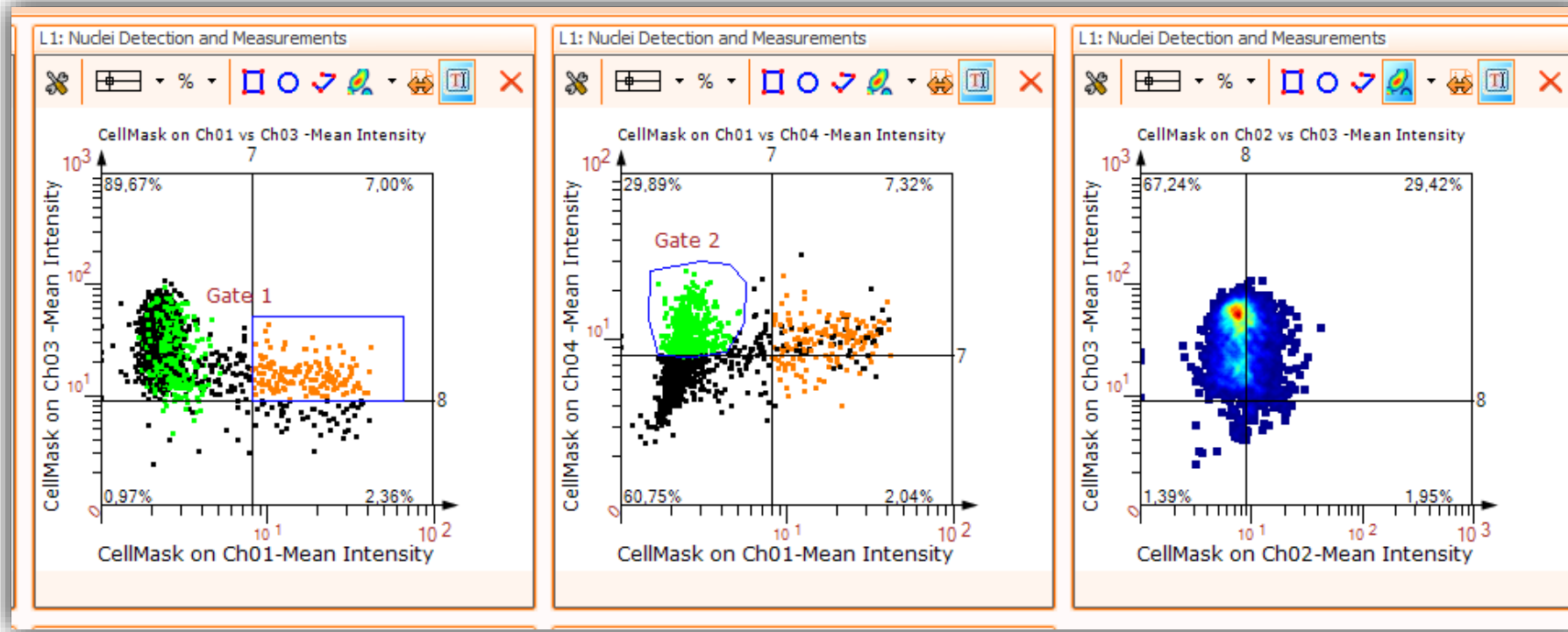
From Image...



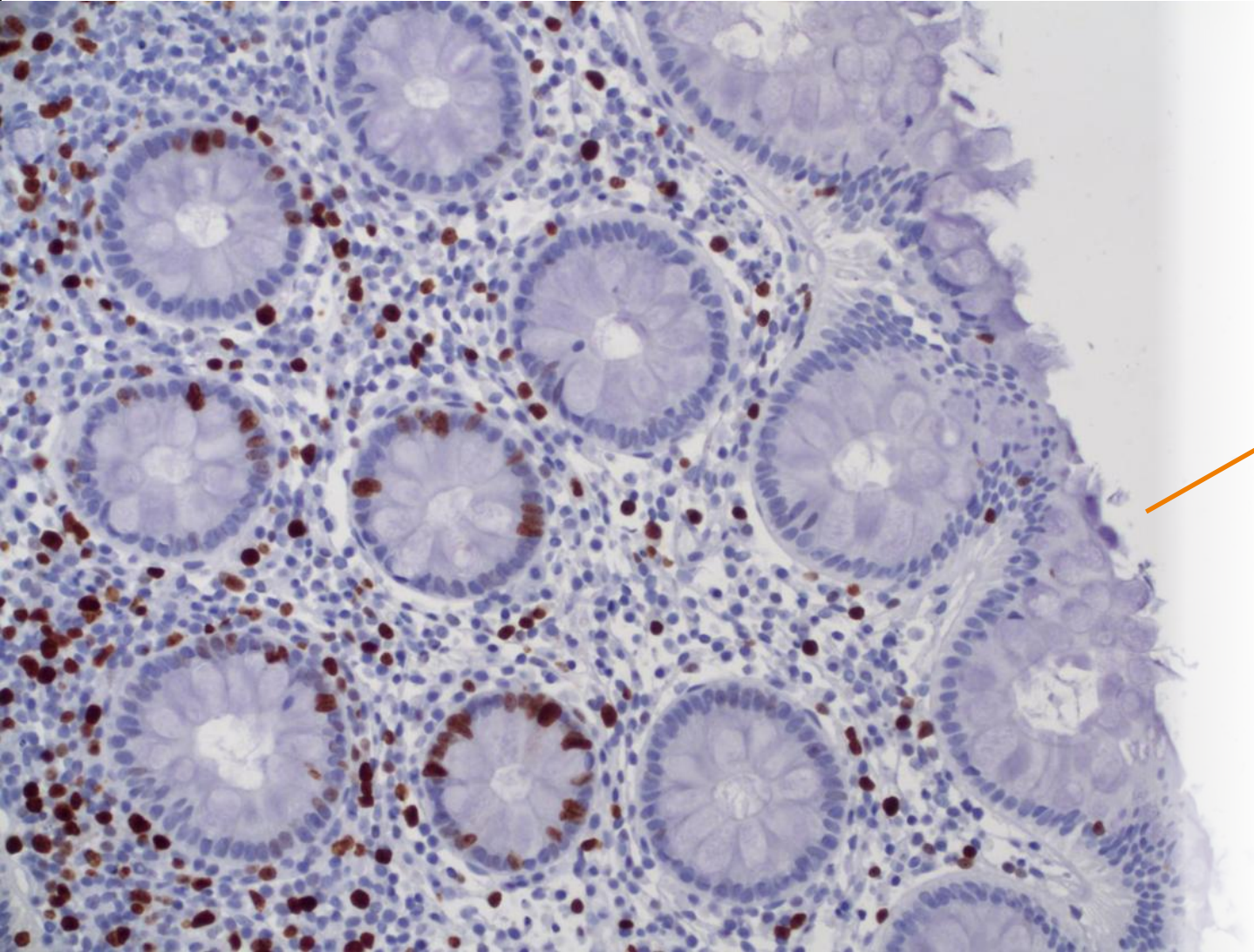
...to Analysis

Each cell is indicated as one dot

The reactivity of two channels is plotted on the x- and y-axis

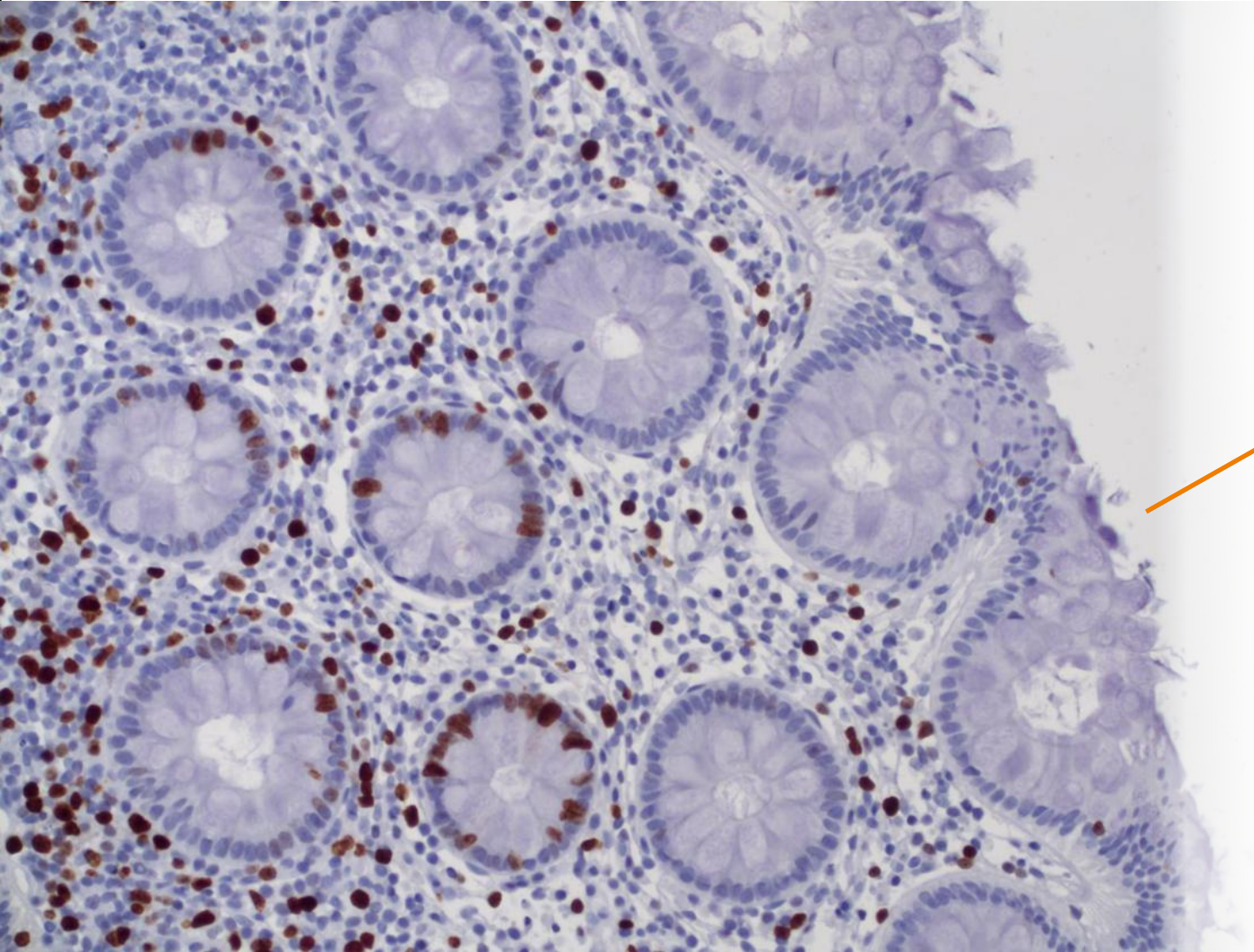


Why we need TissueFAXS™ Cytometry?



How many of the blue nuclei are also stained in brown (in %)?

Why we need TissueFAXS™ Cytometry?

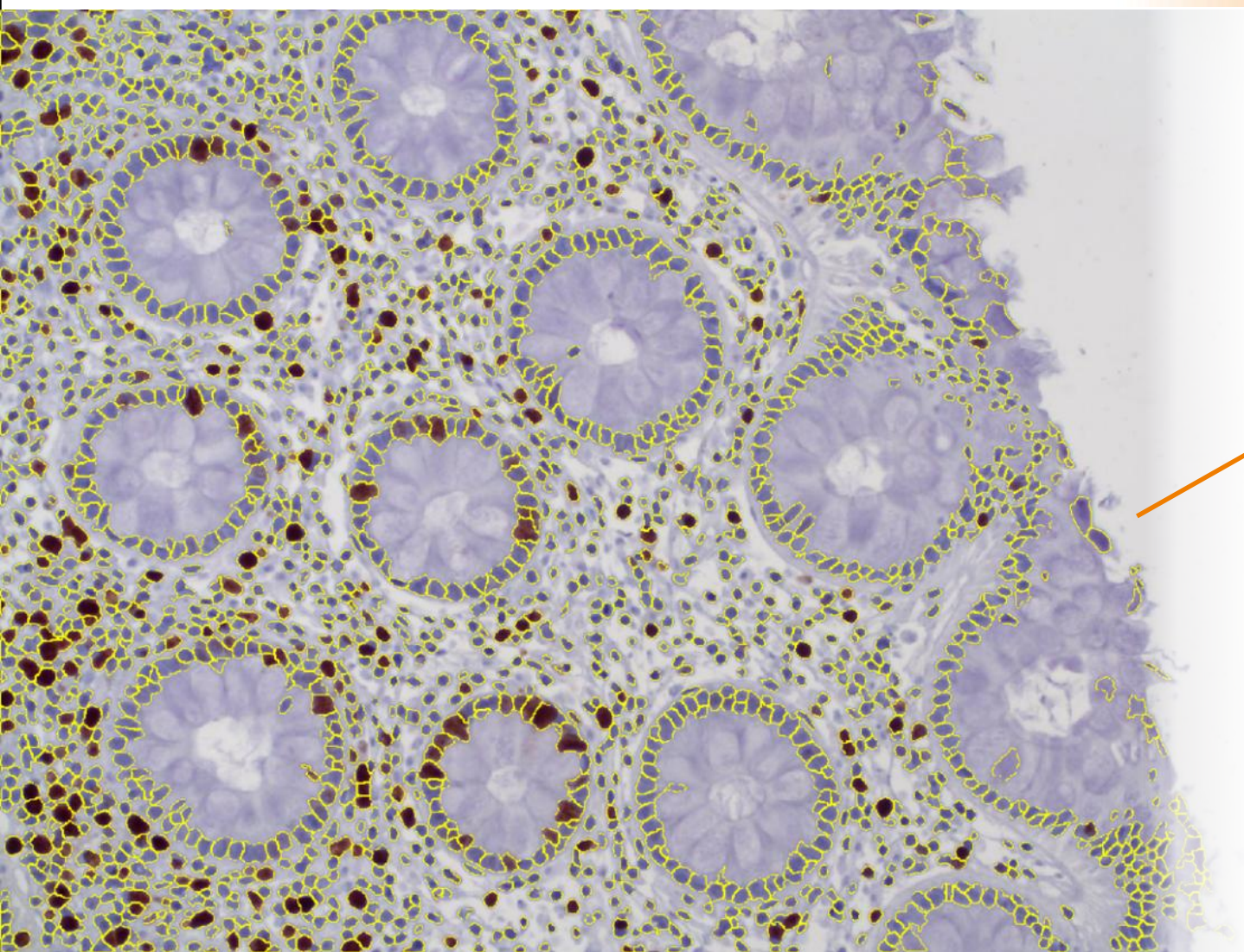


How many of the blue nuclei are also stained in brown (in %)?

Expert's estimations:

1% - 40%

HistoQuest Quantification

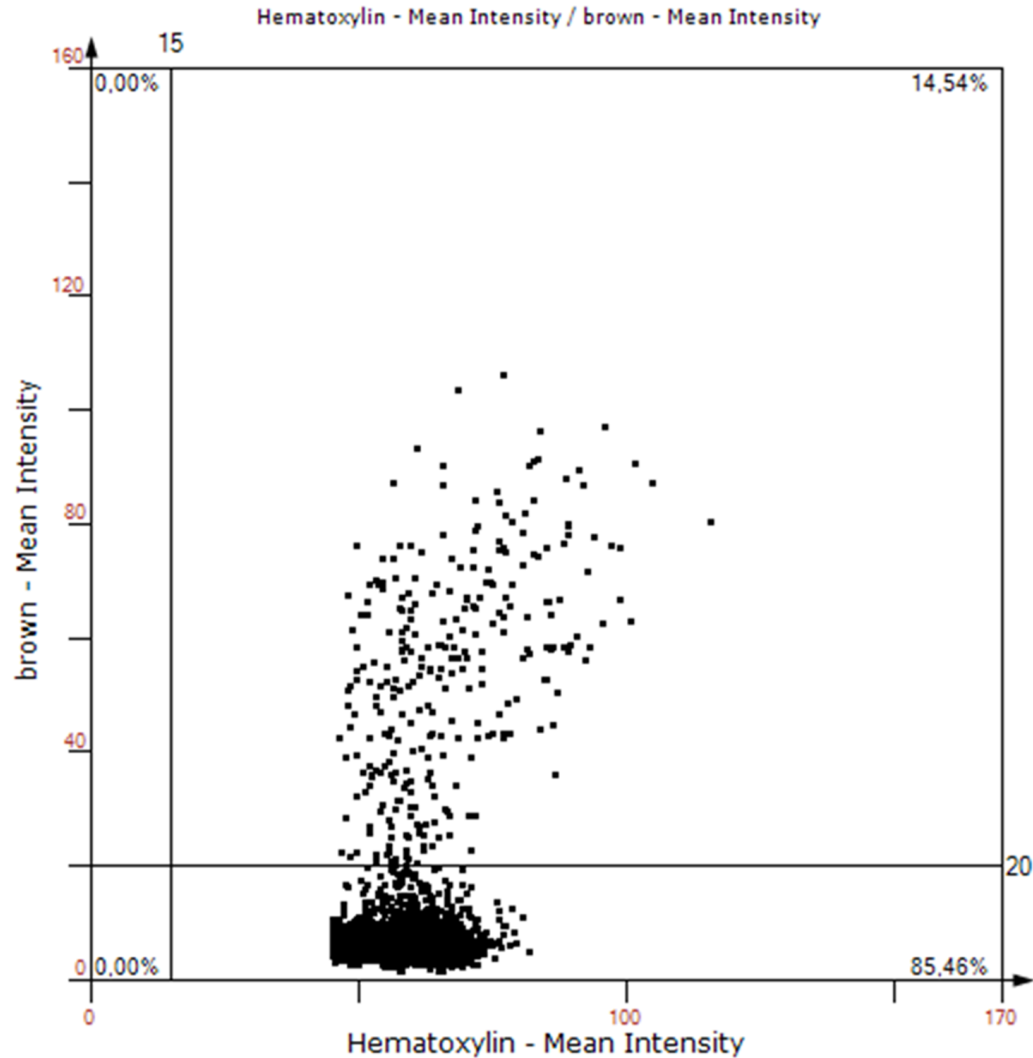


How many of the blue nuclei are also stained in brown (in %)?

Expert's estimations:

1% - 40%

= Reliable Data



How many of the blue nuclei are also stained in brown (in %)?

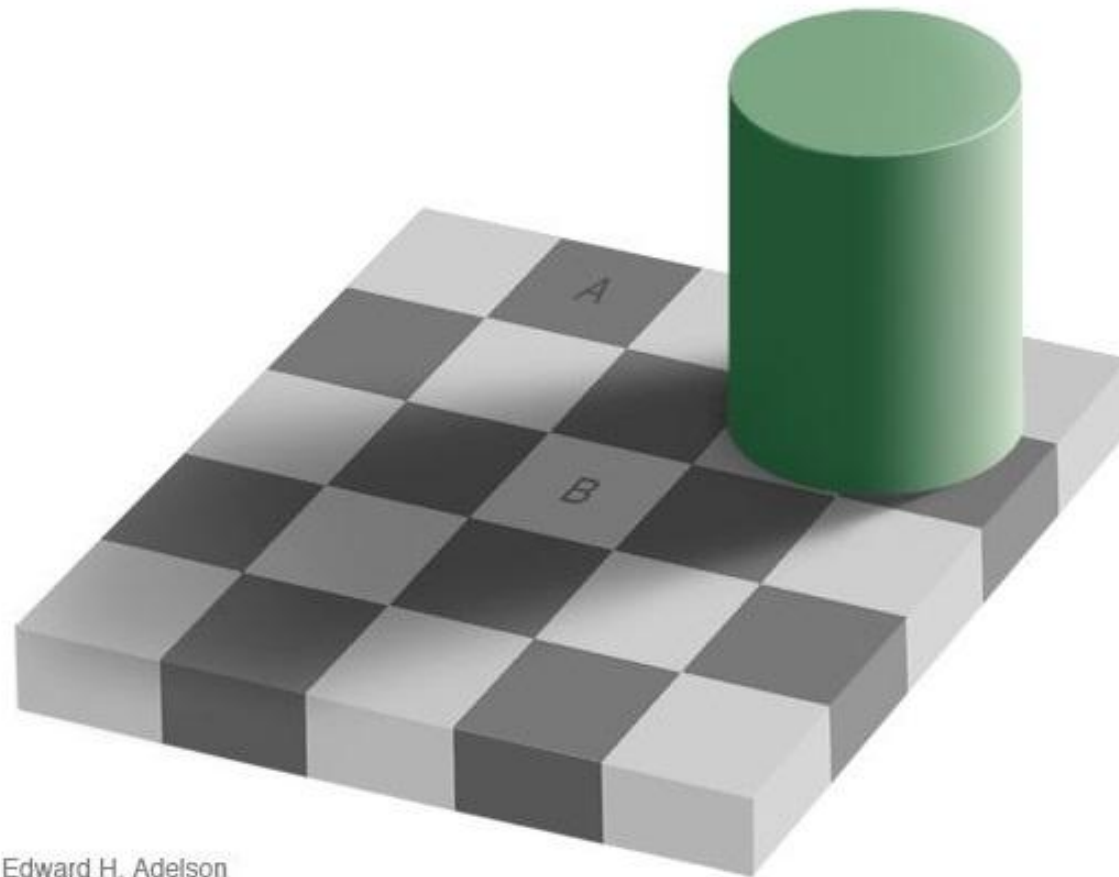
Expert's estimations:

1% - 40%

Observer independent measurement:
14.54%

Why we need TissueFAXS™ Cytometry?

Relative Staining Intensity = gray value(GV)

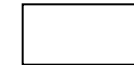


Edward H. Adelson

GV = 0: BLACK



GV = 255: WHITE

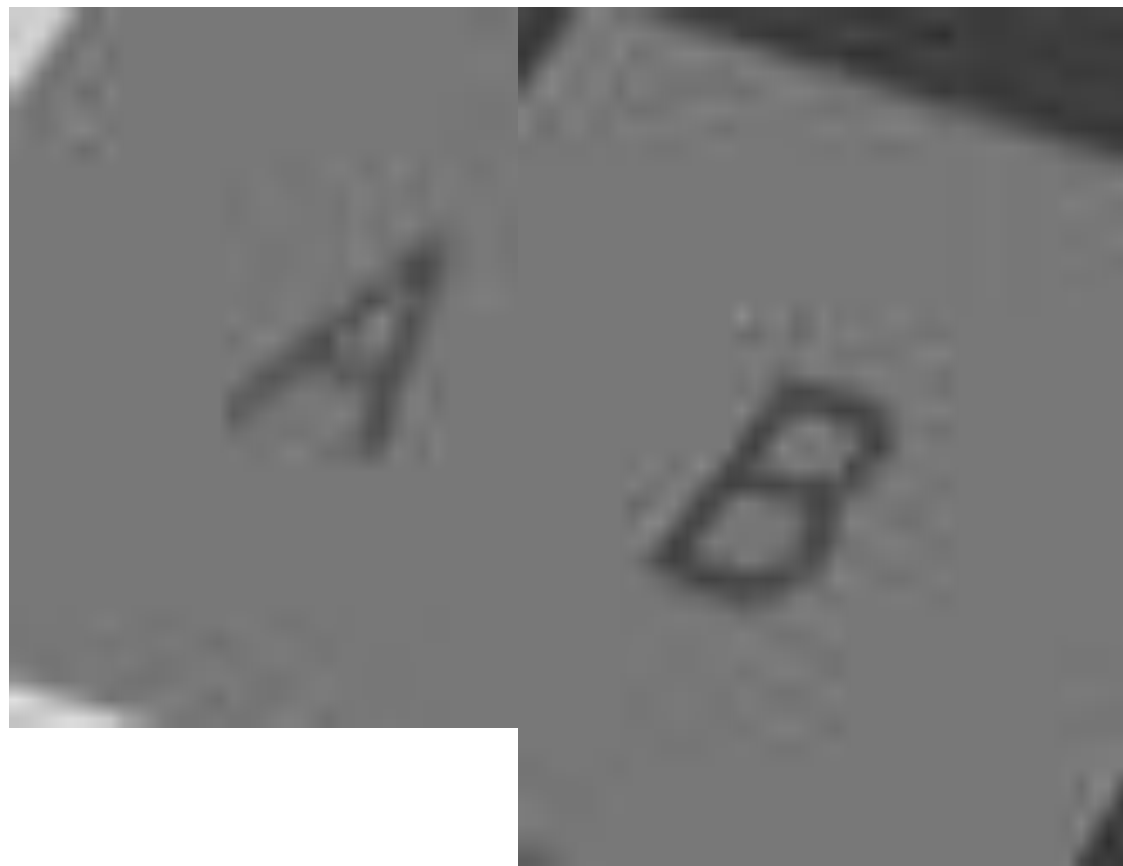


What is the difference in GV
between A and B?

1. $\Delta = 0$
2. $\Delta \leq 20$
3. $\Delta \leq 50$
4. $\Delta \leq 100$
5. $\Delta > 100$

Compare frames A and B – which one is brighter and how much is it brighter (select answer to the right)?

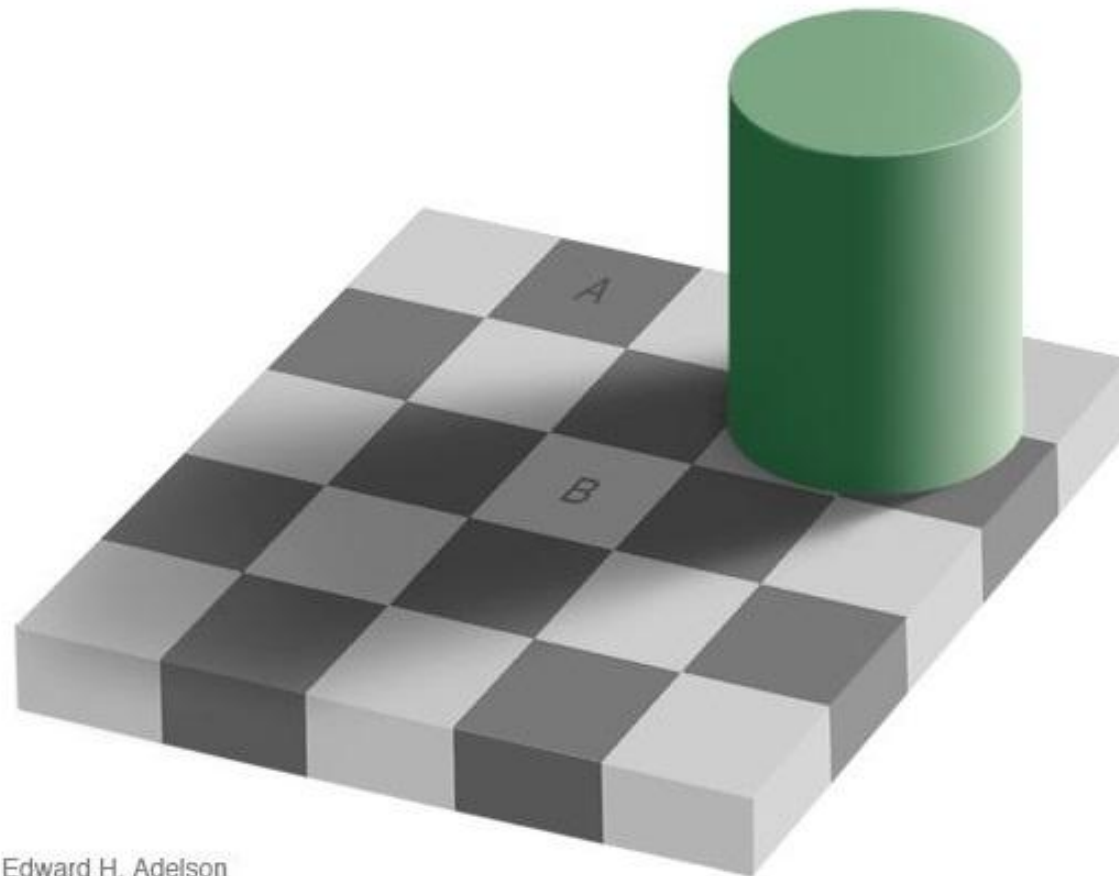
Estimation versus Measurement



$\Delta = 0$!!!!

Estimation versus Measurement

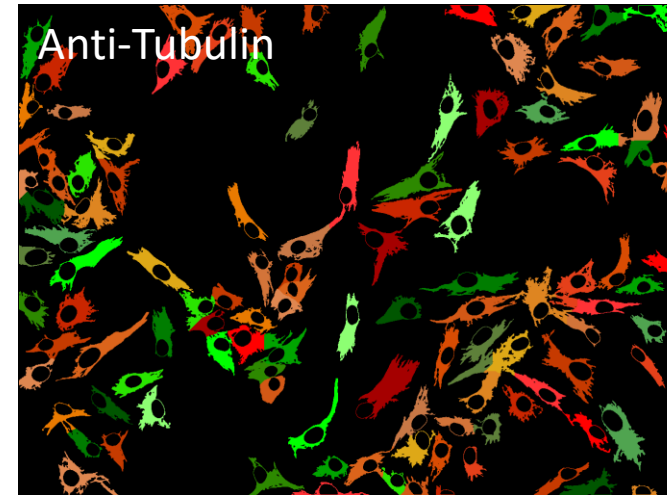
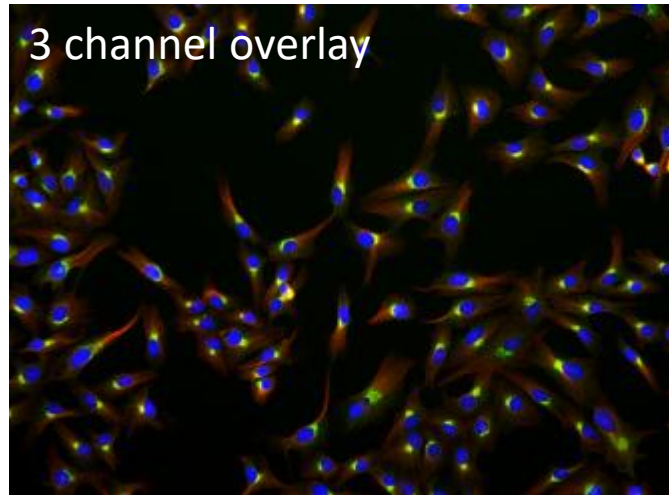
Relative Staining Intensity = gray value(GV)



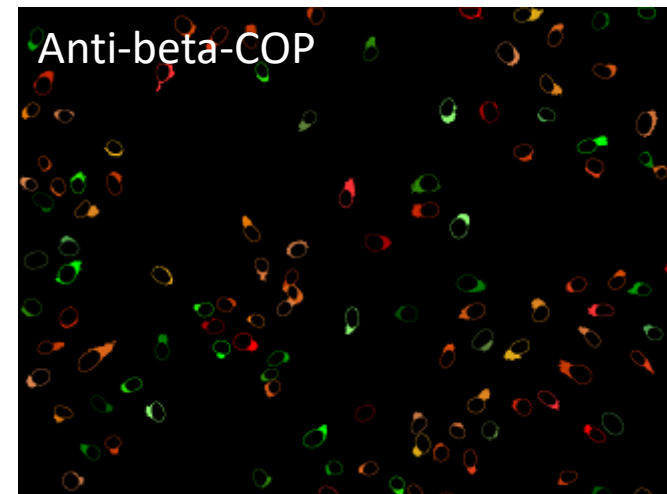
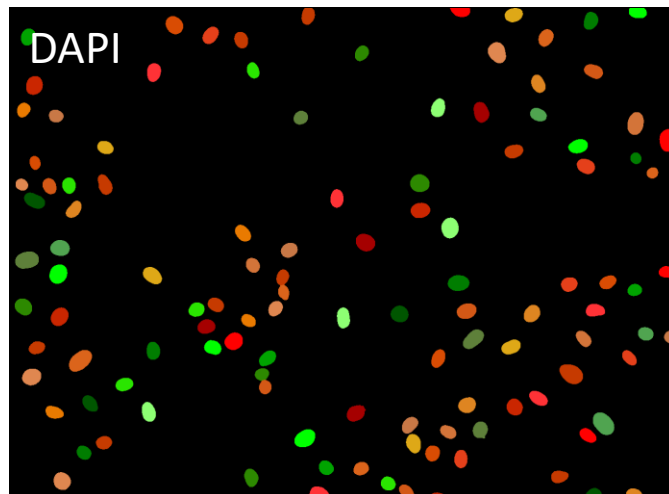
Assume A and B are 2 samples stained for:

- Tumor marker
- Cytokine expression
- Apoptosis
- Proliferation
-

Distinguish between Nucleus and Cytoplasm!



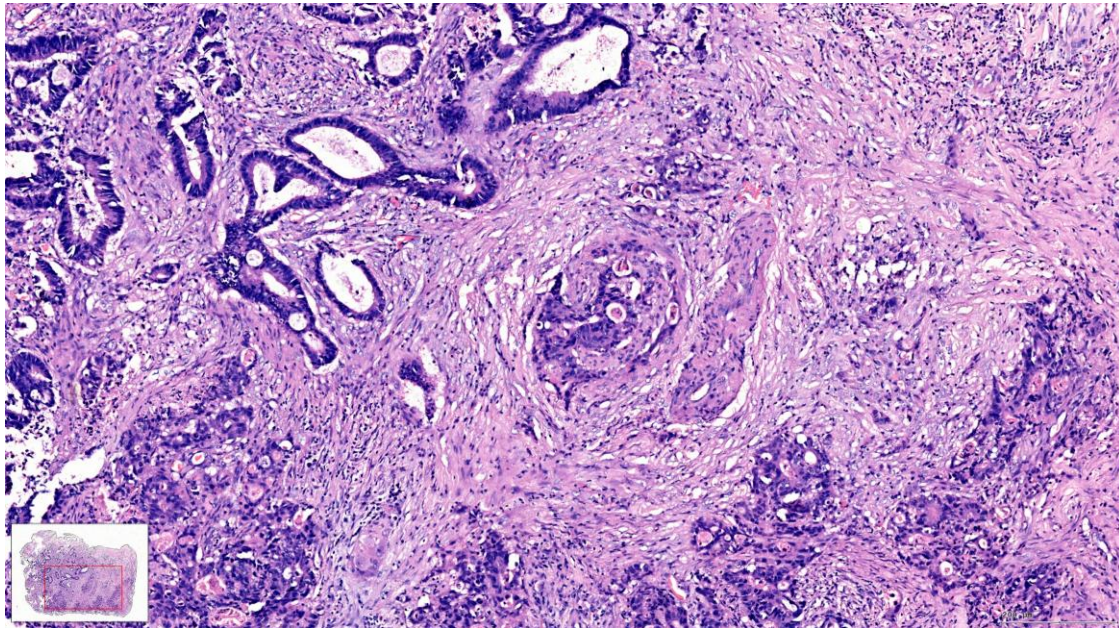
Not only analyze single cells – even analyze subcellular compartments on a single cell basis!



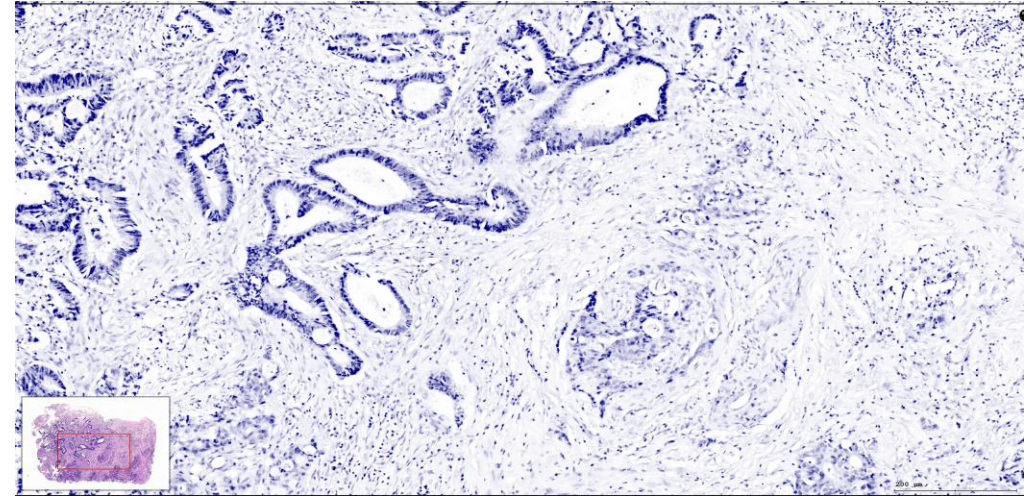
HistoQuest Workflow

1. Click- Color Separation

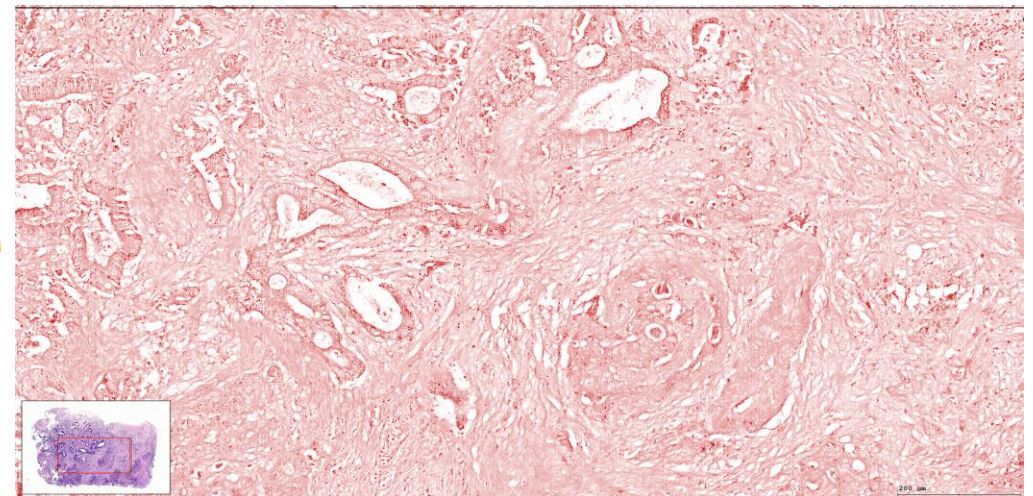
for better cell segmentation results



Hematoxylin shade

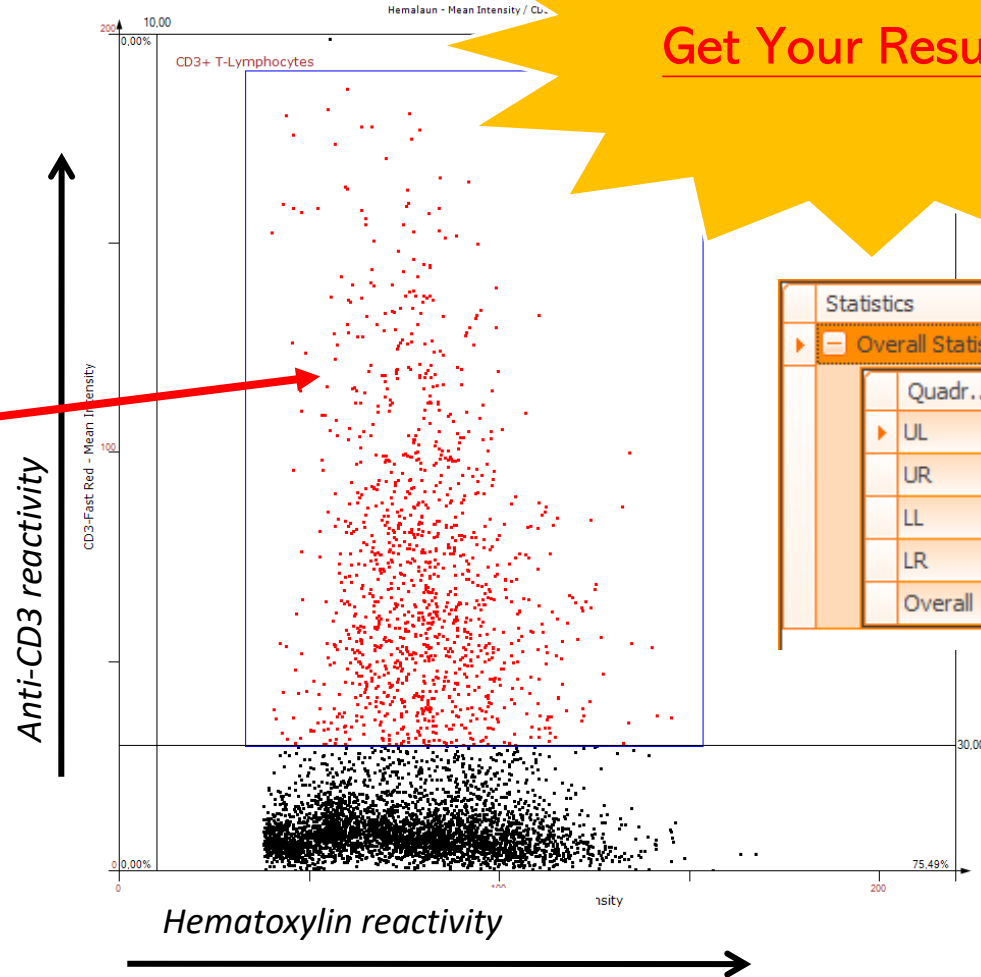
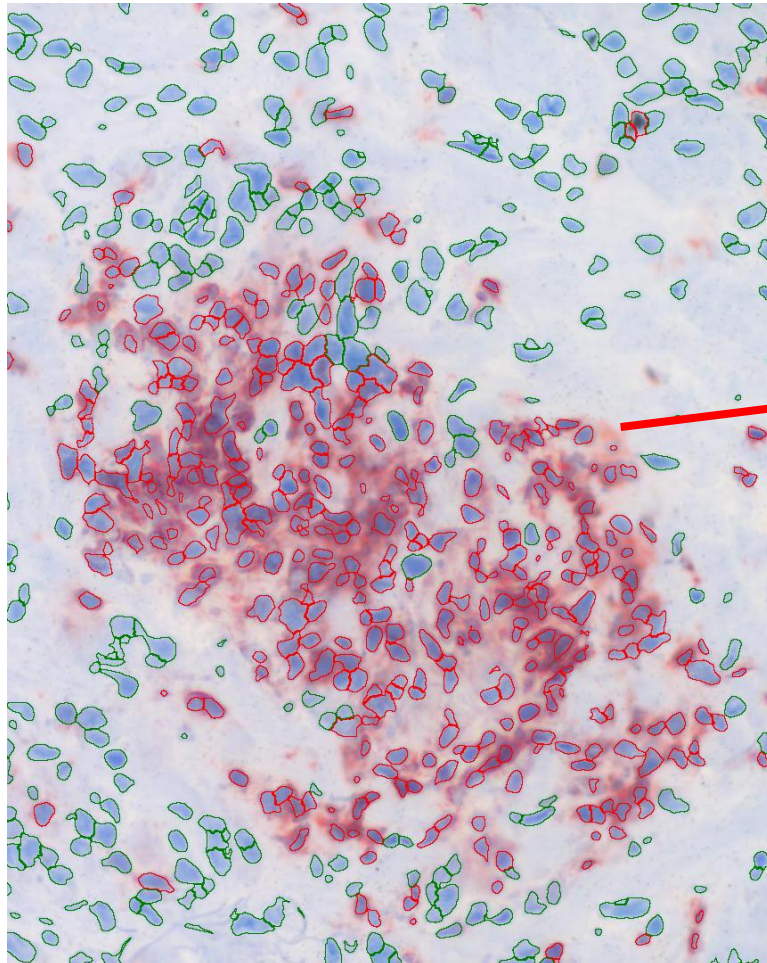


Eosin shade



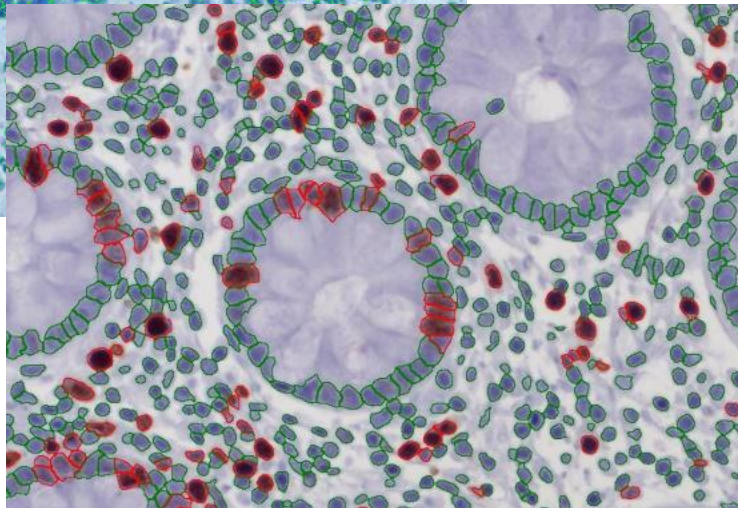
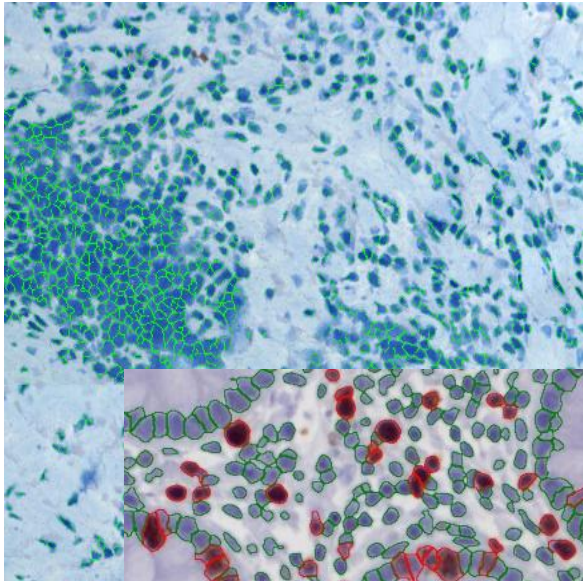
HistoQuest Workflow

2. Automatically identified cells –



HistoQuest Analysis algorithm

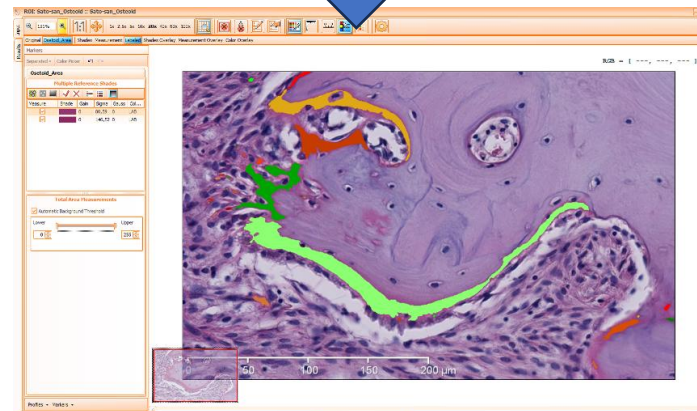
- Single Cell Segmentation



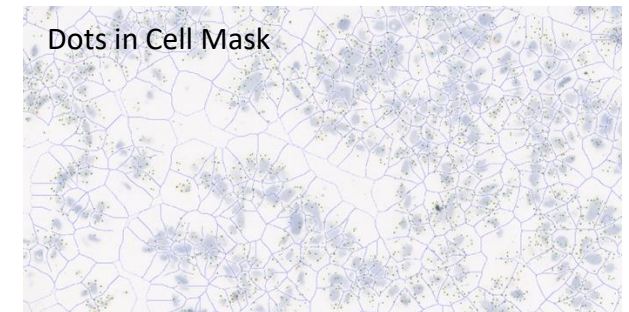
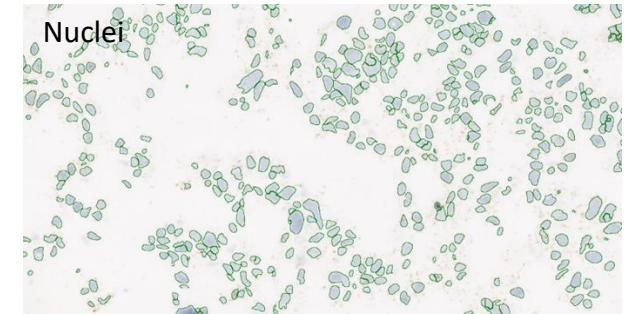
- Total Area Measurement



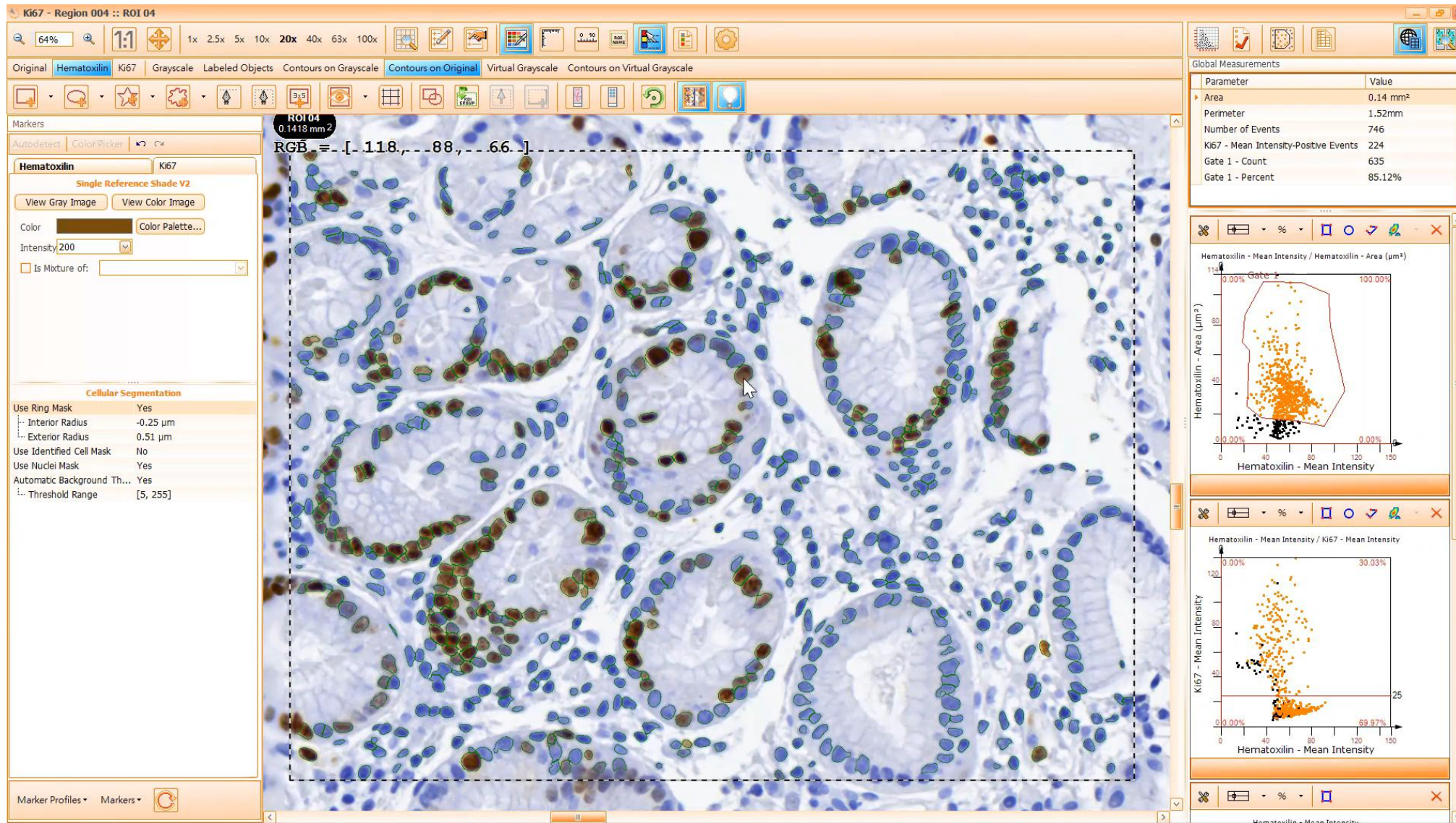
Detection of Red color



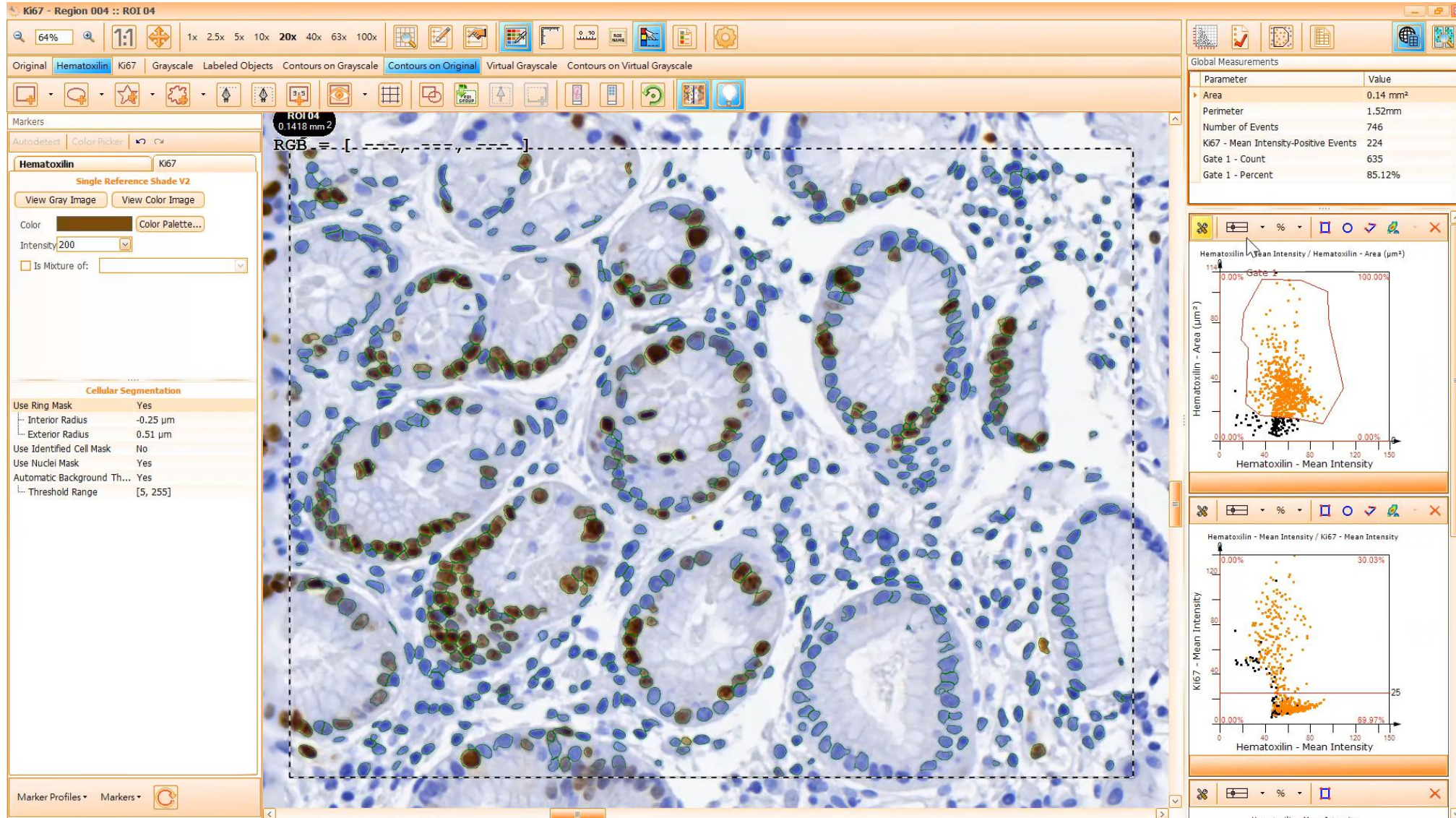
- Dots Detection



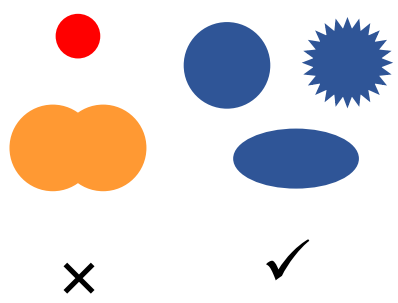
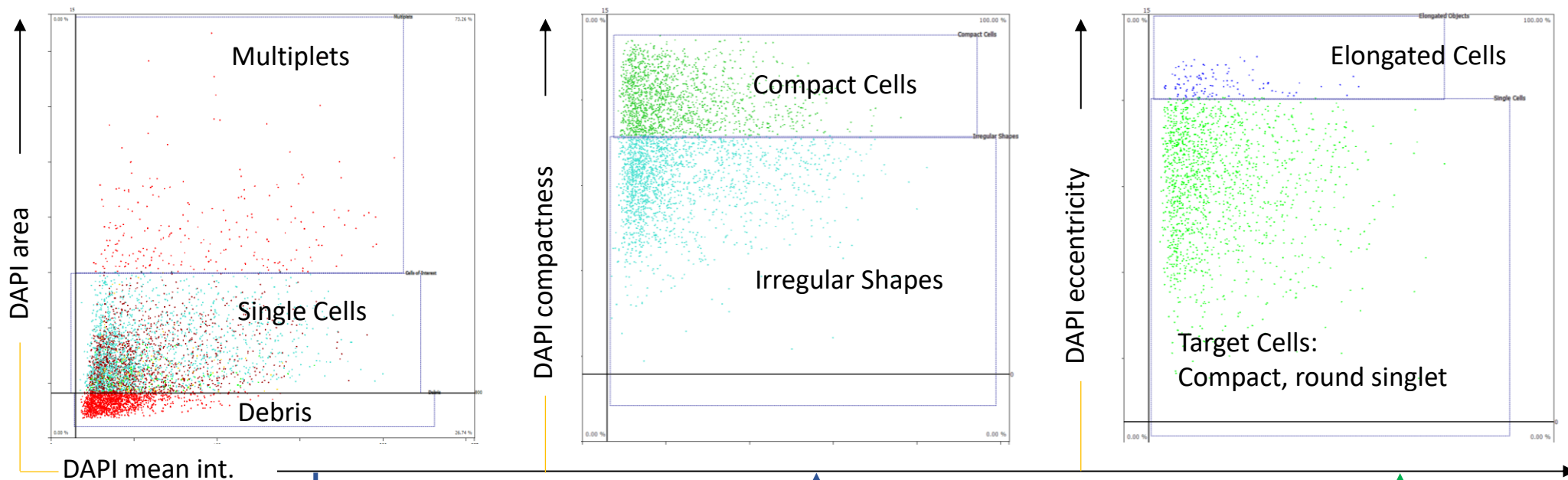
Forward Gating: Seeing is Believing



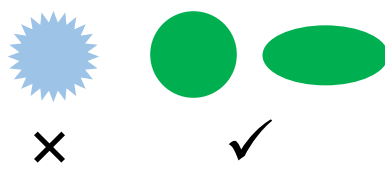
Backward Gating: In-situ analysis



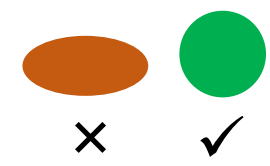
Logical Gating: Precise analysis



Input gate from proper cell size poulation



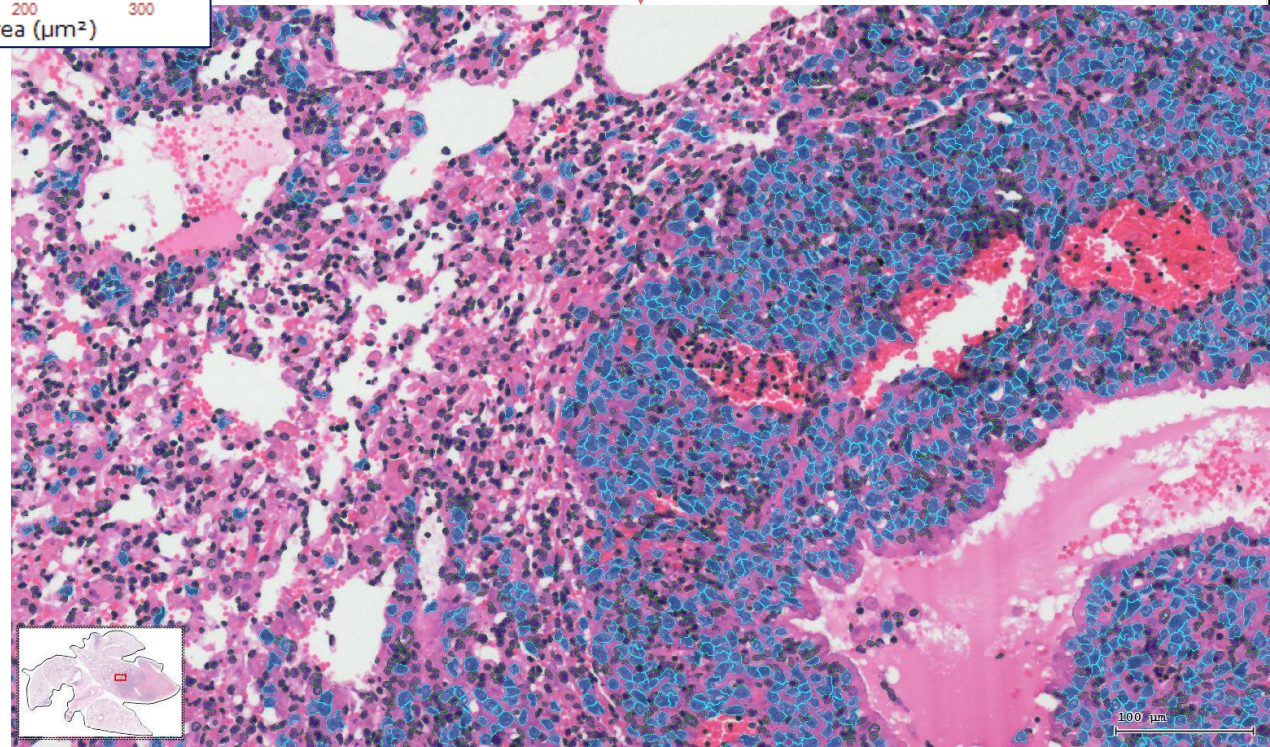
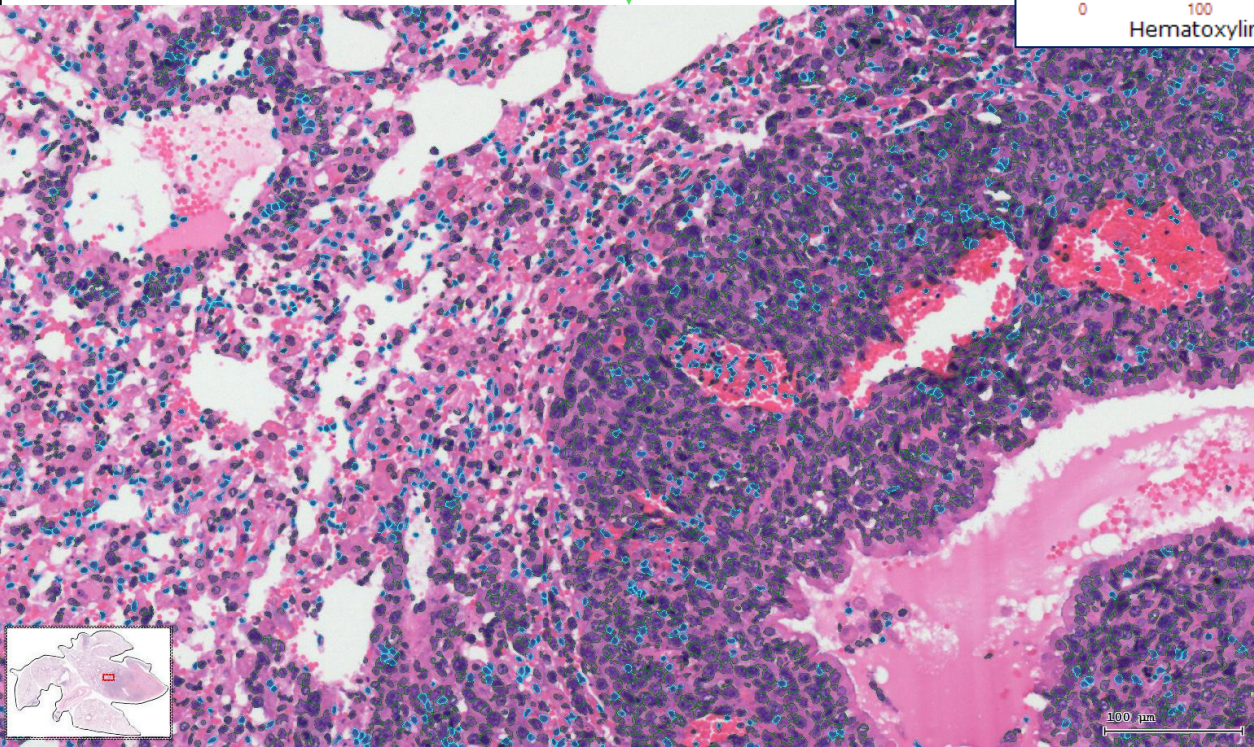
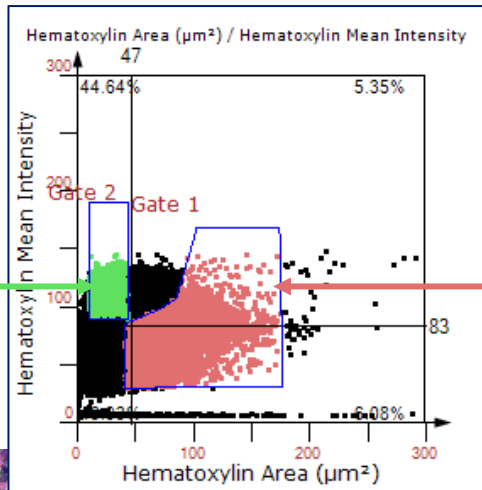
Input gate from singlet and compact cell poulation



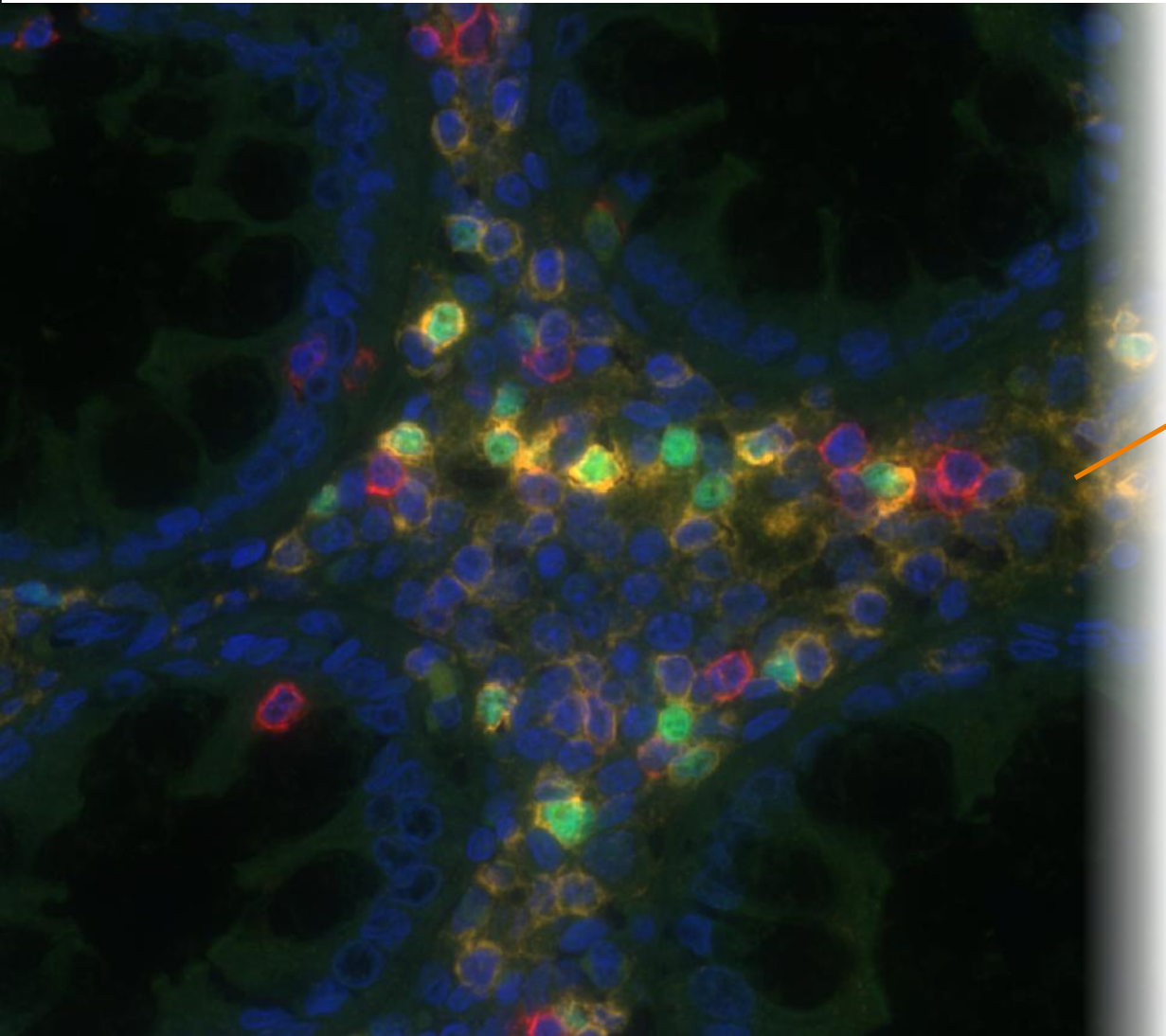
Separate Tumor/ Lymphocytes

Lymphocytes

Tumor Cells



Why we need TissueQuest?

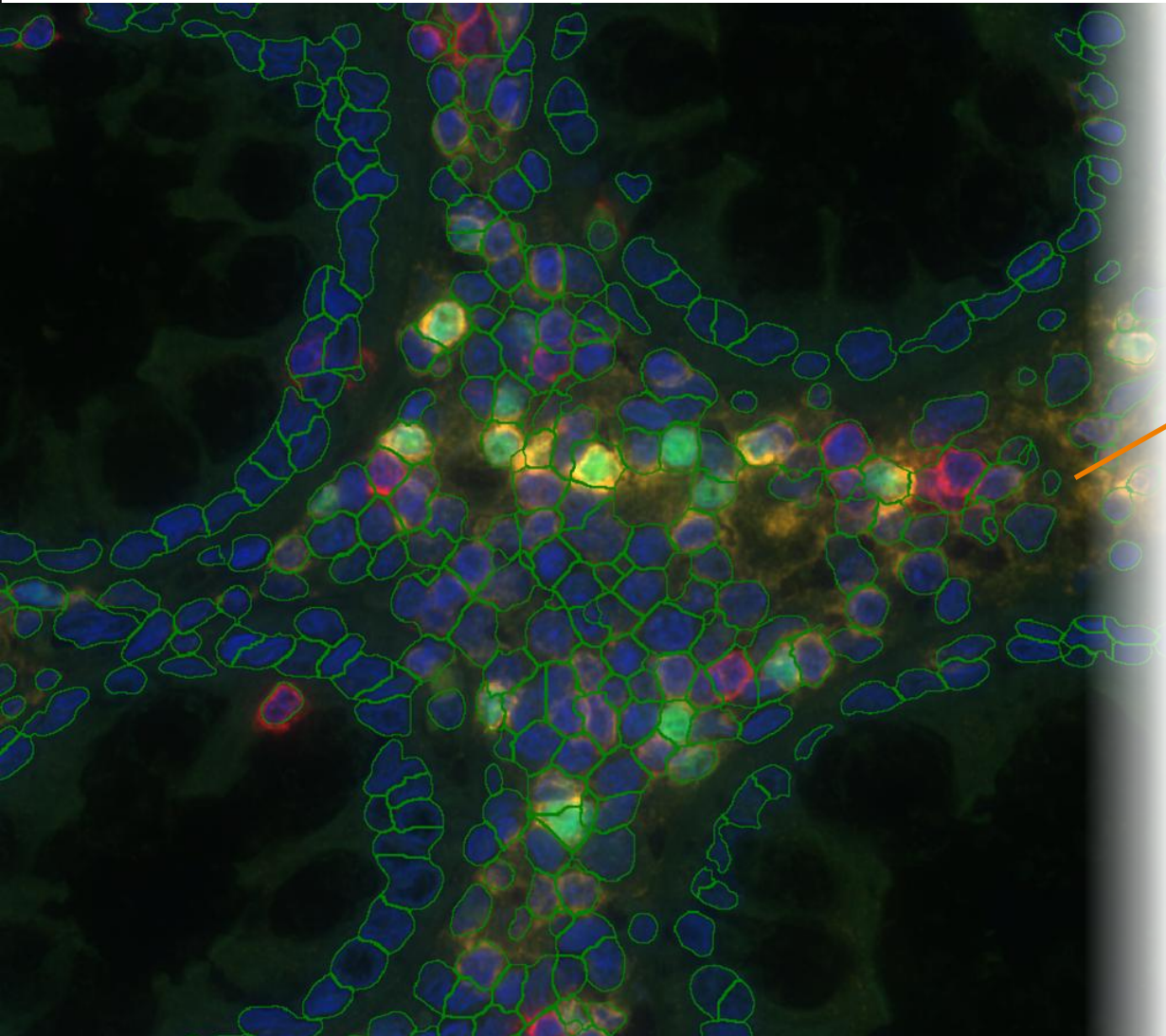


How many **CD4**, **CD8**, **Foxp3** cells are there in this image (in %)?

Colon tissue stained for

- DAPI
- CD4
- CD8
- Foxp3

Why we need TissueQuest?

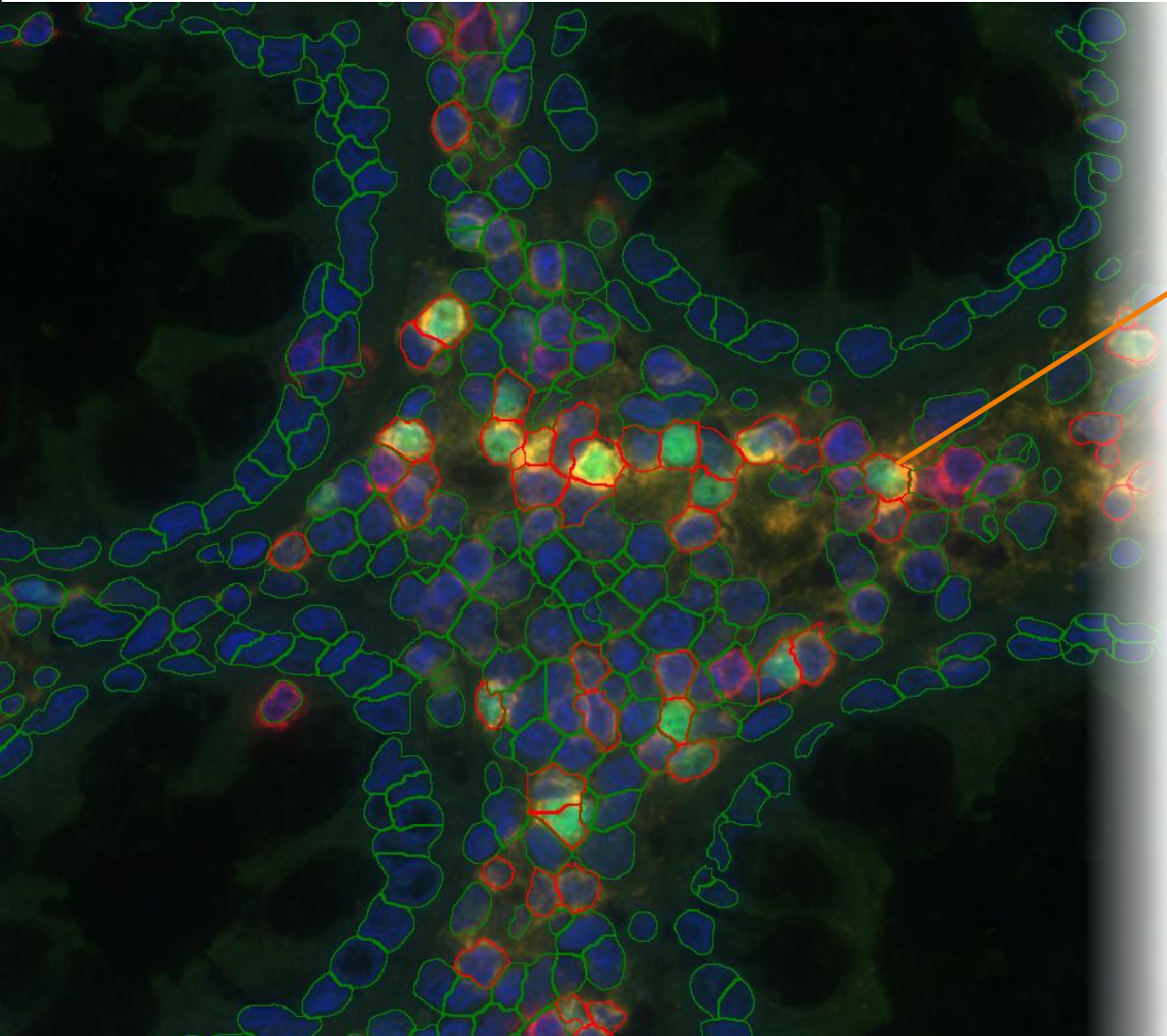


How many **CD4**, **CD8**, **Foxp3** cells are there in this image (in %)?

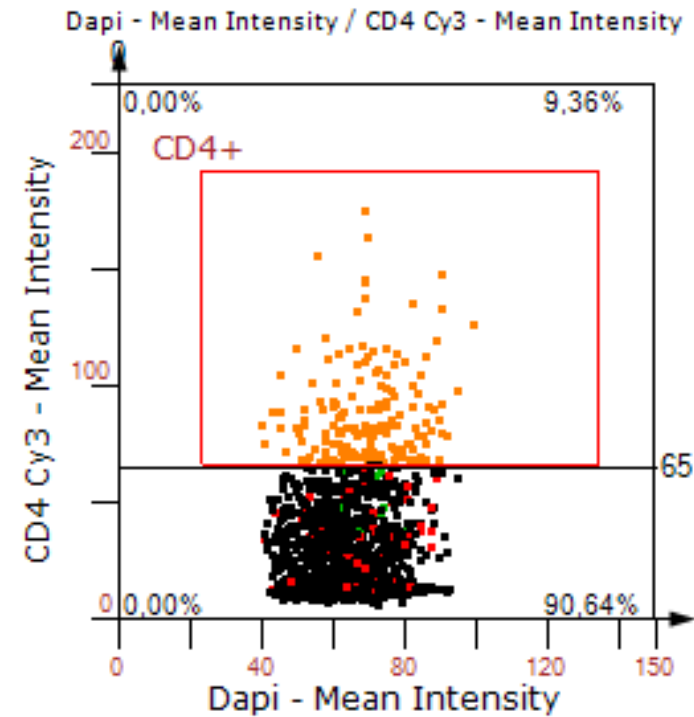
Nuclei detection

- DAPI
- CD4
- CD8
- Foxp3

Why we need TissueQuest?

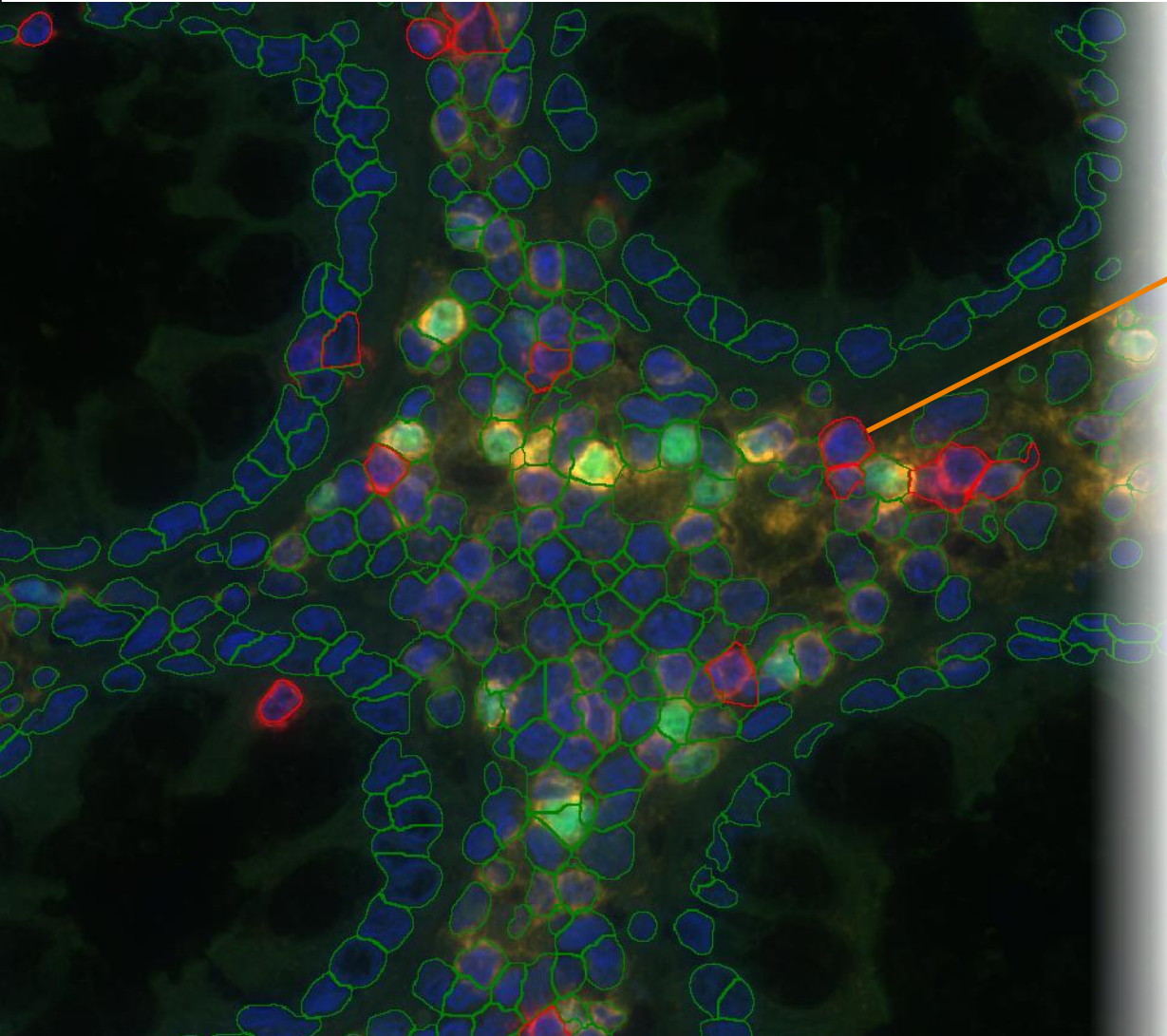


Detection of CD4 positive cells

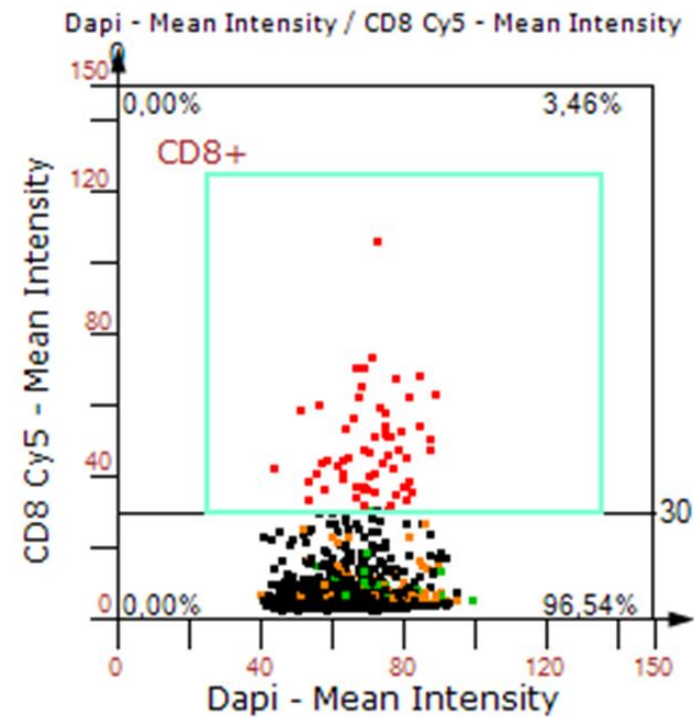


- DAPI
- CD4
- CD8
- Foxp3

Why we need TissueQuest?

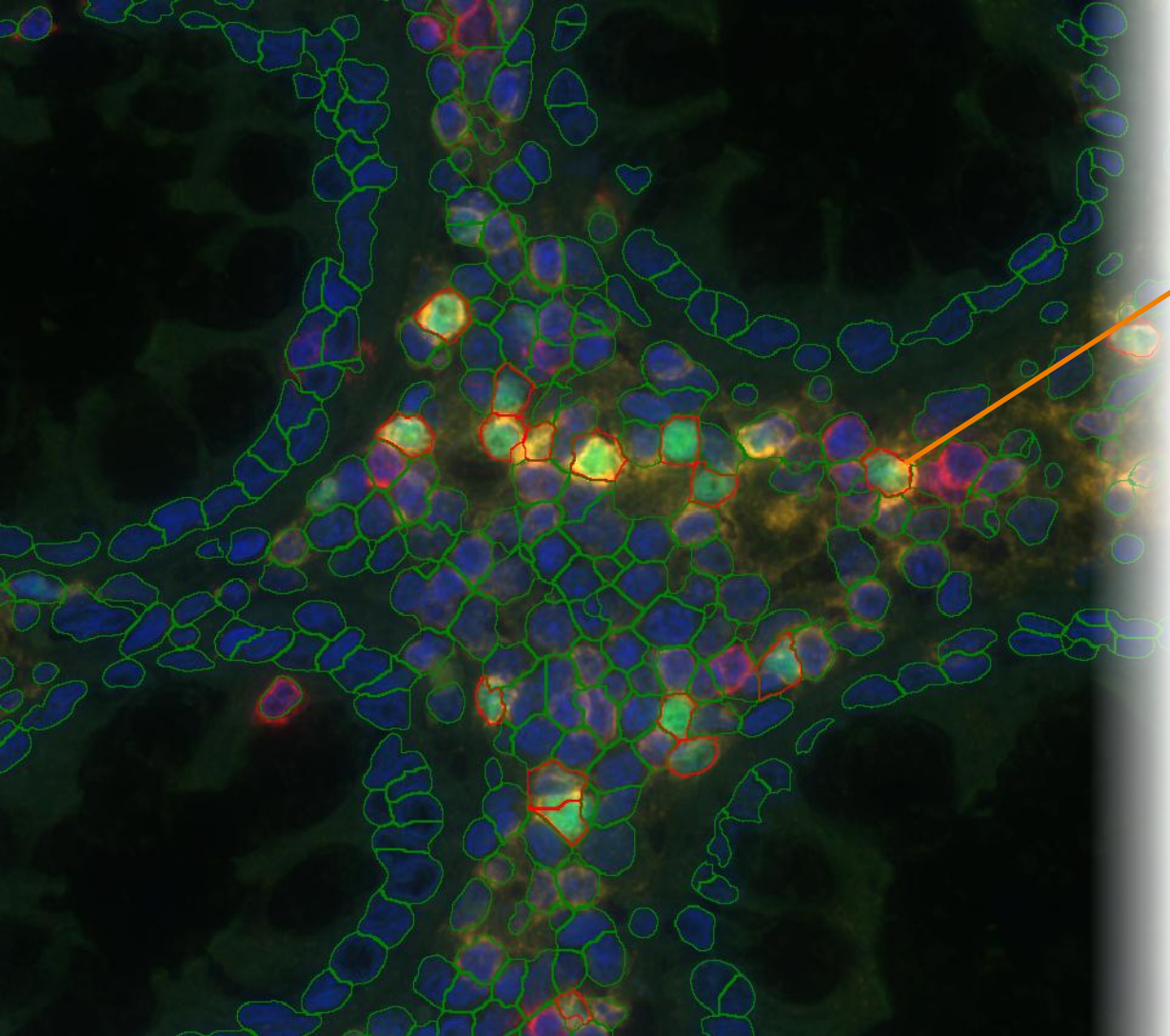


Detection of CD8 positive cells

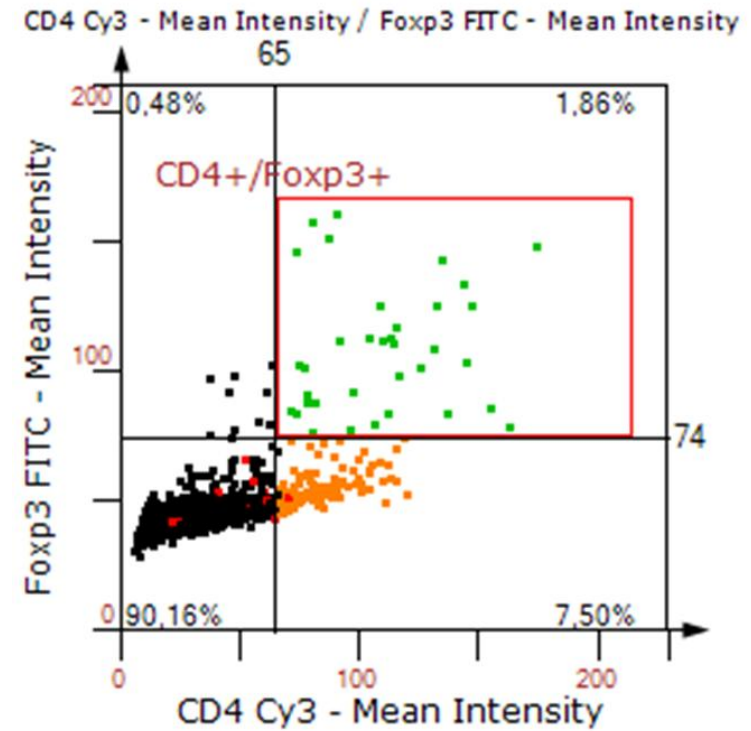


- DAPI
- CD4
- CD8
- Foxp3

Why we need TissueQuest?



Detection of CD4+/Foxp3+ cells

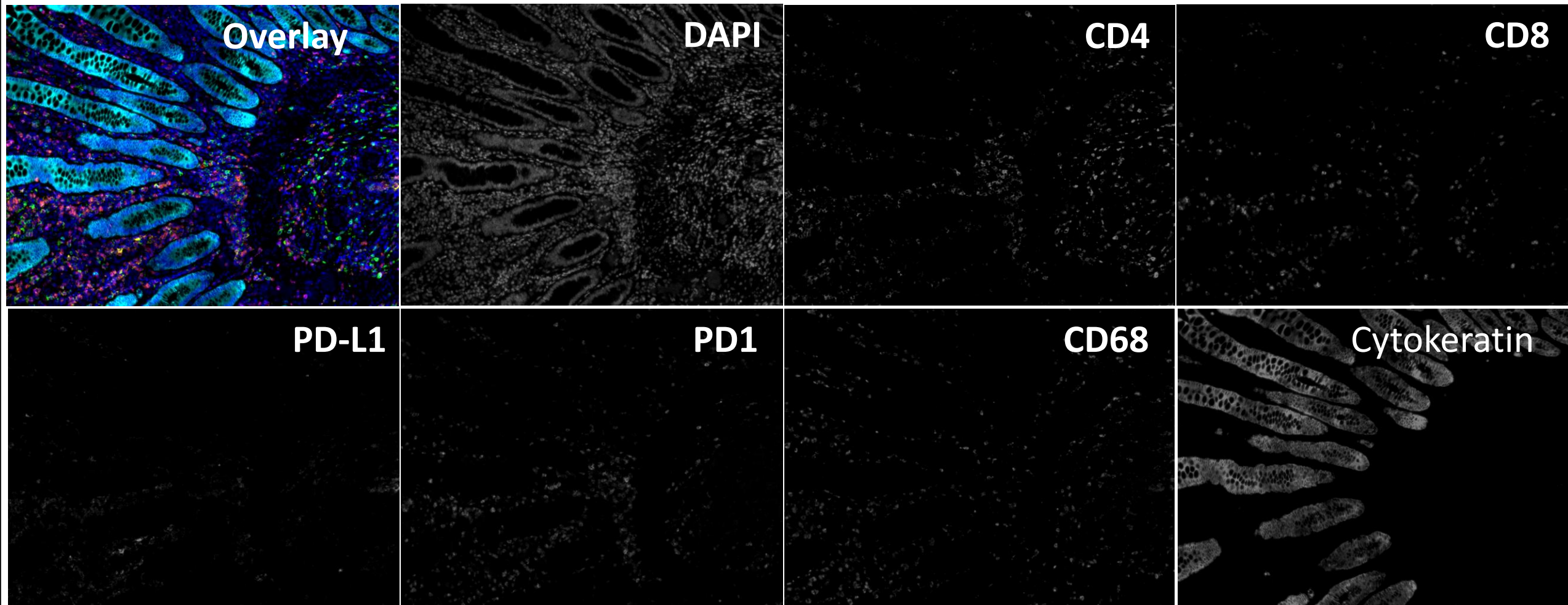


- DAPI
- CD4
- CD8
- Foxp3

TissueQuest Workflow

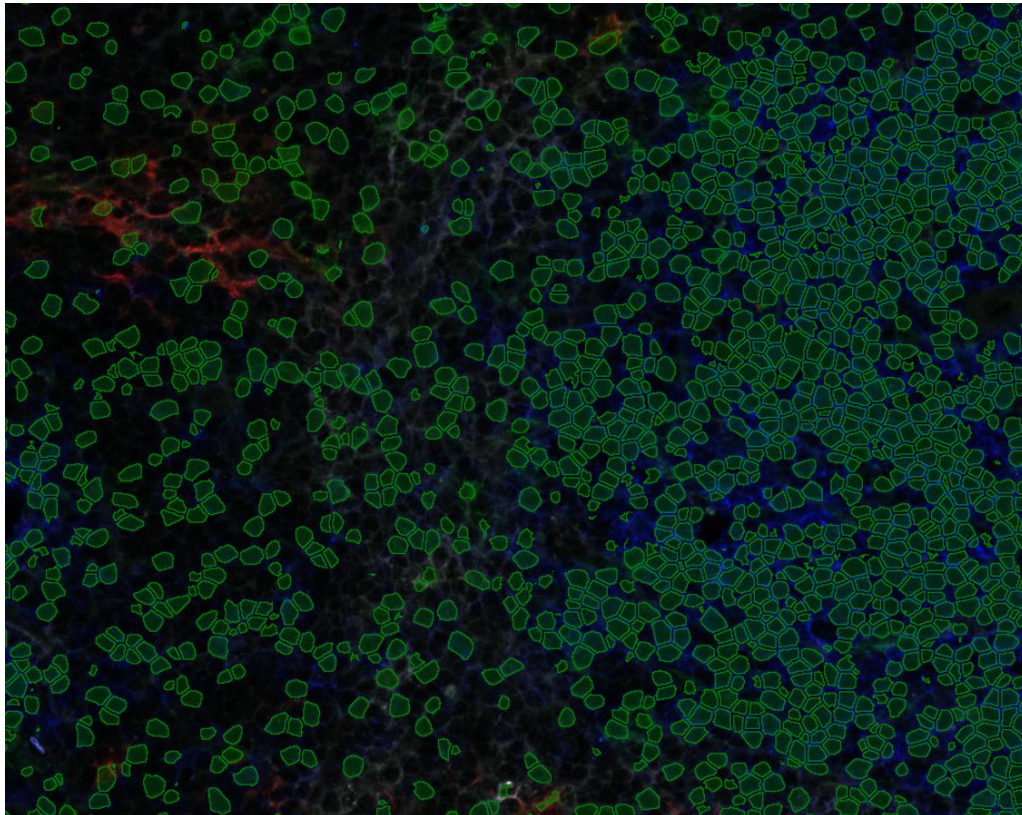
1. Import Single channel images

NO upper limits of marker imported !

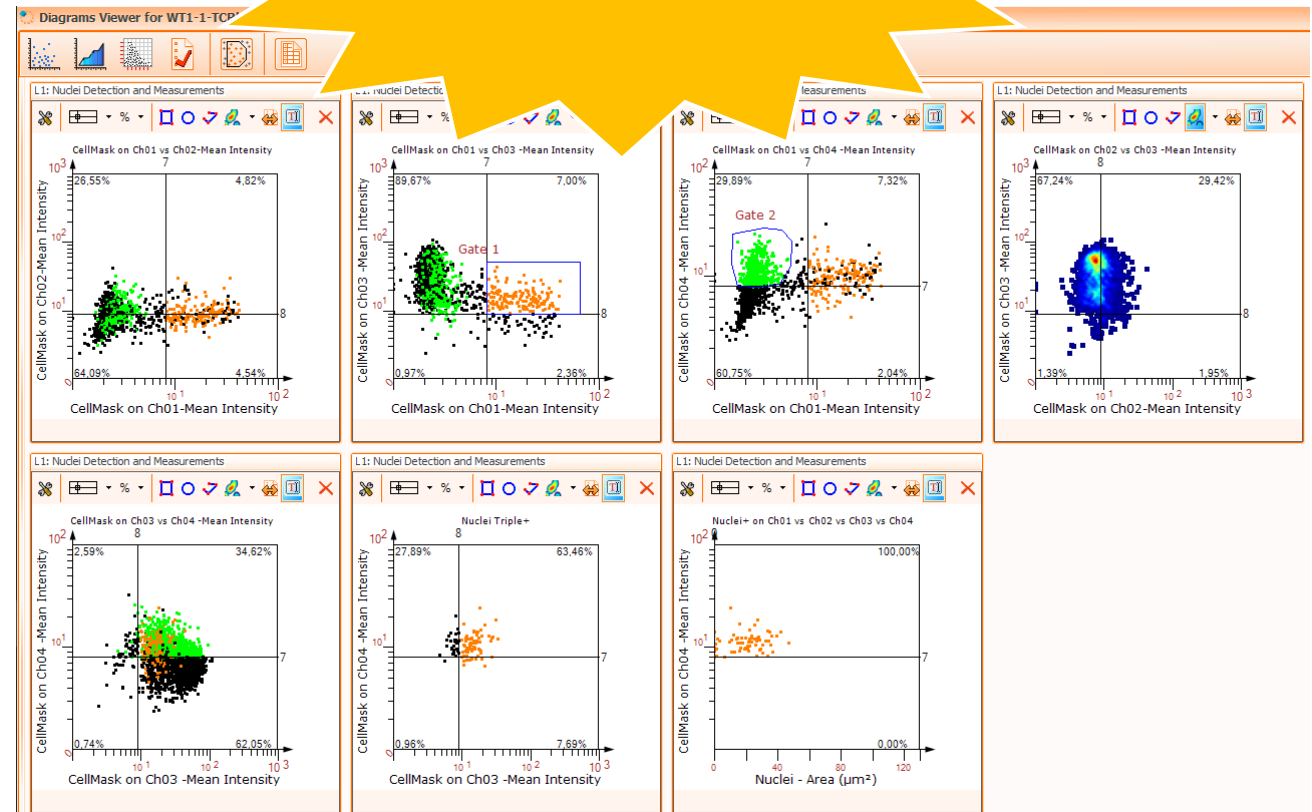


TissueQuest Workflow

2. Automatically identified cells –

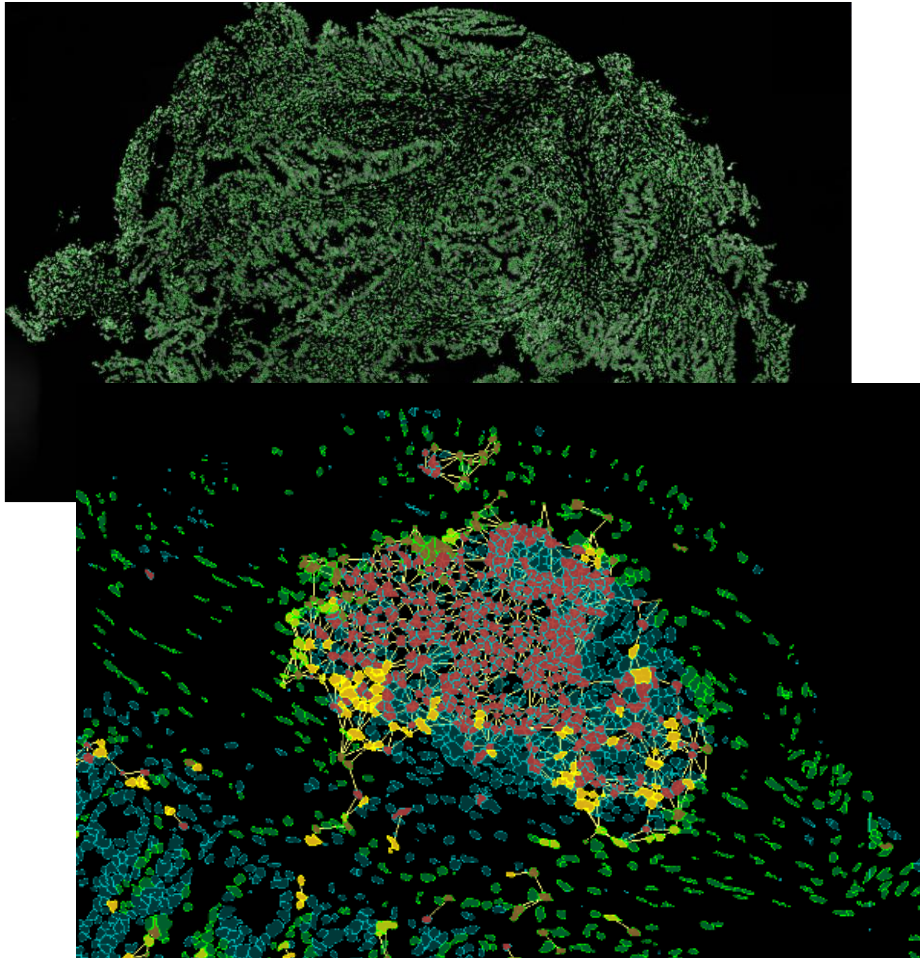


Get Your Results !!

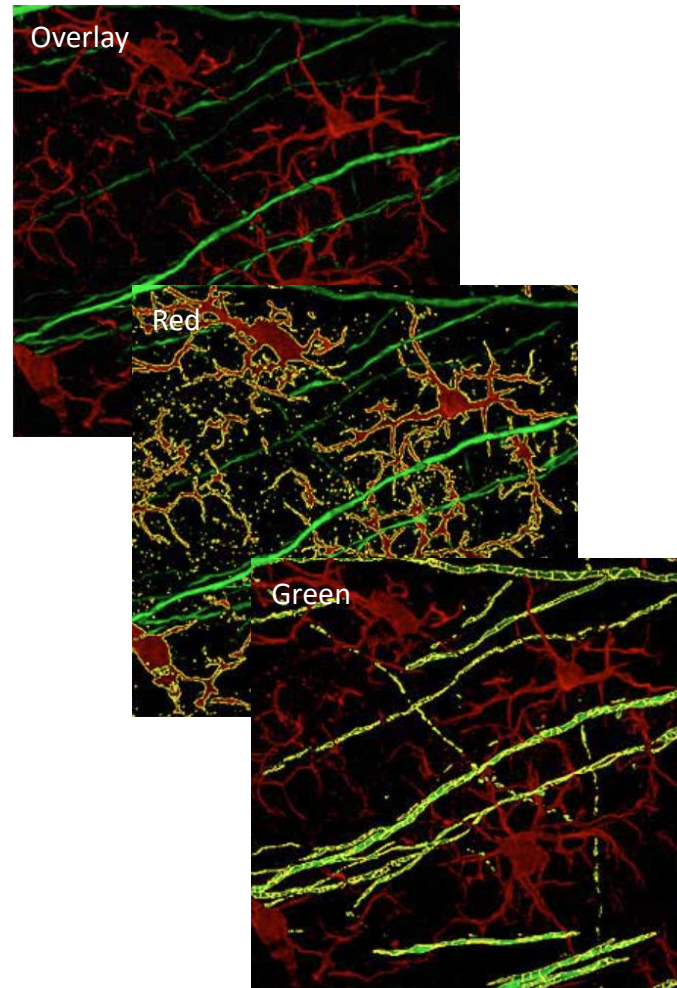


TissueQuest Analysis algorithm

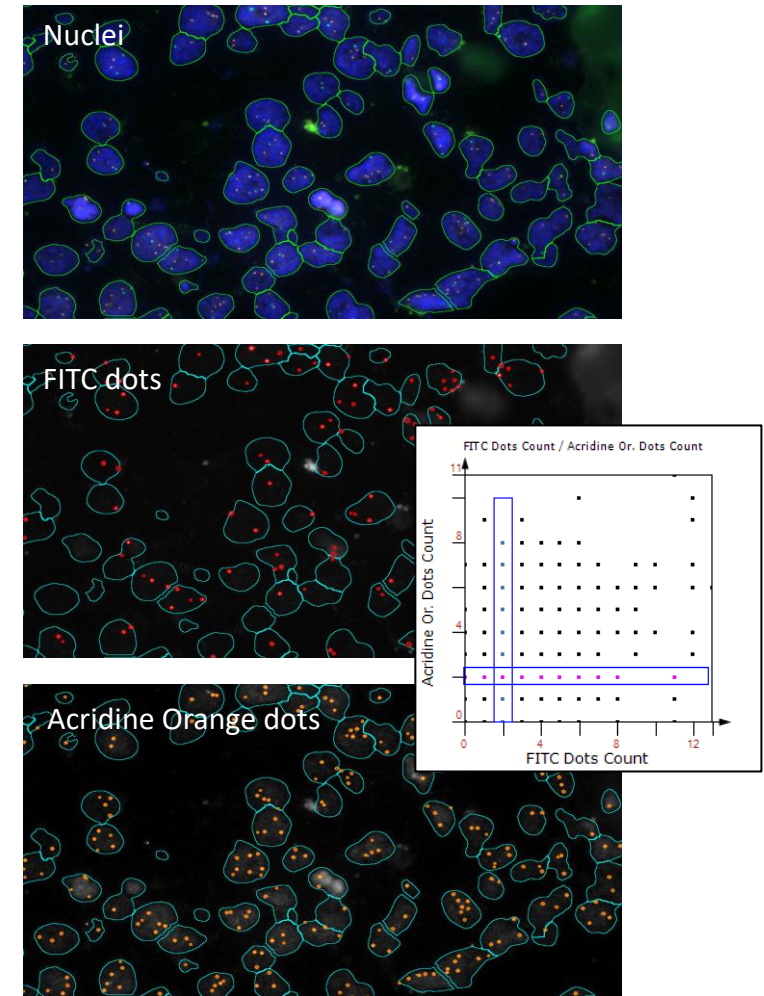
- Single Cell Segmentation



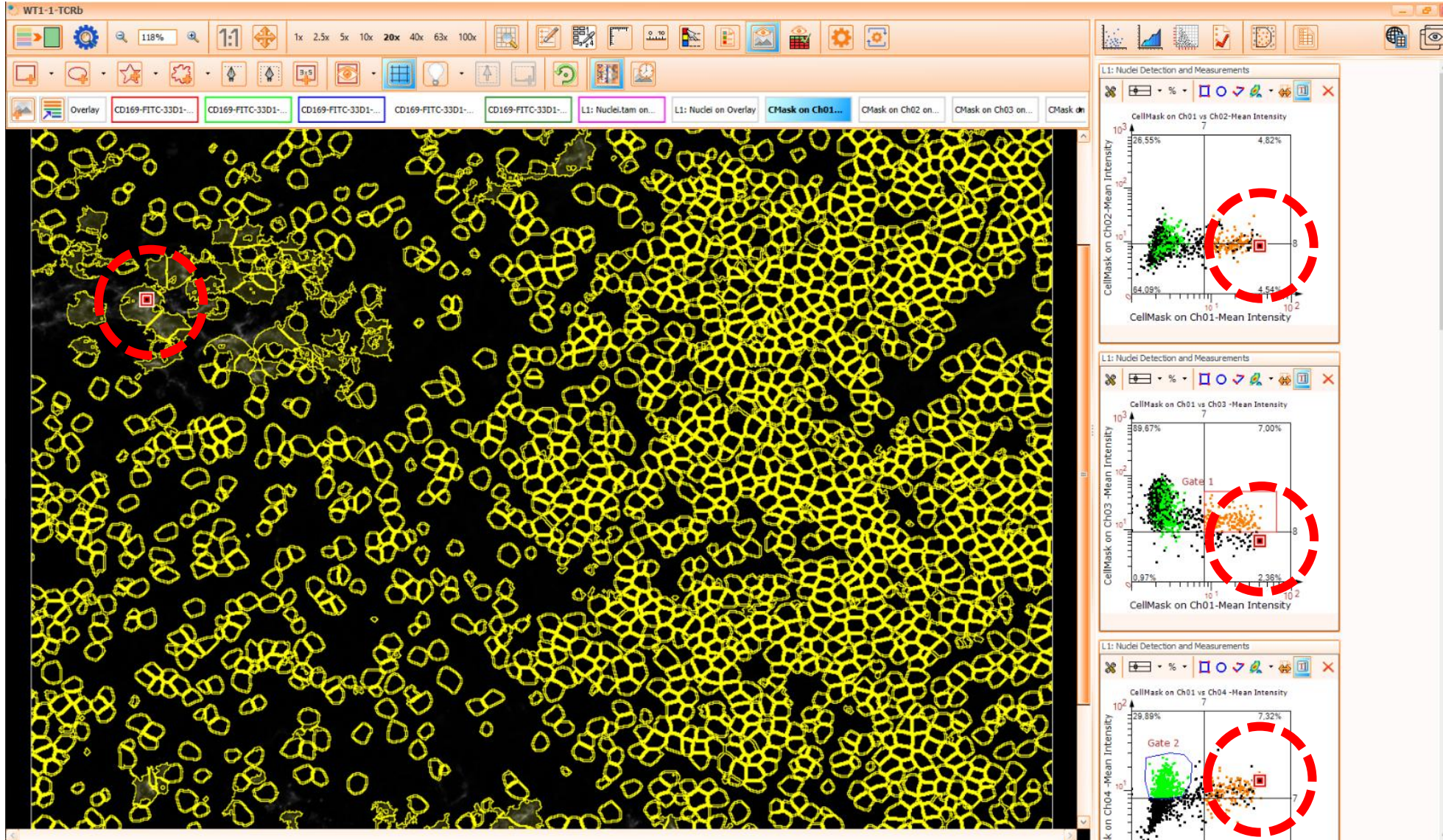
- Total Area Measurement



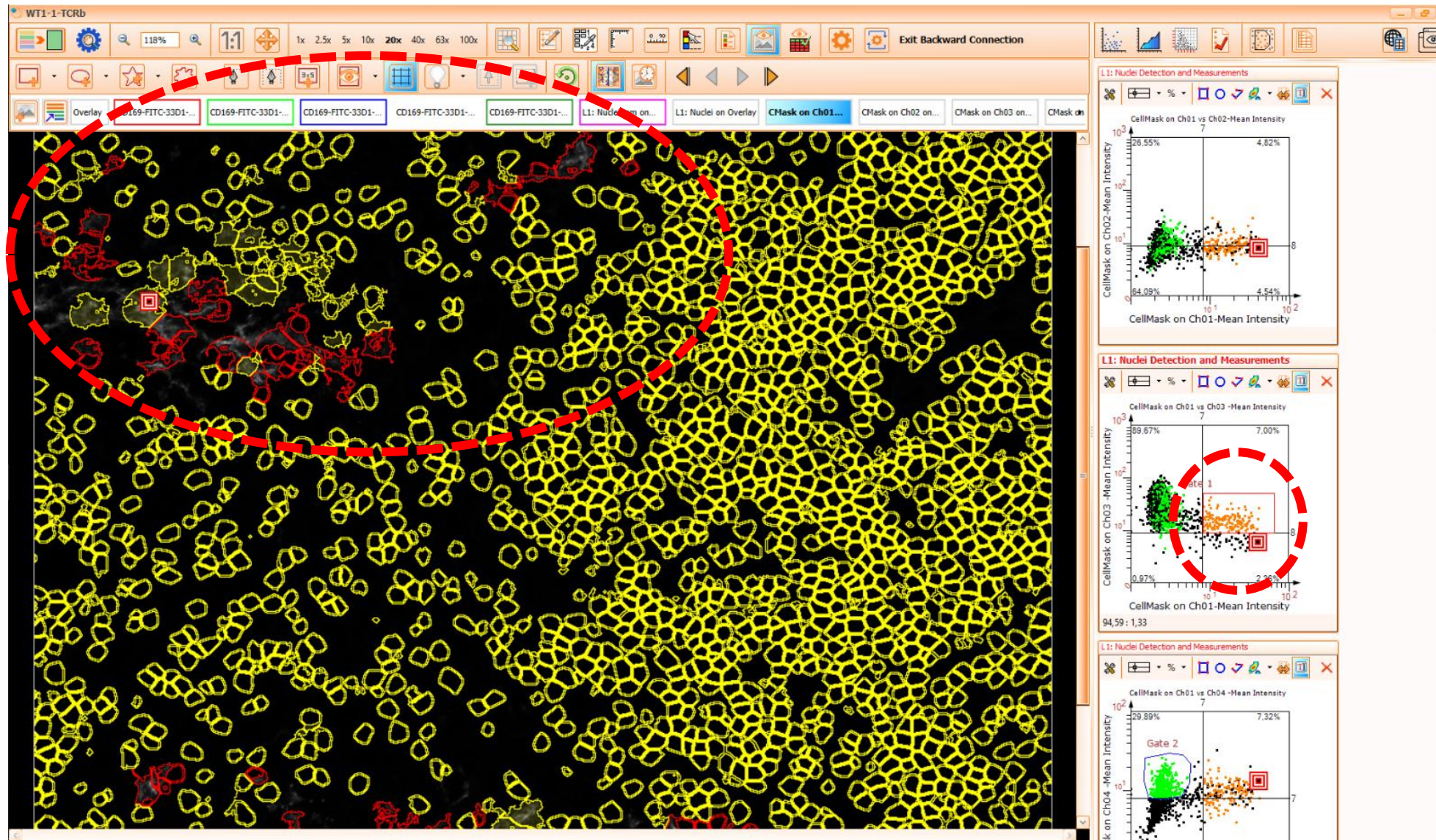
- Dots Detection



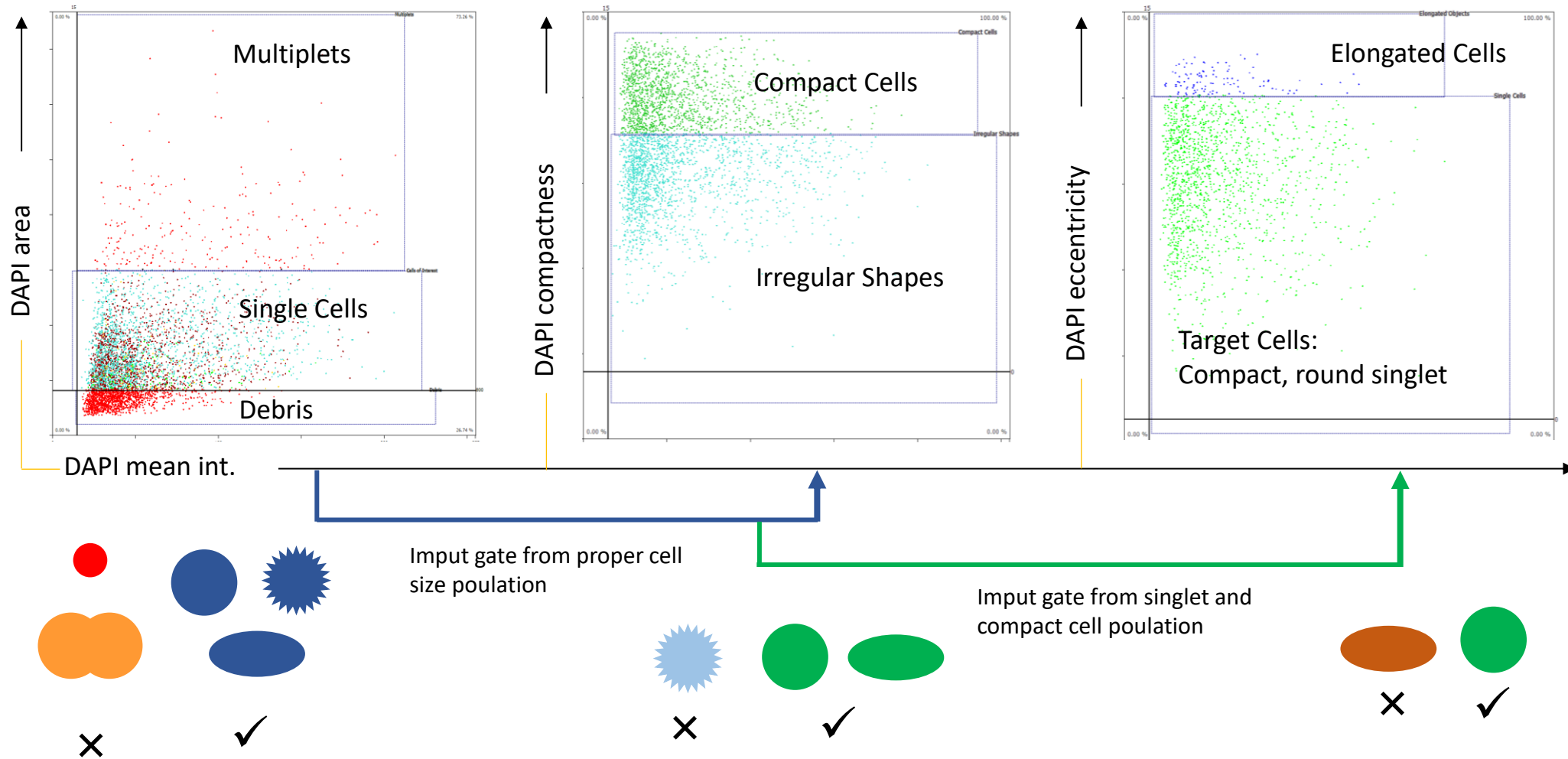
Forward Gating: Seeing is Believing



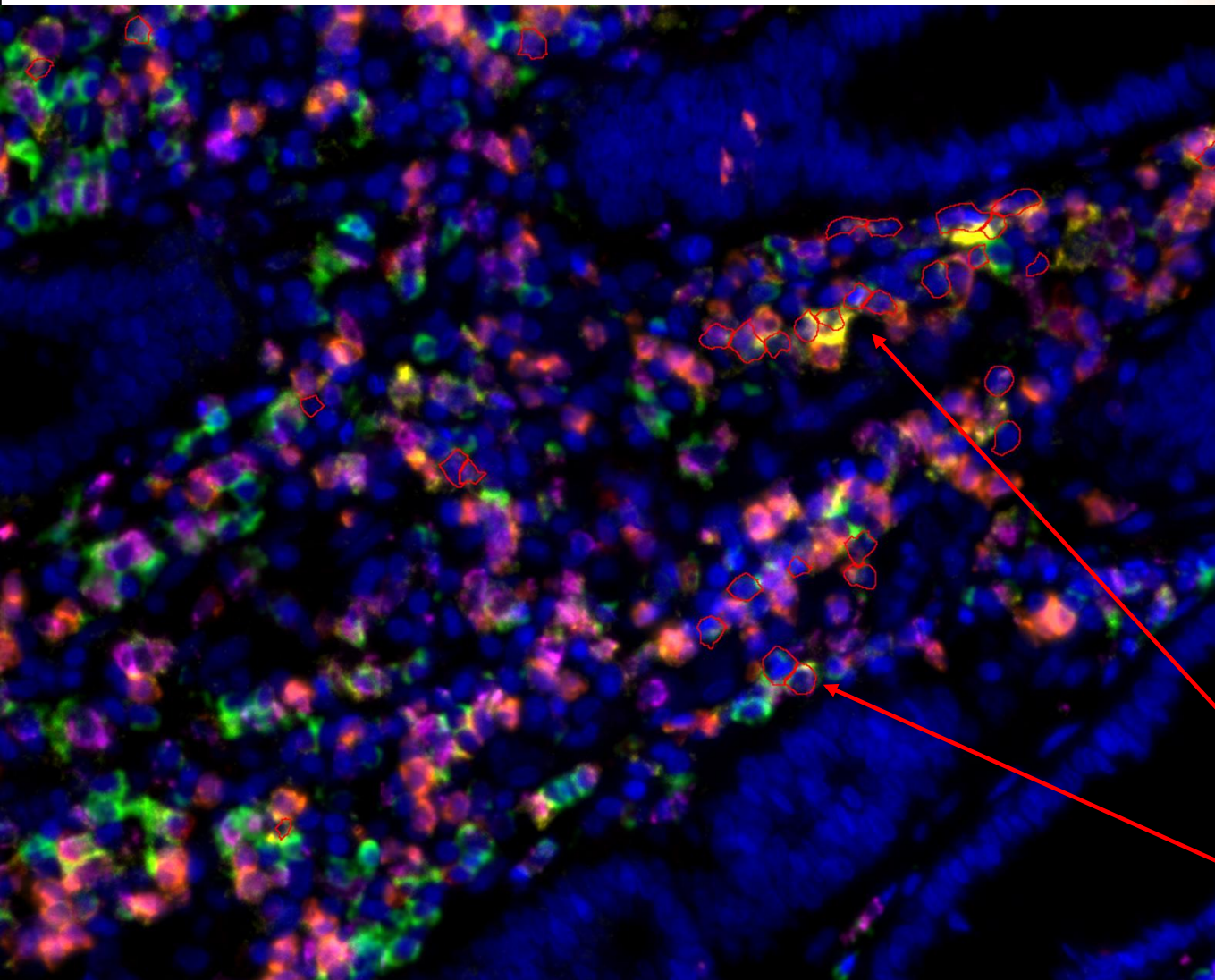
Backward Gating: In-situ analysis



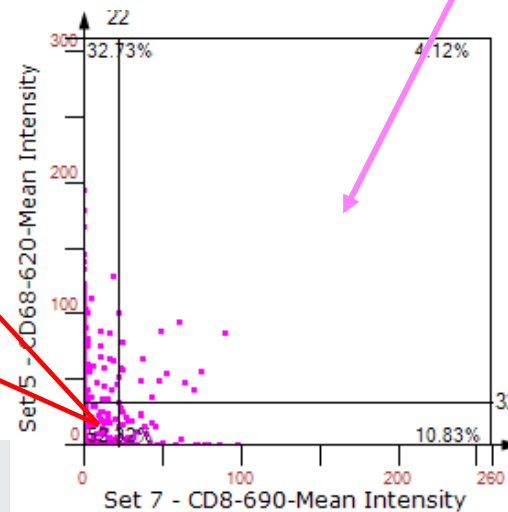
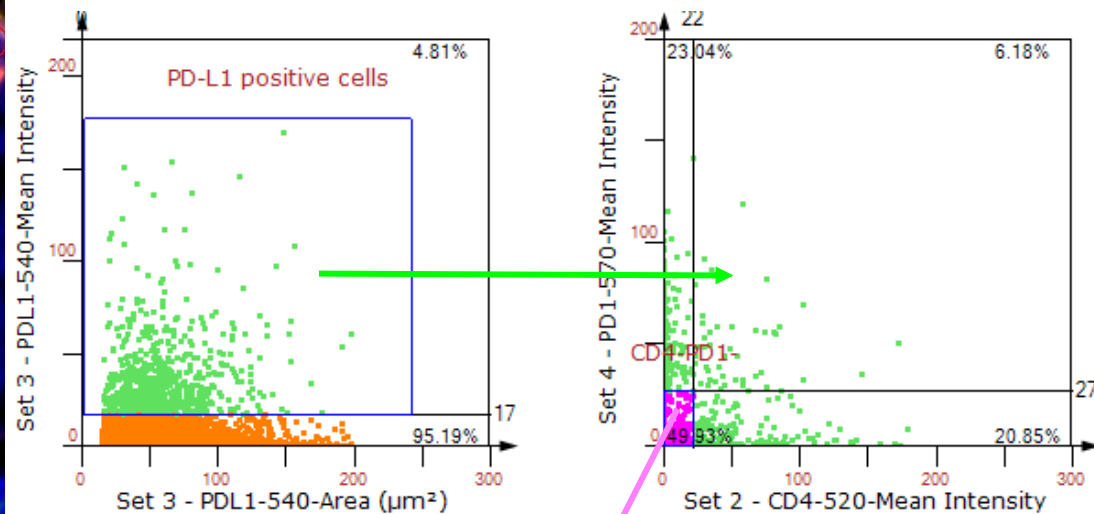
Logical Gating: Precise analysis



7-channel Spatial phenotyping in colon cancer



PD-L1+/CD4-/PD1-/CD8-/CD68-cells

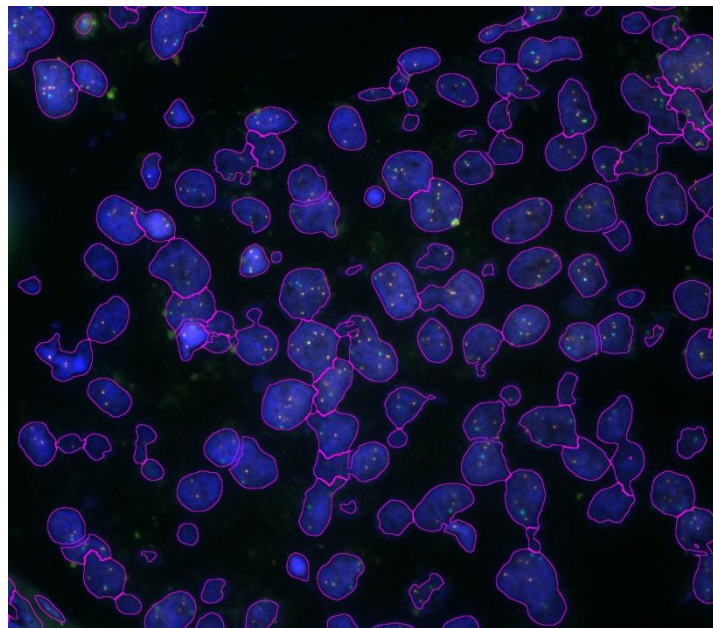
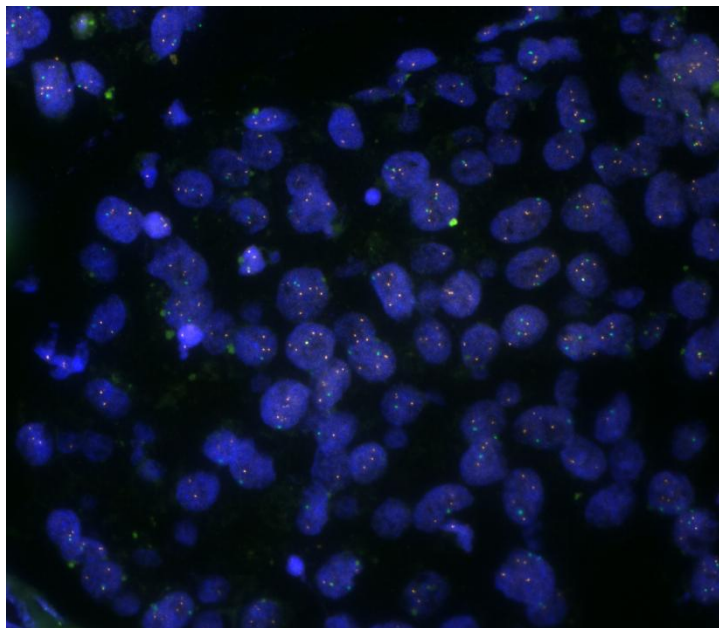


7-channel Spatial phenotyping in colon cancer



TissueFAXS – „DotFinder“ Algorithm

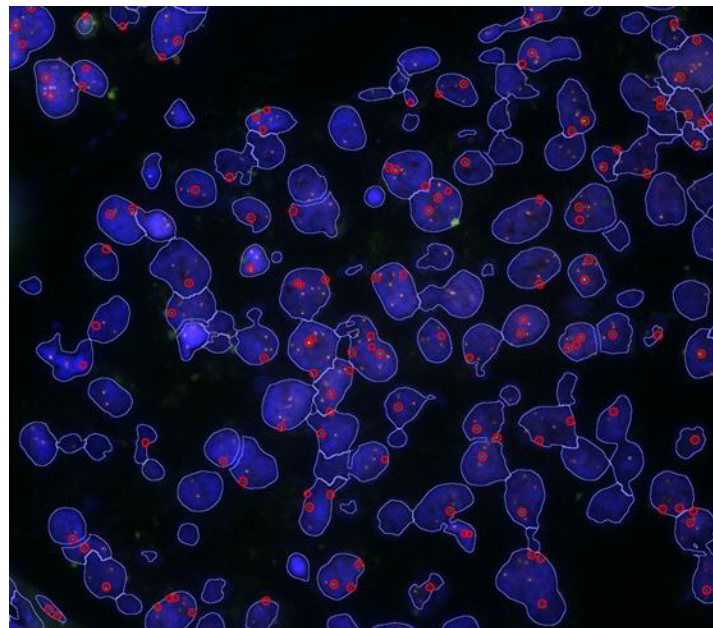
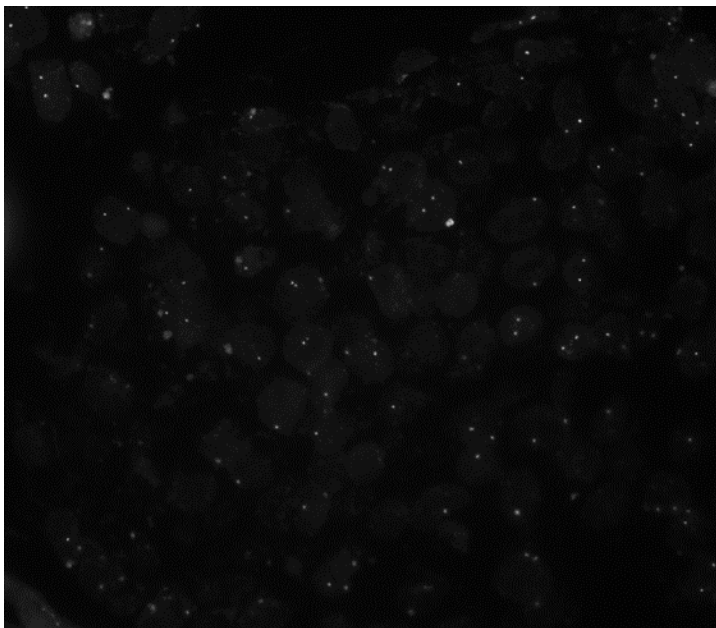
- Nuclear Identification by DAPI



*Identifies and
quantifies dots
optionally in
the nucleus or
in the
cytoplasm*

TissueFAXS – „DotFinder“ Algorithm

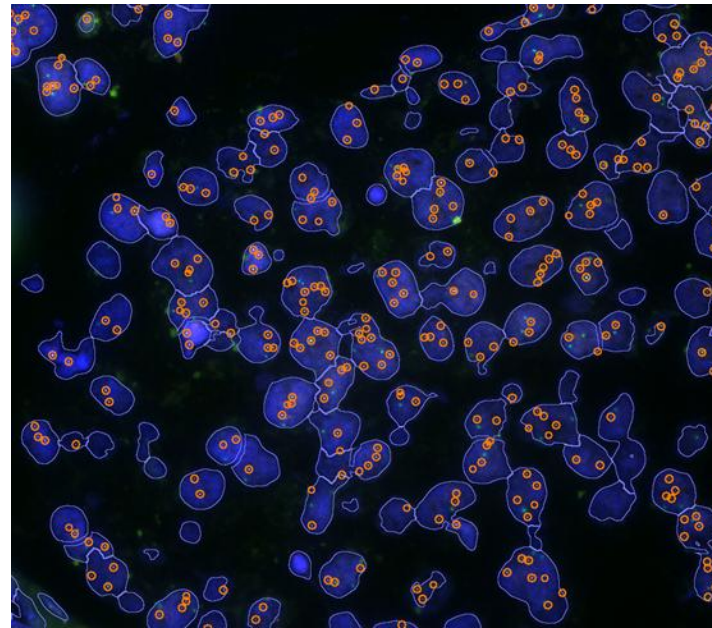
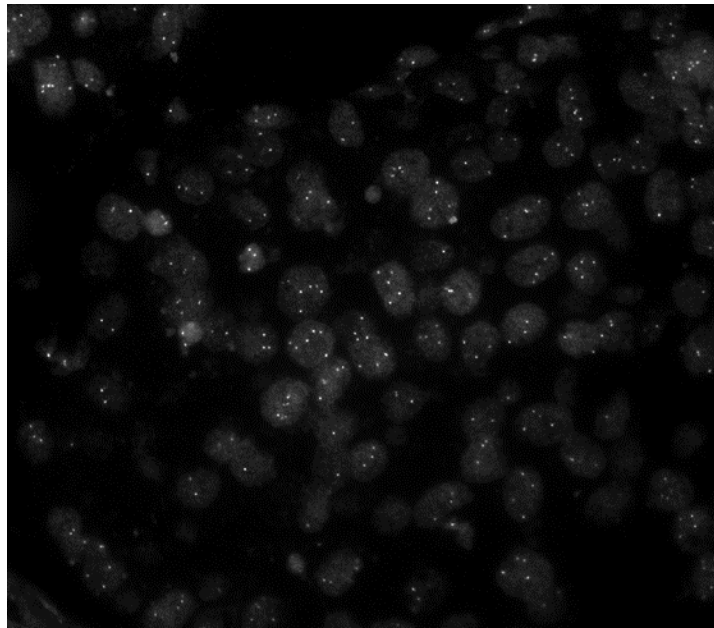
- Green Dots Count



*Identifies and
quantifies dots
optionally in
the nucleus or
in the
cytoplasm*

TissueFAXS – „DotFinder“ Algorithm

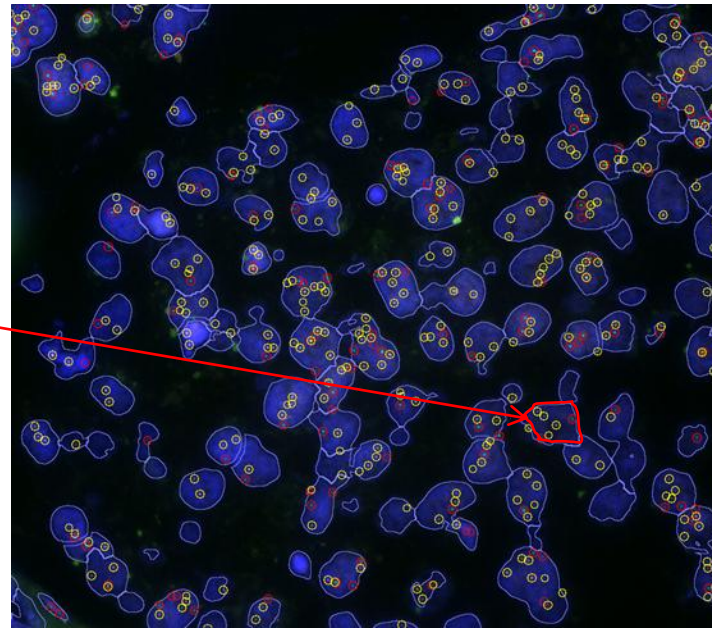
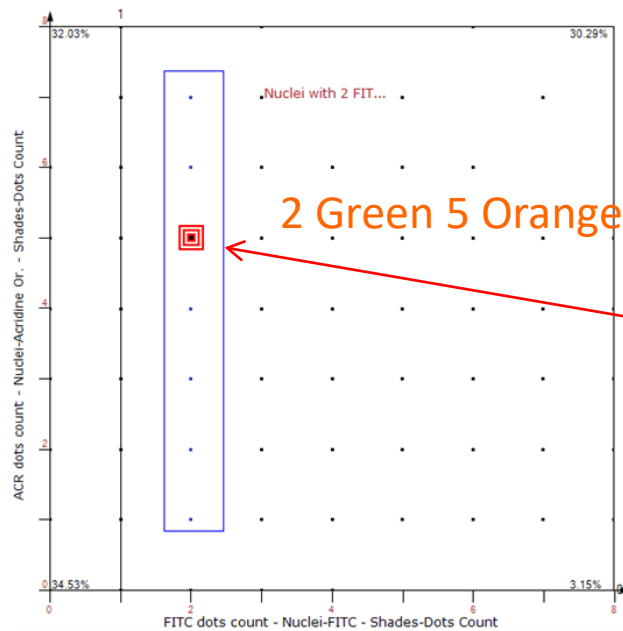
- Orange Dots Count



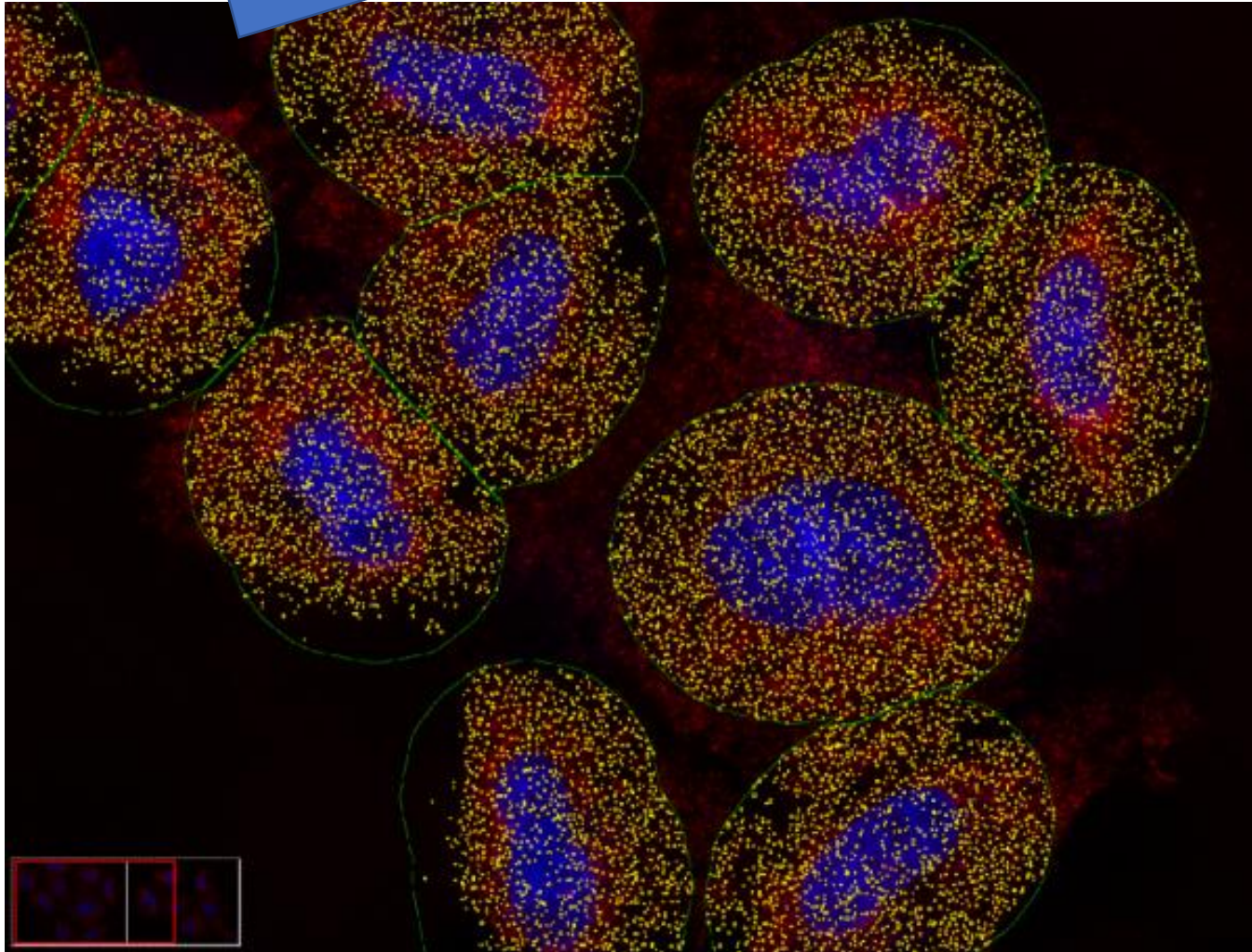
*Identifies and
quantifies dots
optionally in
the nucleus or
in the
cytoplasm*

TissueFAXS – „DotFinder“ Algorithm

- Green dots and Orange dots finding results



Example of detection of all dots from the image with all z-levels merged, within a given range ("ring mask") from the automatically detected nuclear border



*Identifies and
quantifies dots
optionally in
the nucleus or
in the
cytoplasm*

18 Parameters in Each Channel

Manage Measured Parameters and Diagrams

What parameters do you want to measure in your project?

<input checked="" type="checkbox"/> Hematoxylin	<input checked="" type="checkbox"/> Ki67	<input checked="" type="checkbox"/> CD3
<input checked="" type="checkbox"/> Area (μm^2)	<input type="checkbox"/> Area (μm^2)	<input type="checkbox"/> Area (μm^2)
<input checked="" type="checkbox"/> Mean Intensity	<input checked="" type="checkbox"/> Mean Intensity	<input checked="" type="checkbox"/> Mean Intensity
<input type="checkbox"/> Minimum of Intensity	<input type="checkbox"/> Minimum of Intensity	<input type="checkbox"/> Minimum of Intensity
<input type="checkbox"/> Maximum of Intensity	<input type="checkbox"/> Maximum of Intensity	<input type="checkbox"/> Maximum of Intensity
<input type="checkbox"/> Range of Intensity	<input type="checkbox"/> Range of Intensity	<input type="checkbox"/> Range of Intensity
<input type="checkbox"/> Sum Intensity	<input checked="" type="checkbox"/> Sum Intensity	<input type="checkbox"/> Sum Intensity
<input type="checkbox"/> Percentile 25	<input type="checkbox"/> Percentile 25	<input type="checkbox"/> Percentile 25
<input type="checkbox"/> Percentile - Lower Mean 25	<input type="checkbox"/> Percentile - Lower Mean 25	<input type="checkbox"/> Percentile - Lower Mean 25
<input type="checkbox"/> Percentile - Upper Mean 25	<input type="checkbox"/> Percentile - Upper Mean 25	<input type="checkbox"/> Percentile - Upper Mean 25
<input type="checkbox"/> Variance of Intensity	<input type="checkbox"/> Variance of Intensity	<input type="checkbox"/> Variance of Intensity
<input type="checkbox"/> STD of Intensity	<input type="checkbox"/> STD of Intensity	<input type="checkbox"/> STD of Intensity
<input type="checkbox"/> Equivalent Diameter (μm)	<input type="checkbox"/> Equivalent Diameter (μm)	<input type="checkbox"/> Equivalent Diameter (μm)
<input checked="" type="checkbox"/> Perimeter (μm)	<input type="checkbox"/> Perimeter (μm)	<input type="checkbox"/> Perimeter (μm)
<input checked="" type="checkbox"/> Compactness	<input type="checkbox"/> Compactness	<input type="checkbox"/> Compactness
<input checked="" type="checkbox"/> Eccentricity	<input type="checkbox"/> Eccentricity	<input type="checkbox"/> Eccentricity
<input type="checkbox"/> Minimum Width (μm)	<input type="checkbox"/> Minimum Width (μm)	<input type="checkbox"/> Minimum Width (μm)
<input type="checkbox"/> Maximum Length (μm)	<input type="checkbox"/> Maximum Length (μm)	<input type="checkbox"/> Maximum Length (μm)
<input type="checkbox"/> Feret Ratio	<input type="checkbox"/> Feret Ratio	<input type="checkbox"/> Feret Ratio

One Click Report Generator

CYCLIN D1 analysis

Date of analysis 2021/12/1 上午 10:20:32



HistoQuest Report

Experiment name	CYCLIN D1 analysis
Experiment description	mouse 76day, treatment A drug
Product version	7.1.1.117
Location	D:\TissueGnostics\Demo projects\Advanced project\CYCLIN D1
Segmentation method	Nuclear Segmentation
Color Separation method	Single Reference Shade
Institution name	
Department	
Laboratory	

Experiment Markers

Marker name: Cyclin D Original

Segmentation Method Parameters

Parameter	Value
Use Ring Mask	Yes
Interior Radius	-0.24µm
Exterior Radius	0.48µm
Use Identified Cell Mask	Outside
Max Growing Steps	10.63µm
Skip Steps	0µm
Use Nuclei Mask	Yes
Automatic Background Threshold	No
Background Threshold	25

CYCLIN D1 analysis

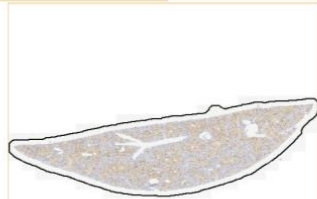
Date of analysis 2021/12/1 上午 10:20:32



Project Items

Cyclin D1 - Region 003: Region 003

Objective:	20x
Rows count:	11
Columns count:	23
FOV's count:	169
FOV Size:	0.494561821 mm/ 0.371493489 mm
Area:	23.309628 mm ²
Status:	Processed (169 out of 169 FOVs)
Comment:	



1. Global Measurements

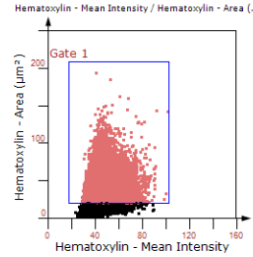
Area	23.31 mm ²
Perimeter	25.36 mm

CYCLIN D1 analysis

Date of analysis 2021/12/1 上午 10:20:32



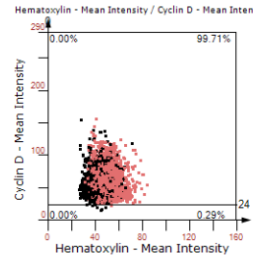
Hematoxylin - Mean Intensity / Hematoxylin - Area (µm²)



Overall Statistics

Quadrant	Mean of Hematoxylin - Mean Intensity	Mean of Hematoxylin - Area (µm ²)	Count
Overall	46.834	29.442	63016
Gate 1	49.308	39.384	40406

Hematoxylin - Mean Intensity / Cyclin D - Mean Intensity



Overall Statistics

CYCLIN D1 analysis

Date of analysis 2021/12/1 上午 10:20:32



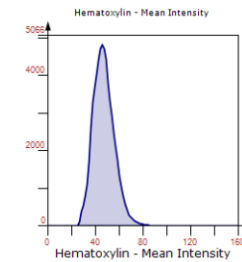
Quadrant	Mean of Hematoxylin - Mean Intensity	Mean of Cyclin D - Mean Intensity	Count	Percent	No./mm ²
UL	0.000	0.000	0	0.00%	0.000
UR	46.837	59.758	62832	99.71%	2695.538
LL	0.000	0.000	0	0.00%	0.000
LR	45.886	20.351	184	0.29%	7.894
Overall	46.834	59.643	63016	100.00%	2703.432
Gate 1	49.308	60.598	40406	64.12%	1733.447

Gate 1

Quadrant	Mean of Hematoxylin - Mean Intensity	Mean of Cyclin D - Mean Intensity	Count	Percent	No./mm ²
UL	0.000	0.000	0	0.00%	0.000
UR	49.305	60.657	40346	99.85%	1730.873
LL	0.000	0.000	0	0.00%	0.000
LR	51.291	21.313	60	0.15%	2.574
Overall	49.308	60.598	40406	100.00%	1733.447

3. Histograms

Hematoxylin - Mean Intensity



Overall Statistics

Quadrant	Mean of Hematoxylin - Mean Intensity	Count	Percent	No./mm ²
Overall	46.834	63016	100.00%	2703.432

List Mode data

.xls
.pdf
.csv

Raw Data

Raw Data for >> L1: Nuclei Detection and Measurements - WT1-1-TCRb (showing 2158 events of 2158)

Region of Interest	Event Label	Nuclei - Area (µm ²)	Cell Mask on Ch01-Mean Intensity	Cell Mask on Ch02-Mean Intensity	Cell Mask on Ch03 -Mean Intensity	Cell Mask on Ch04 -Mean Intensity
WT1-1-TCRb	1	27,815020	11,329150	10,631090	10,749190	6,527500
WT1-1-TCRb	2	14,400680	2,031250	12,618320	20,226320	3,375000
WT1-1-TCRb	3	16,965190	2,687500	8,520000	13,859840	4,368932
WT1-1-TCRb	4	1,282253	12,402170	16,790080	7,812500	7,812500
WT1-1-TCRb	5	20,516040	9,810526	10,609290	9,630435	5,685897
WT1-1-TCRb	6	1,874061	2,086957	4,724138	15,433570	5,615385
WT1-1-TCRb	7	7,200341	1,037037	3,035088	11,039220	3,596491
WT1-1-TCRb	8	0,098635	0,000000	0,000000	8,619047	0,000000
WT1-1-TCRb	9	15,485670	8,237569	6,570470	12,660310	6,241830
WT1-1-TCRb	10	30,774060	27,528660	6,506849	3,182390	5,700000
WT1-1-TCRb	11	3,057679	7,818182	9,570867	11,864200	6,627451
WT1-1-TCRb	12	4,635836	1,032967	2,934783	13,834530	3,895833
WT1-1-TCRb	13	22,291470	1,380952	4,432748	9,372929	5,701149
WT1-1-TCRb	14	16,570650	32,963730	6,098655	3,281879	5,006098
WT1-1-TCRb	15	17,162460	28,250000	5,806084	3,744048	5,812500
WT1-1-TCRb	16	2,761775	0,430769	2,092308	12,301550	2,215385
WT1-1-TCRb	17	33,733100	11,070340	10,024020	15,986500	7,244240
WT1-1-TCRb	18	23,869620	1,335165	10,074350	19,553890	4,484099
WT1-1-TCRb	19	43,399310	4,058219	12,280590	12,653280	6,217391
WT1-1-TCRb	20	37,086690	7,294798	12,414400	10,793850	8,077491
WT1-1-TCRb	21	35,703800	1,566038	4,864662	12,529410	6,541582
WT1-1-TCRb	22	8,088055	1,339623	5,272000	11,262030	6,915888
WT1-1-TCRb	23	4,142662	17,764710	5,868132	4,400000	5,809091
WT1-1-TCRb	24	34,226280	1,200000	6,844193	11,881440	4,331950
WT1-1-TCRb	25	10,455290	5,668141	8,246106	6,691730	5,523809
WT1-1-TCRb	26	20,220140	26,350470	5,678049	11,550360	5,252381
WT1-1-TCRb	27	30,774060	3,561856	6,432727	15,170680	5,711111
WT1-1-TCRb	28	18,839250	5,039326	14,198020	9,916335	6,834783
WT1-1-TCRb	29	0,197270	3,000000	3,090909	9,615385	2,666667
WT1-1-TCRb	30	9,666211	8,946428	6,372549	5,729508	7,732394
WT1-1-TCRb	31	13,217060	11,660960	4,046980	10,242860	3,912752
WT1-1-TCRb	32	3,846758	1,661972	3,208333	9,172317	3,766234
WT1-1-TCRb	33	20,022870	2,111111	8,754717	11,497780	7,965742
WT1-1-TCRb	34	3,452218	20,209880	3,087719	11,543210	3,070175
WT1-1-TCRb	35	9,764846	2,060345	7,436620	13,388130	4,262295
WT1-1-TCRb	36	25,743680	19,181340	5,745690	18,572970	6,127946
WT1-1-TCRb	37	6,509897	1,552083	7,964844	2,927835	4,605769
WT1-1-TCRb	38	15,978840	2,000000	6,568306	11,795670	7,839591
WT1-1-TCRb	39	20,417410	5,477612	8,608866	14,336830	8,662516
WT1-1-TCRb	40	21,403750	1,538462	7,497041	18,265170	7,279570
WT1-1-TCRb	41	33,634470	11,302630	6,707921	12,825780	7,912477
WT1-1-TCRb	42	19,332420	1,312500	3,707865	11,497200	6,325000
WT1-1-TCRb	2158	AVG=17,87	AVG=3,00	AVG=7,49	AVG=35,51	AVG=6,87

Collapse All Expand All

Export To Excel... Export To PDF... Export To CSV... Export To ICEFormat... View Backward Data for filtered events Exit Backward Connection Load default columns Show Column Chooser Close

Statistics Report

.xls
.pdf
.csv

The screenshot displays the 'Statistics Report' software interface. A 'New Column' dialog box is open in the foreground, allowing users to define a new column. The dialog includes options for data source (Global Measurements, From Histogram, From Scattergram, Propagated Gate, Computed Parameters, Computed with Scalar, Scoring), a text field for 'Add column name:', a 'Use existing scattergrams from first sample' checkbox, a 'Select Layer:' dropdown (set to 'L1: Nuclei Detection and Measurements'), a 'Select unit:' dropdown (set to 'Percent'), a 'Select existing scattergram:' dropdown (set to 'CellMask on CH03 vs CH04 -Mean Intensity'), and a 'Select implicit gate:' dropdown (set to 'Upper Right').

The background shows the main application window with an 'Input' section on the left containing a tree view of 'Groups of compatible Sample Links' and 'Nuclei Analysis' with 'WT1-1-TCRb' selected. The 'Statistical Data' section on the right contains a table of statistical metrics.

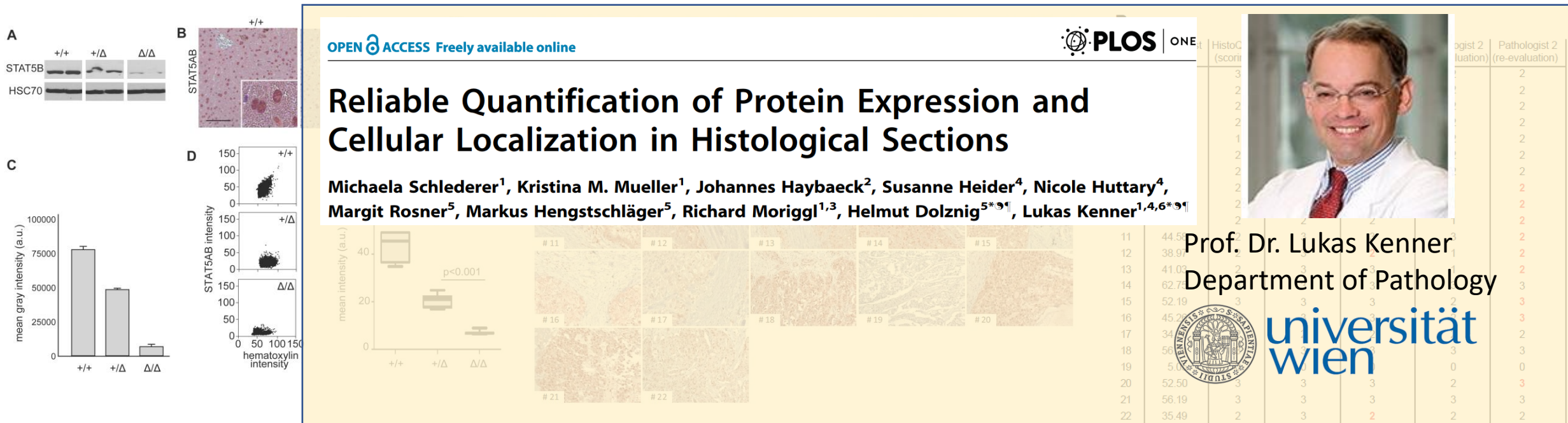
Group	Name	Type
WT1-1-TCRb	No of Ch02+ Nuclei	Existing Scattergram
WT1-1-TCRb	No of Ch01+ Nuclei	Existing Scattergram
WT1-1-TCRb	No of Ch03+ Nuclei	Existing Scattergram
WT1-1-TCRb	No of Ch04+ Nuclei	Existing Scattergram
WT1-1-TCRb	No of Ch01+&Ch02+ Nuclei	Existing Scattergram
WT1-1-TCRb	No of Ch01+&Ch03+ Nuclei	Existing Scattergram
WT1-1-TCRb	No of Ch01+Ch04+ Nuclei	Existing Scattergram
WT1-1-TCRb	No of Ch02+Ch03+ Nuclei	Existing Scattergram
WT1-1-TCRb	No of Ch02+Ch04+ Nuclei	Existing Scattergram
WT1-1-TCRb	No of Ch03+Ch04+ Nuclei	Existing Scattergram
WT1-1-TCRb	No of Ch01+Ch02+Ch03+ Nuclei	Existing Scattergram
WT1-1-TCRb	No of Ch01+Ch02+Ch03+Ch04+ Nuclei	Existing Scattergram
WT1-1-TCRb	Total No of Nuclei	Existing Scattergram
WT1-1-TCRb	Triple positive cells	Existing Scattergram

At the bottom of the application window, there are buttons for 'Fill Report Data', 'Clear Report Data', 'Export To PDF...', 'Export to CSV...', 'Export to Excel...', and 'Close'.

Is TG Accurate?





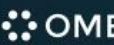






Compared with western blotting & pathologists quantification

- High correlation with WB results
- Align with pathologists
- **Reproducibility & Standardization**



Import Images from 3rd parties

- .aqproj
- .vmic
- .czi
- .ndpi
- .svs
- .mrxs
- .vsi
- .jpg/jpeg
- .tif/tiff
- .bmp
- .png

 <p>TissueFAXS</p> <p>■ Import multiple projects</p>	 <p>PreciPoint (GTIF; VMIC)</p> <p>■ Import multiple projects</p>
 <p>StrataFAXS II</p> <p>■ Import multiple projects</p>	 <p>Zeiss (CZI)</p> <p>■ Import multiple projects</p>
 <p>OME-TIFF</p> <p>■ Import multiple projects</p>	 <p>Hamamatsu NanoZoomer (NDPI)</p> <p>■ Import multiple projects</p>
<p>BigTIFF</p> <p>BigTIFF</p>	 <p>Aperio (SVS)</p> <p>■ Import multiple projects</p>
 <p>Folder</p>	 <p>3DHISTECH Pannoramic / Mirax (MRXS)</p> <p>■ Import multiple projects</p>
 <p>TISSUEGNOSTICS PRECISION THAT INSPIRES</p>	 <p>Olympus (VSI)</p> <p>■ Import multiple projects</p>

Publications

Signalling Molecules in Apoptosis

Death receptor 5 mediated-apoptosis contributes to cholestatic liver disease

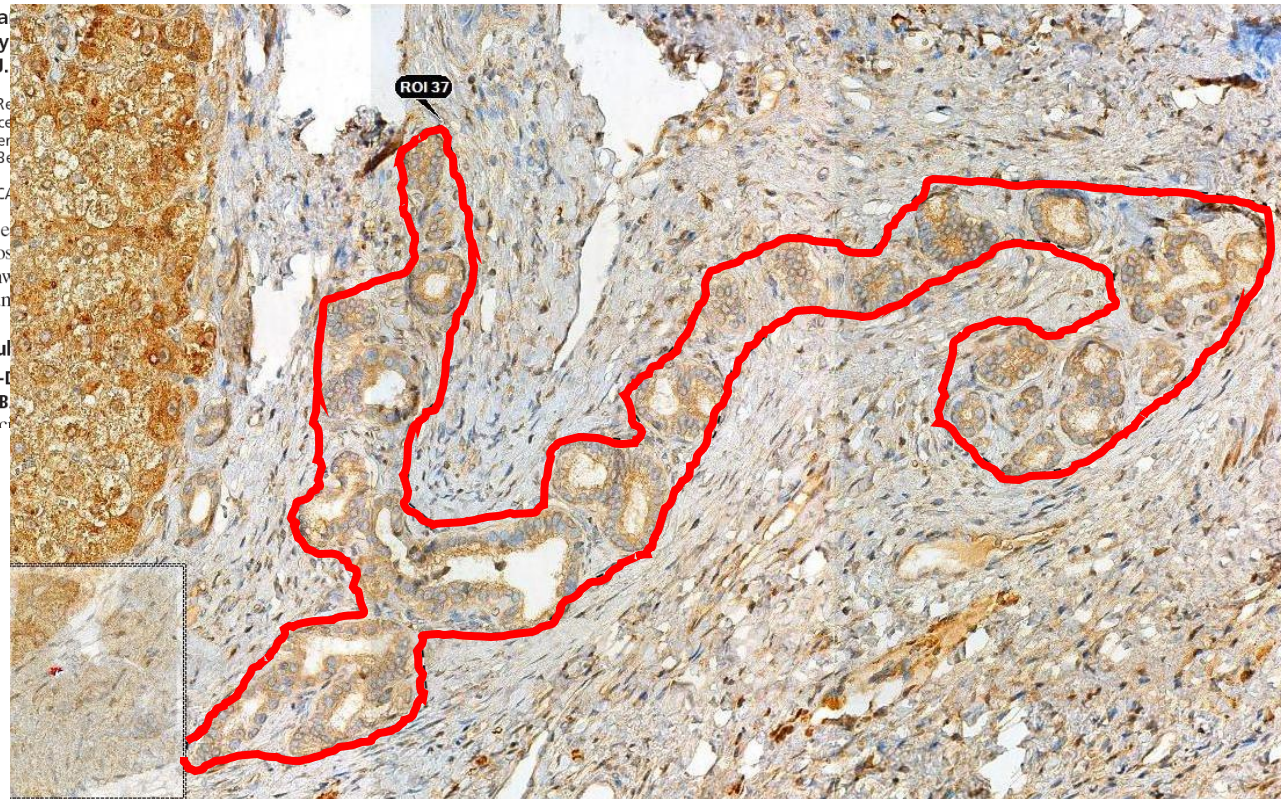
Kazuyoshi Takeda*^{†‡§}, Yuko Kojima^{§¶}, Kenichi Ikejima[¶], Kenichi Hatanaka[¶], Tomonori Aoyama[¶], Steffen Frese^{††}, Hiroko Ikeda^{**}, Nicole M. Hayashi[¶], Noriyoshi Sueyoshi[¶], Nobuhiro Sato[¶], Yasuni Nakanuma^{**}, Mark J. Gores^{††}

*Department of Immunology, [†]Division of Biomedical Imaging Research, Biomedical Research Center, [‡]Department of Pathology, [§]Department of Cell Biology, [¶]Department of Surgery, ^{††}University School of Medicine, Hongo 2-1-1, Bunkyo-ku, Tokyo 113-8421, Japan; ^{**}Cancer Research Institute, East Melbourne, Victoria 3002, Australia; ^{**}Human Pathology, Kanazawa University School of Medicine, Kanazawa, Japan; ^{††}Department of Clinical Research, Laboratory of Thoracic Surgery, University of

Edited by Leonard A. Herzenberg, Stanford University School of Medicine, Stanford, CA

Chronic cholestasis often results in premature death from liver failure with fibrosis; however, the molecular mechanisms contributing to biliary cirrhosis are not demonstrated. In this article, we show that the death signal mediated by TNF-related apoptosis-inducing ligand (TRAIL) receptor 2/death receptor 5 (DR5) may be a key regulator of cholestatic liver injury. Agonistic anti-DR5 monoclonal antibody treatment triggered cholangiocyte apoptosis, and subsequently induced cholangitis and cholestatic liver injury in a mouse strain-specific manner. TRAIL- or DR5-deficient

cholestatic liver injury
fibrosis
pathway
mechanism
Result
Anti-DR5
Not B
toxic

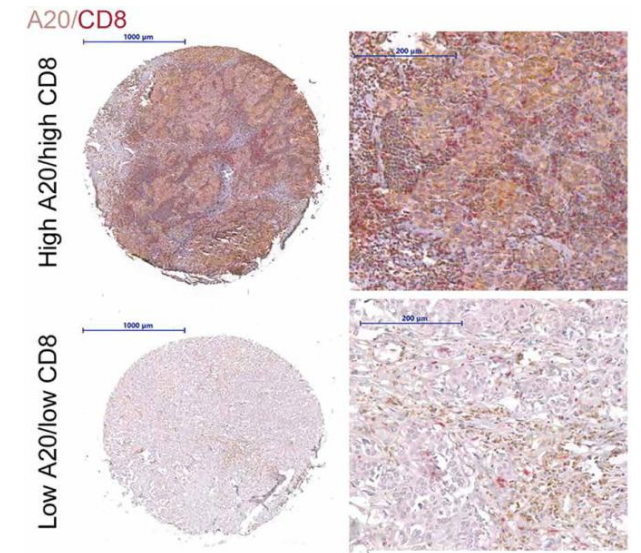
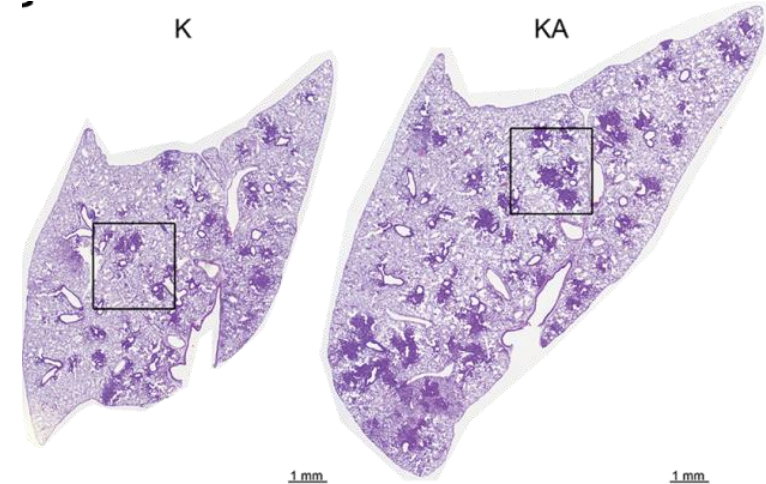
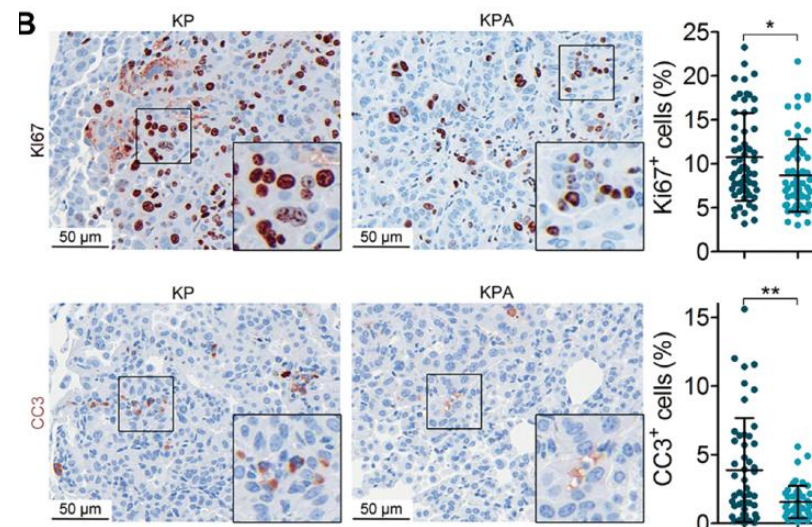
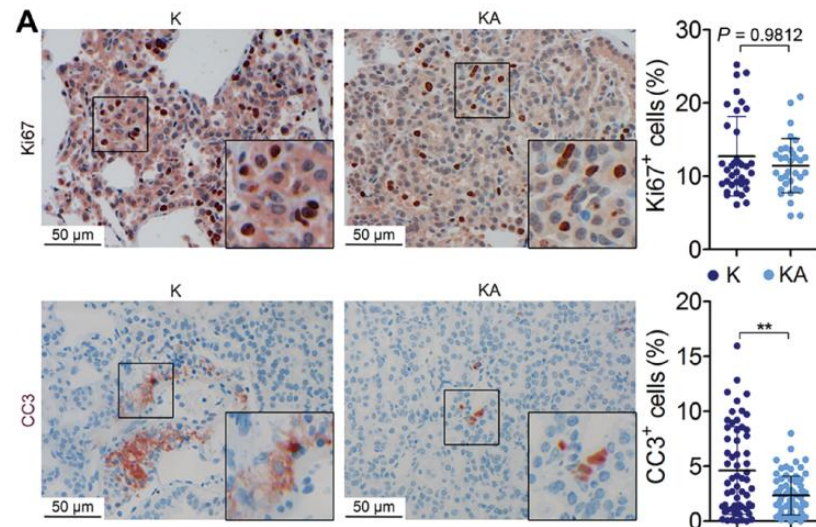


Region-specific analysis of liver tissue by HistoQuest as key feature in tissue analysis

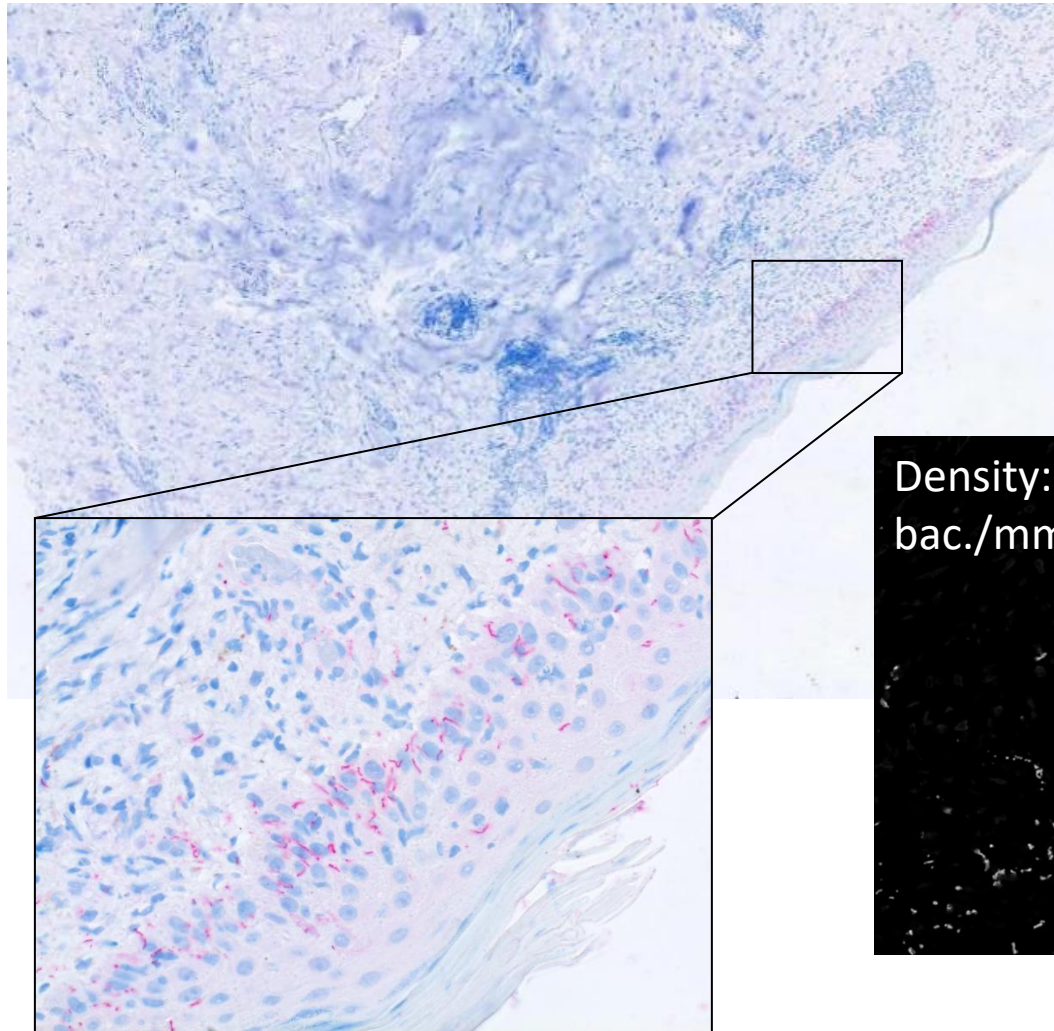
Immune Escape of Lung Cancer

Science Translational Medicine

Down-regulation of A20 promotes immune escape of lung adenocarcinomas



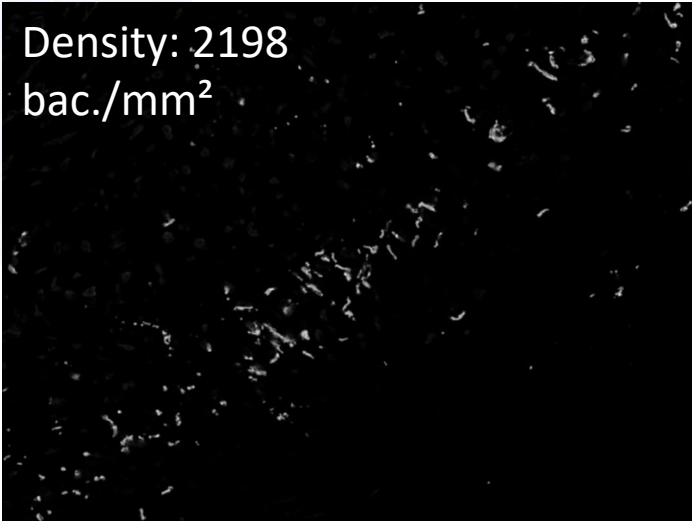
Counting of Bacteria (Spirochaeta)



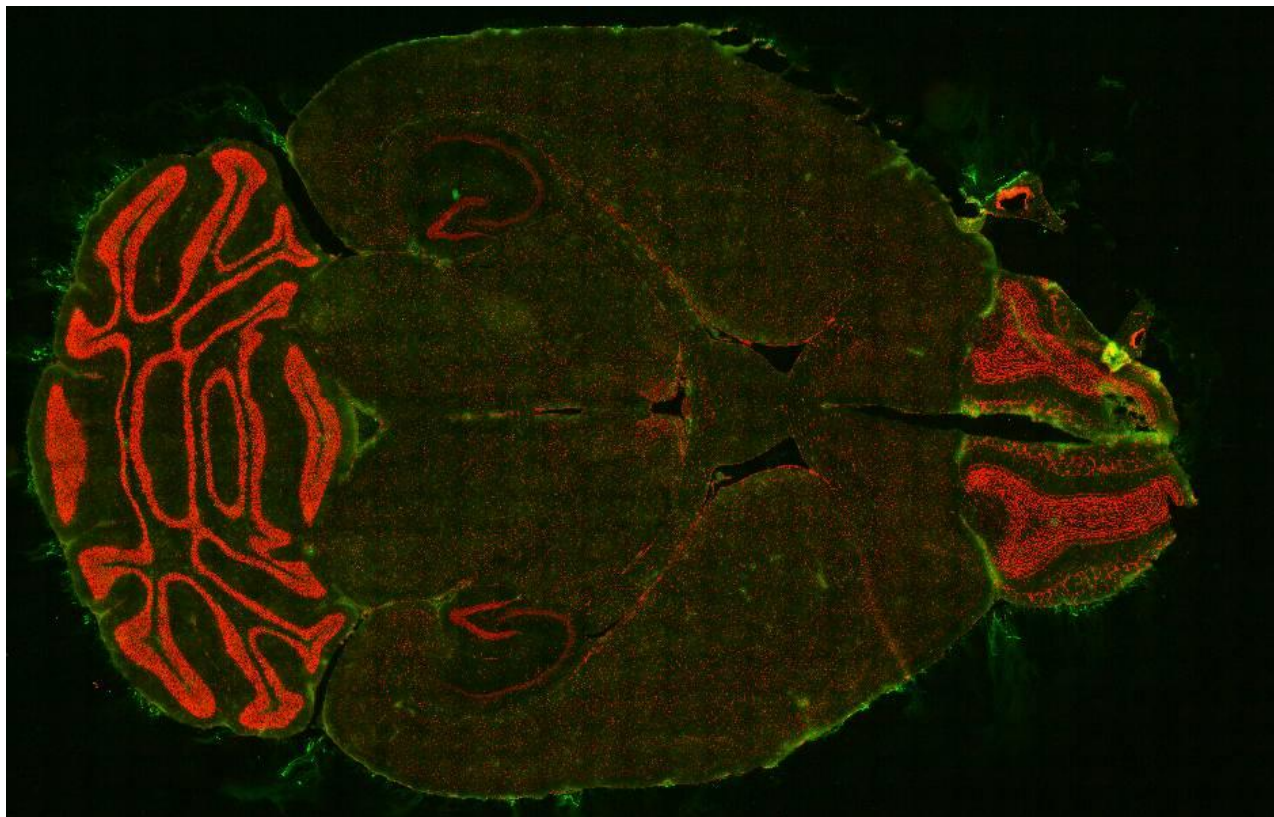
Aim:

Automatically count bacteria per mm² in infected skin.

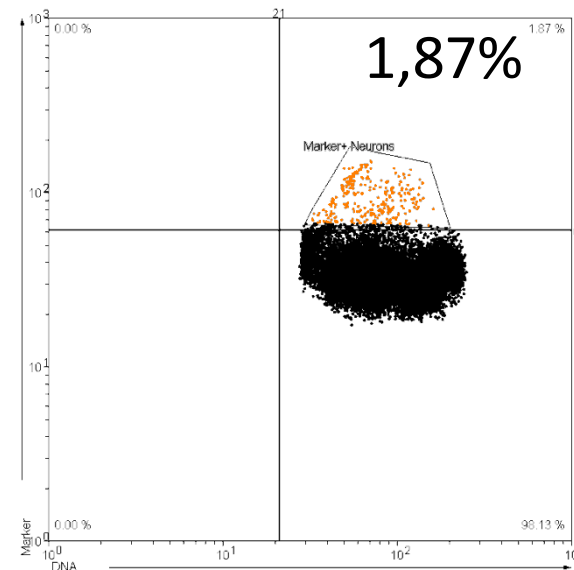
Density: 2198
bac./mm²

A fluorescence microscopy image showing the same area as the histological images. The bacteria appear as bright, glowing, corkscrew-shaped structures against a dark background, demonstrating the automated counting process.

Measurement of Neuronal Markers



Red: nuclei (Propidium Iodide)
Green: neuronal marker

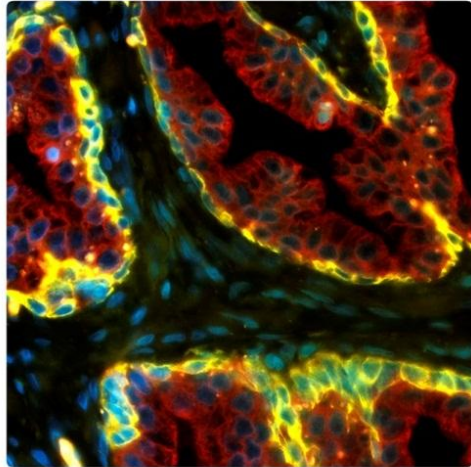


All events in scattergram					
	X-Mean	Y-Mean	Events	#/mm ²	%
UL	0.00	0.00	0	0.00	0.00
UR	70.83	84.02	374	2423.47	1.87
LL	0.00	0.00	0	0.00	0.00
LR	84.12	32.62	19660	127394.17	98.13
SUM	83.88	33.58	20034	129817.64	100.00

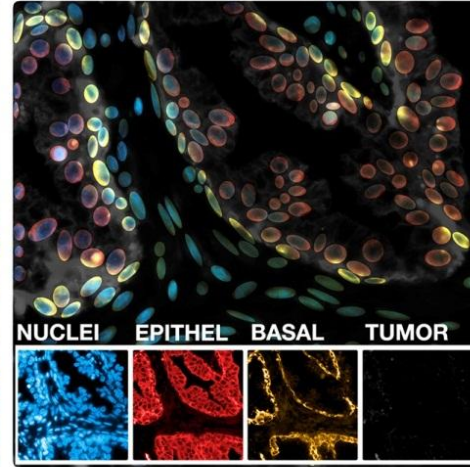
Marker+Neurons					
	X-Mean	Y-Mean	Events	#/mm ²	%
UL	0.00	0.00	0	0.00	0.00
UR	72.45	90.92	285	1846.76	100.00
LL	0.00	0.00	0	0.00	0.00
LR	0.00	0.00	0	0.00	0.00
SUM	72.45	90.92	285	1846.76	100.00

Early Identification of Malignant Transformation

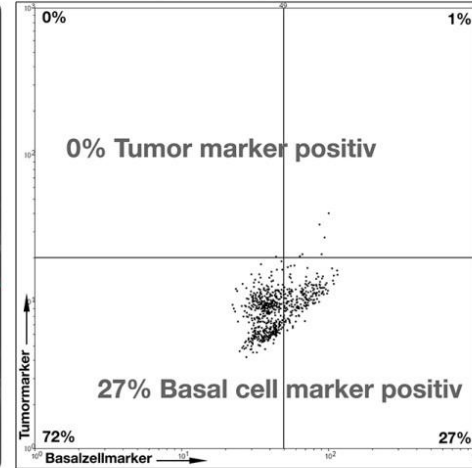
NORMAL AREA



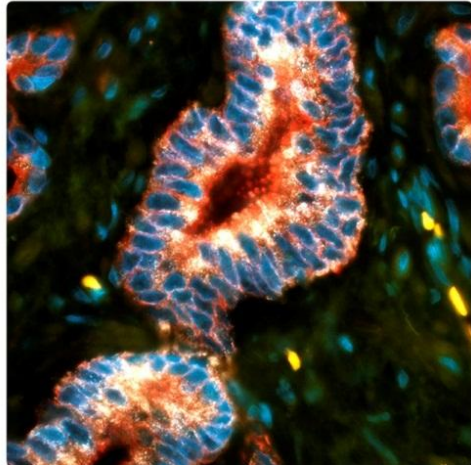
CELL RECOGNITION



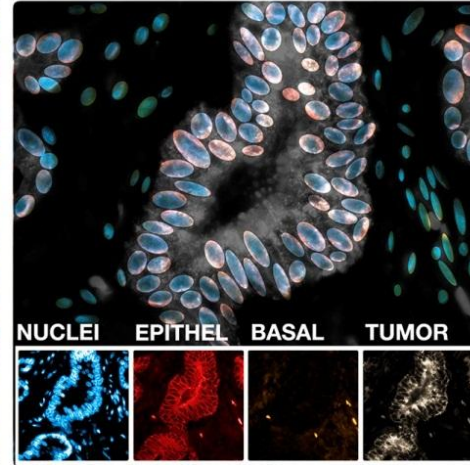
SCATTERGRAM



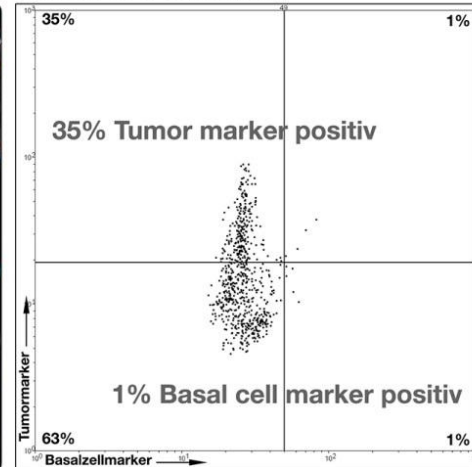
TUMOR AREA



CELL RECOGNITION



SCATTERGRAM



Aim:

Provide an automated and observer independent data basis for clinical diagnosis of prostate cancer based on a specific tumor marker and changes in the composition of prostatic glands characterized by different types of cytokeratin expressed by epithelial cells.

Tissues are Where Immune Cells Function

nature

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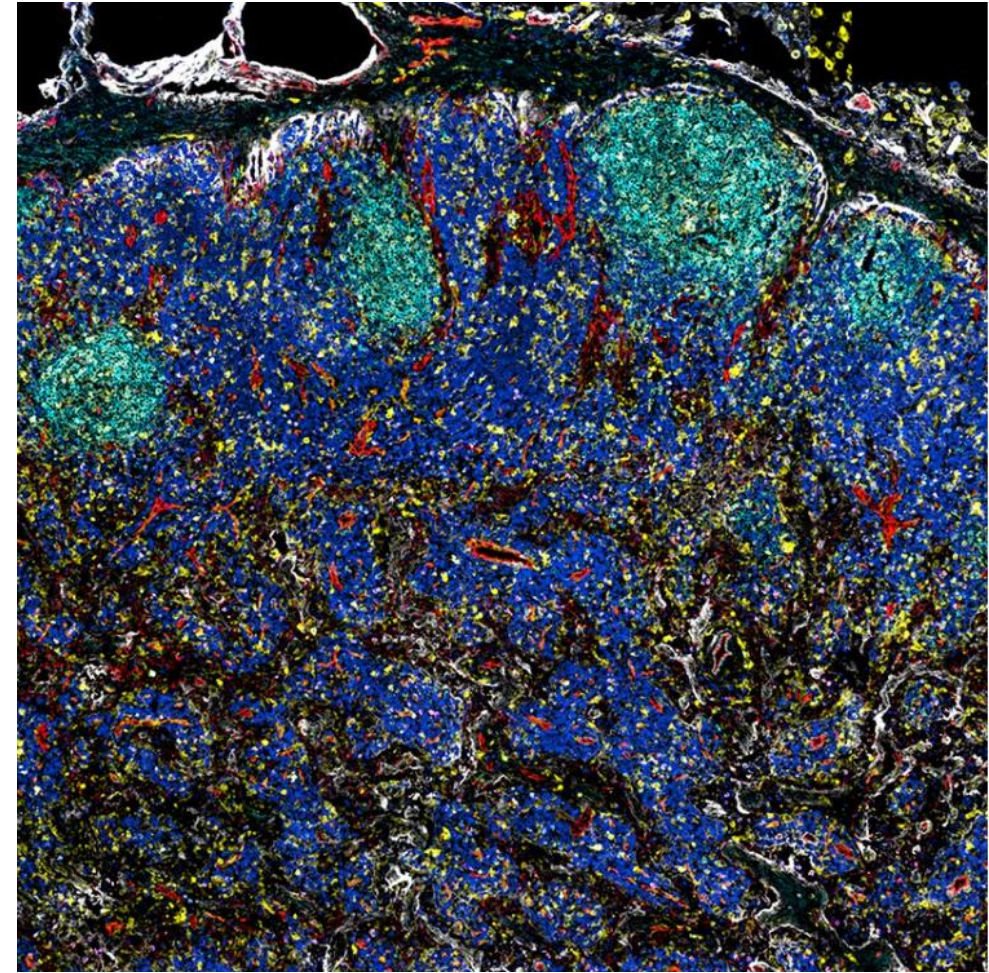
nature > comment > article

COMMENT | 25 May 2021

Tissues, not blood, are where immune cells function

COVID has shown we must study immunity in the whole body – let's sort the logistics to acquire the right samples.

Donna L. Farber 



Immune cells have designated sites. In this lymph node, colours indicate cell types, such as B cells (light blue) and T cells (dark blue and green).

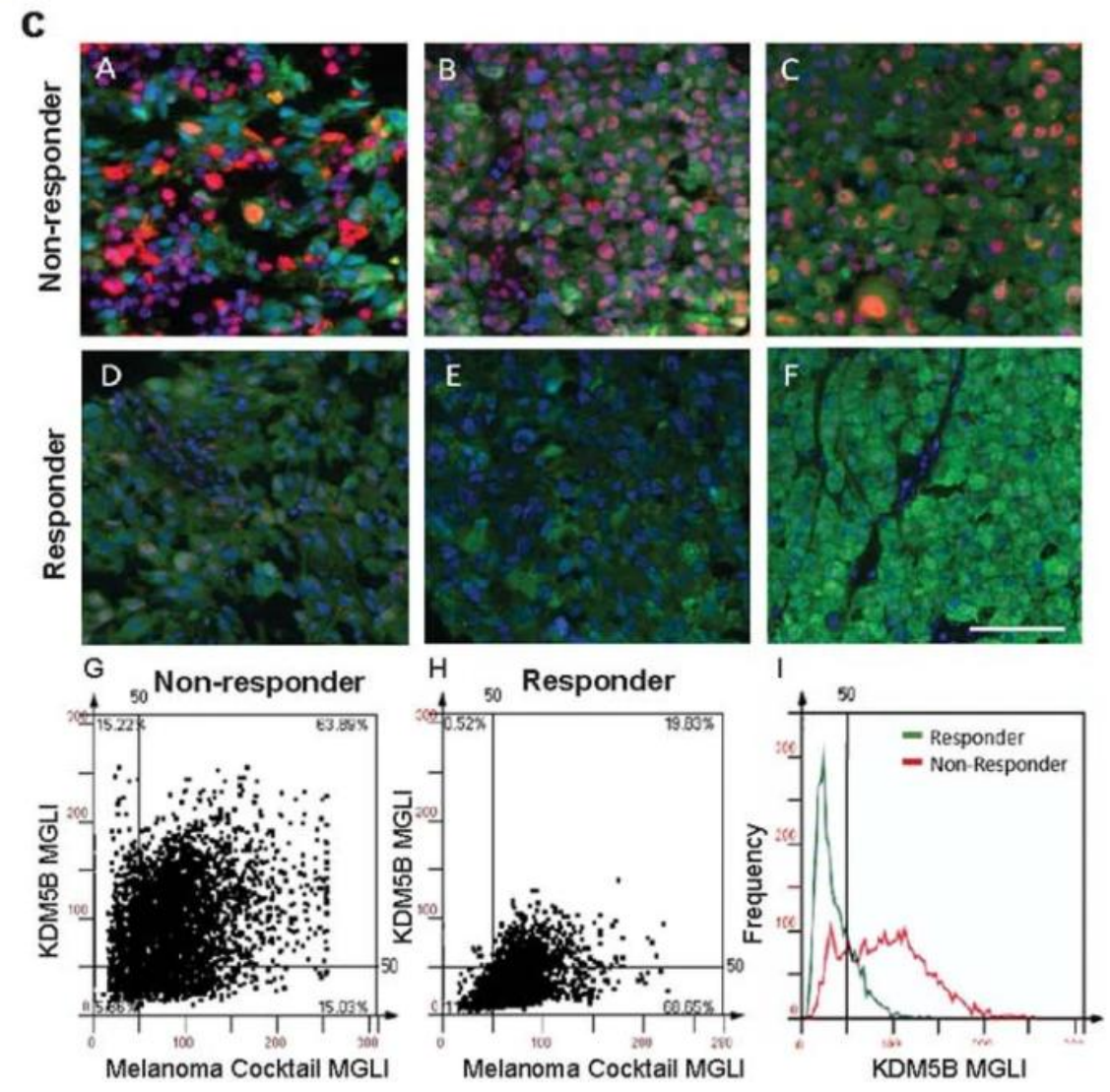
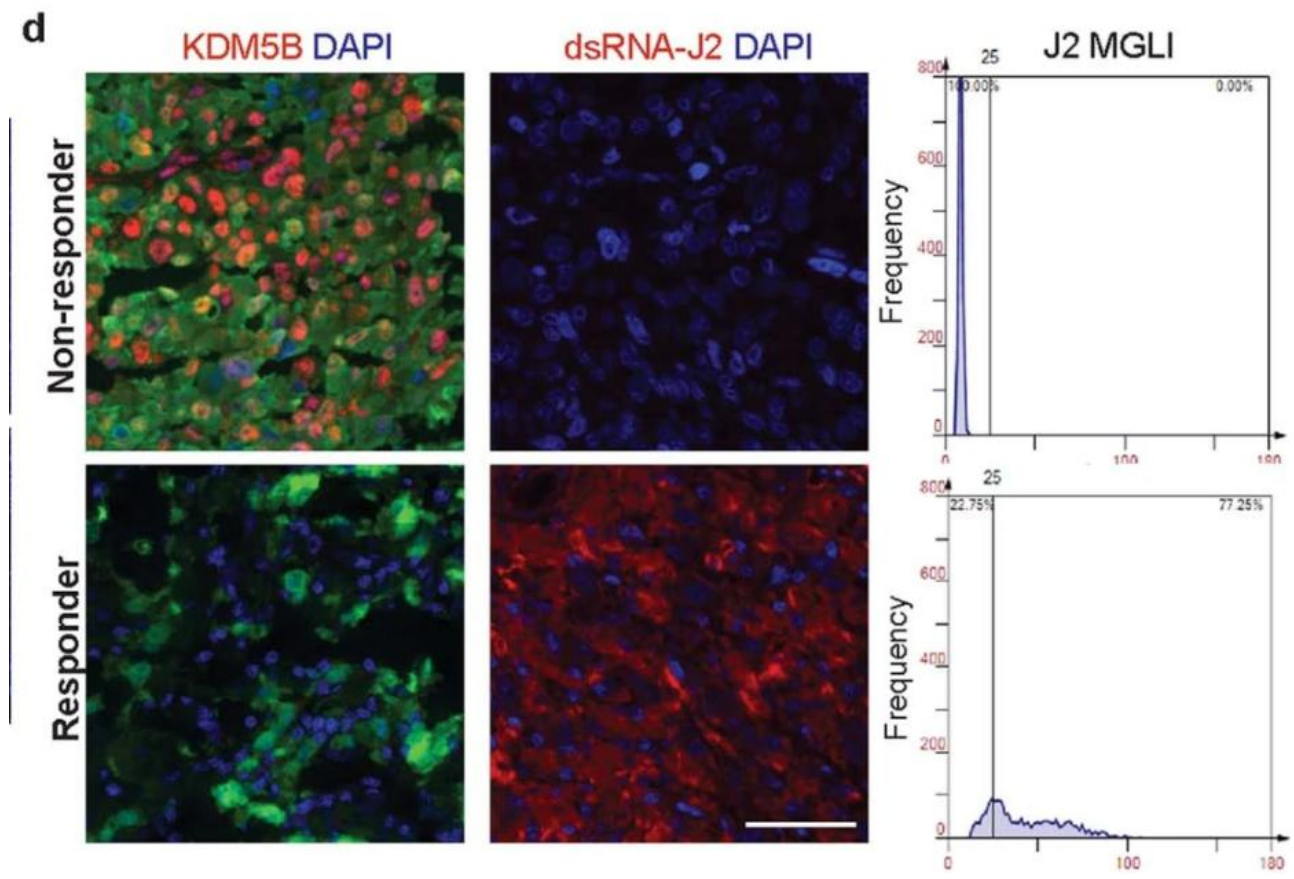
Credit: Andrea Radtke

Epigenetic Therapies Boost Anti-tumour Immune Responses

nature

Article | Published: 20 October 2021

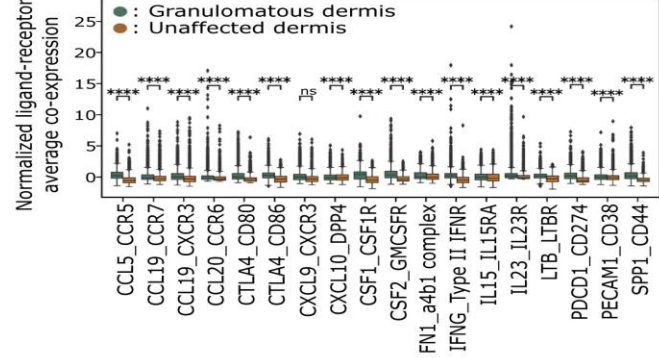
KDM5B promotes immune evasion by recruiting SETDB1 to silence retroelements



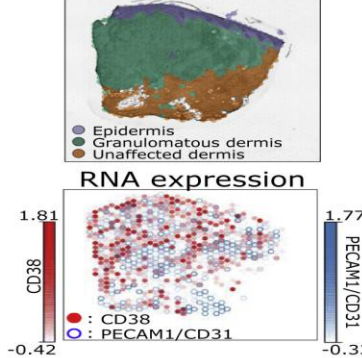
Single-cell and spatial transcriptomics reveal aberrant lymphoid developmental programs driving granuloma formation



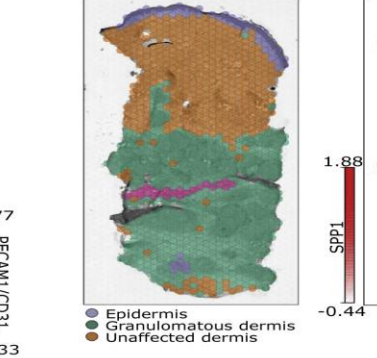
A Co-expression of selected ligand-receptor pairs within spatial transcriptomic clusters



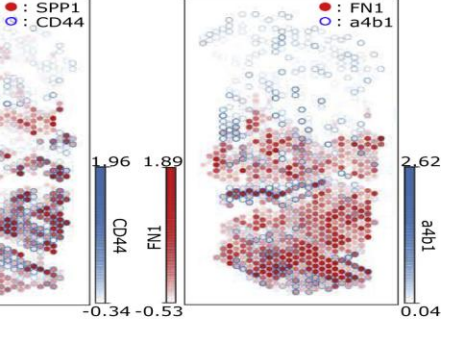
B Spatial transcriptomic clusters



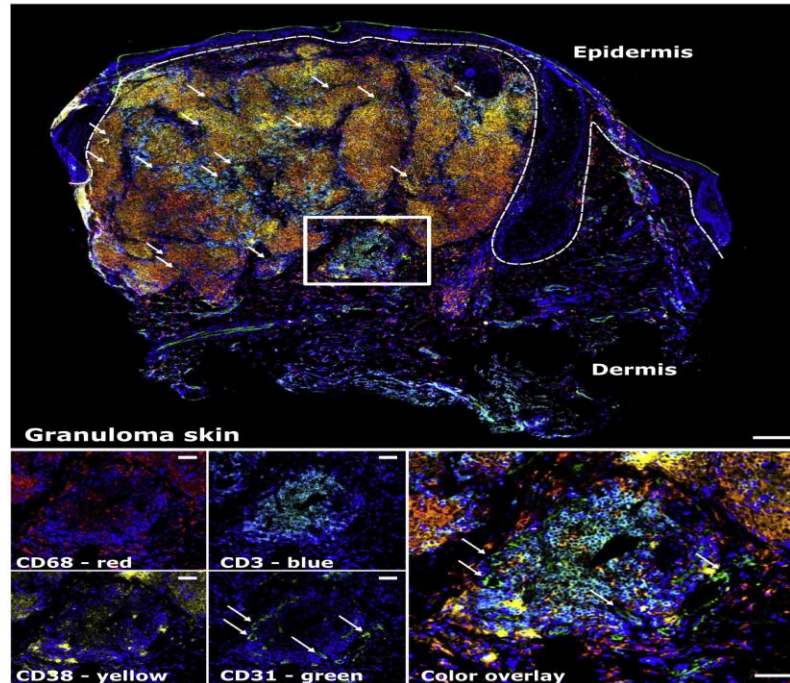
D Spatial transcriptomic clusters



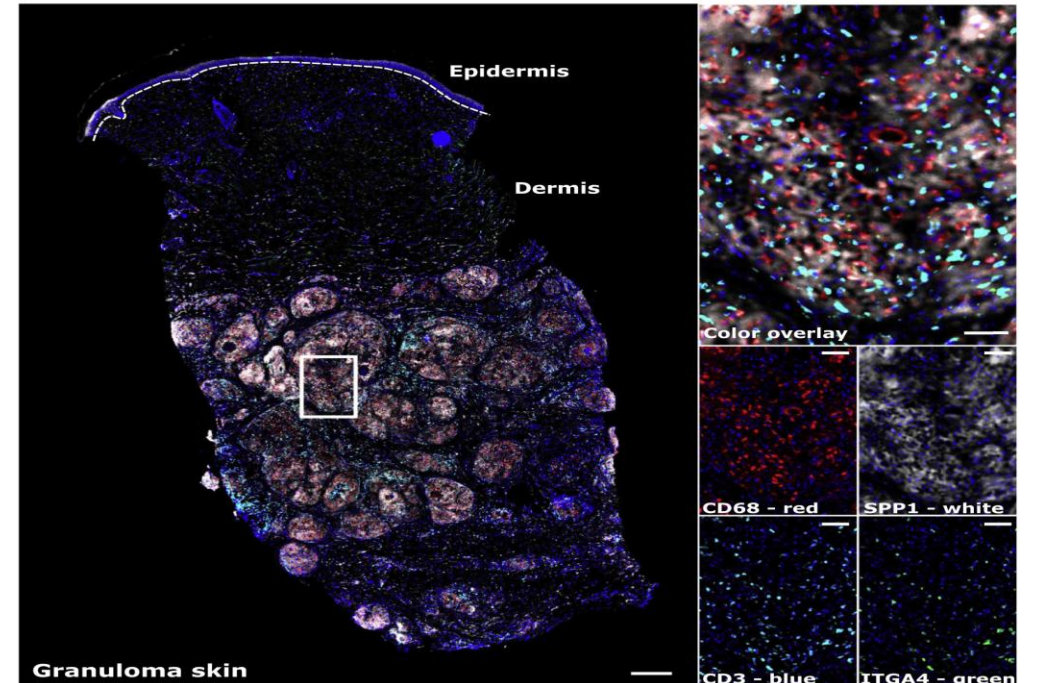
RNA expression



C Protein expression of selected ligand-receptor pairs



E Protein expression of selected ligand-receptor pairs



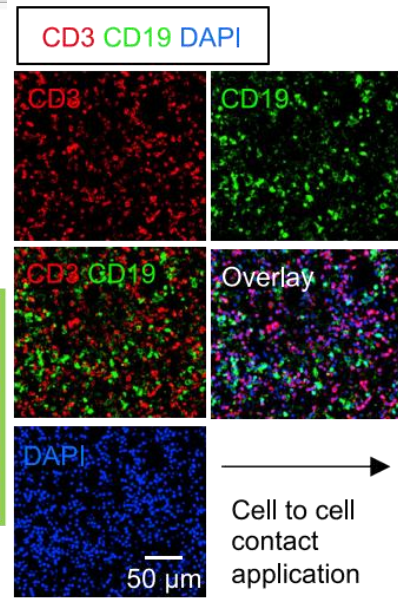
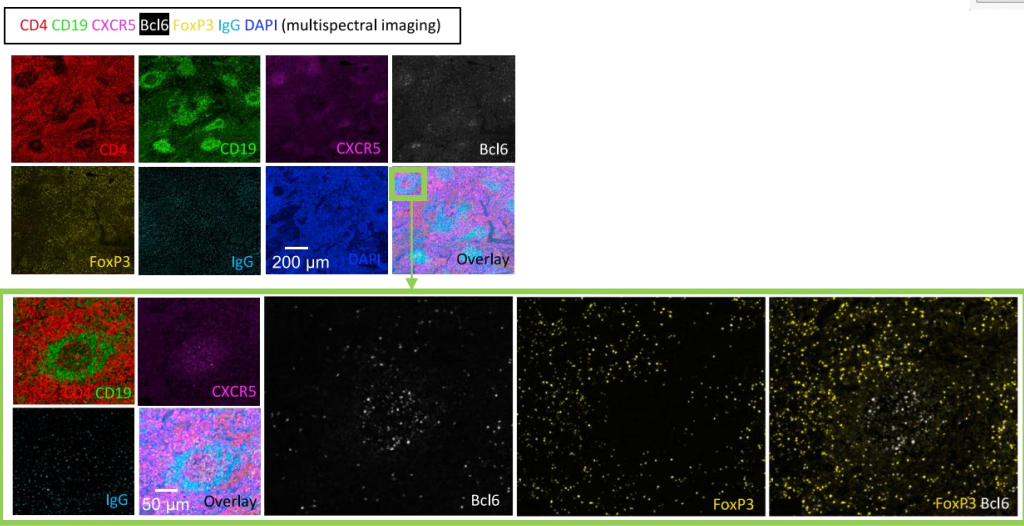
Immune Response in COVID-19

Cell

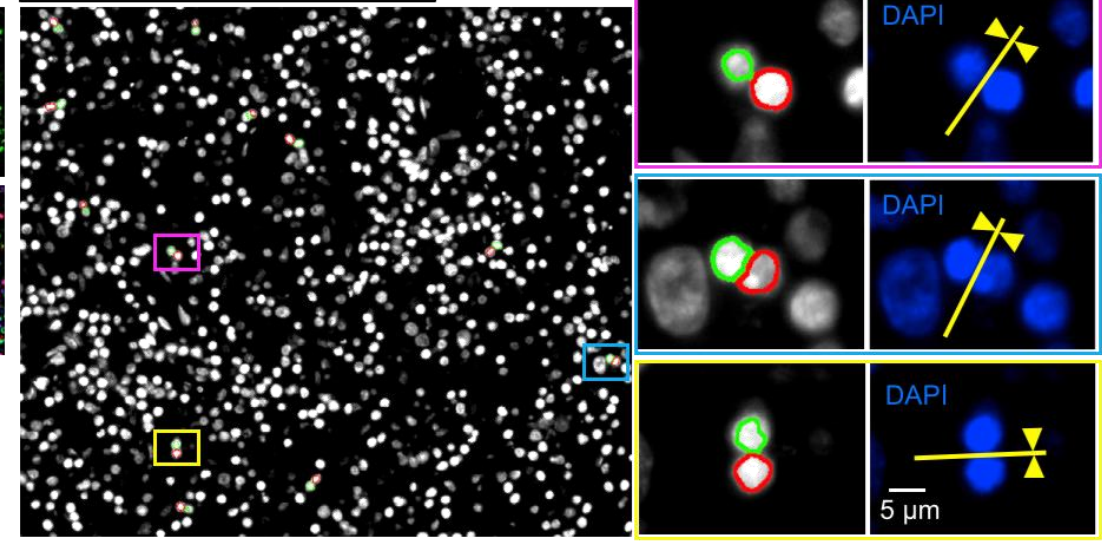
Loss of Bcl-6-Expressing T Follicular Helper Cells and Germinal Centers in COVID-19



Dr. Thomas Diefenbach



CD3+ T cell in T-B conjugate
CD19+ B cell in T-B conjugate

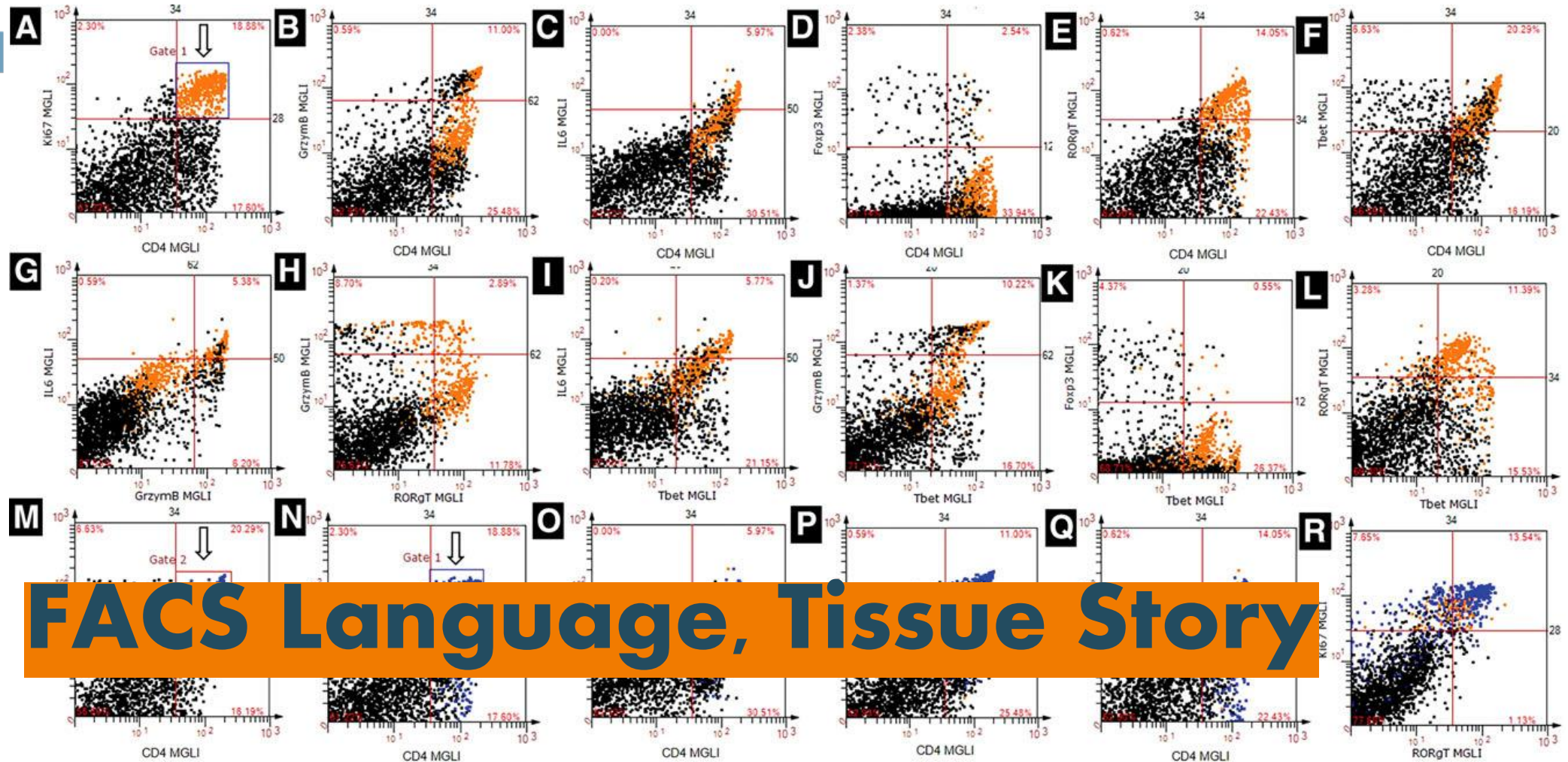


Phenotyping immune cells in situ

Cytometry
PART A



Journal of Quantitative
Cell Science



FACS Language, Tissue Story

Intracellular Parasites (Dot Analysis)

RESEARCH ARTICLE

An Emerging Approach for Parallel Quantification of Intracellular Protozoan Parasites and Host Cell Characterization Using TissueFAXS Cytometry

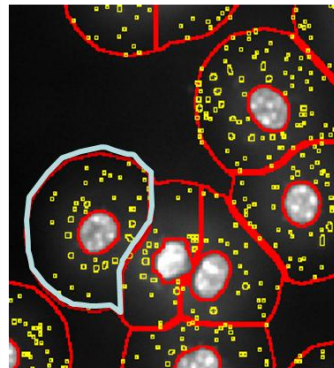
Maximilian Schmid¹, Bianca Dufner¹, Julius Dürk¹, Konstanze Bedal¹, Kristina Stricker¹, Lukas Ali Prokoph¹, Christoph Koch¹, Anja K. Wege², Henner Zirpel³, Ger van Zandbergen^{2,4}, Rupert Ecker⁵, Bogdan Boghiu⁵, Uwe Ritter^{1*}

1 Institute of Immunology, University of Regensburg, Regensburg, Germany, **2** Department of Gynecology and Obstetrics, University of Regensburg, Regensburg, Germany, **3** Division of Immunology, Paul-Ehrlich-Institute, Langen, Germany, **4** Institute of Immunology, University Medical Center of the Johannes Gutenberg University of Mainz, Mainz, Germany, **5** TissueGnostics GmbH, Vienna, Austria

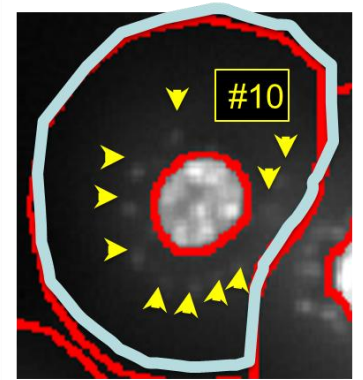
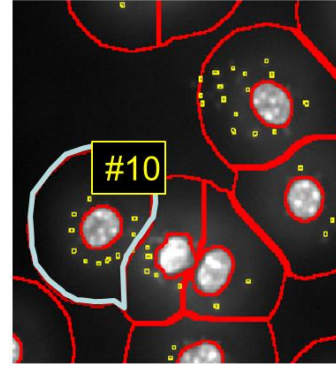
+ *L. major*



Setting #1 (high sensitivity)



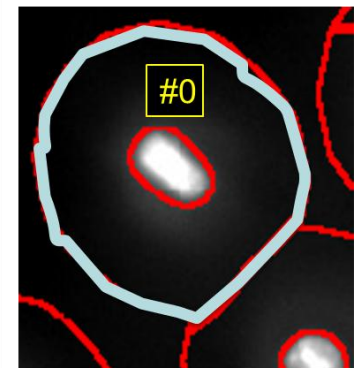
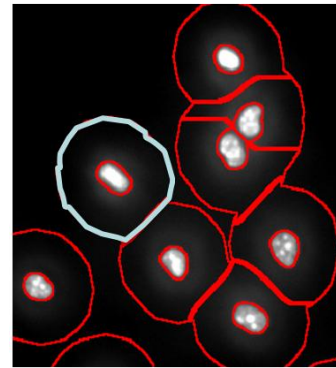
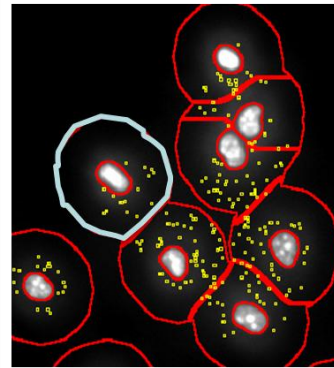
Setting #2 (low sensitivity)



"DAPI-positive parasites"

DAPI-positive parasites

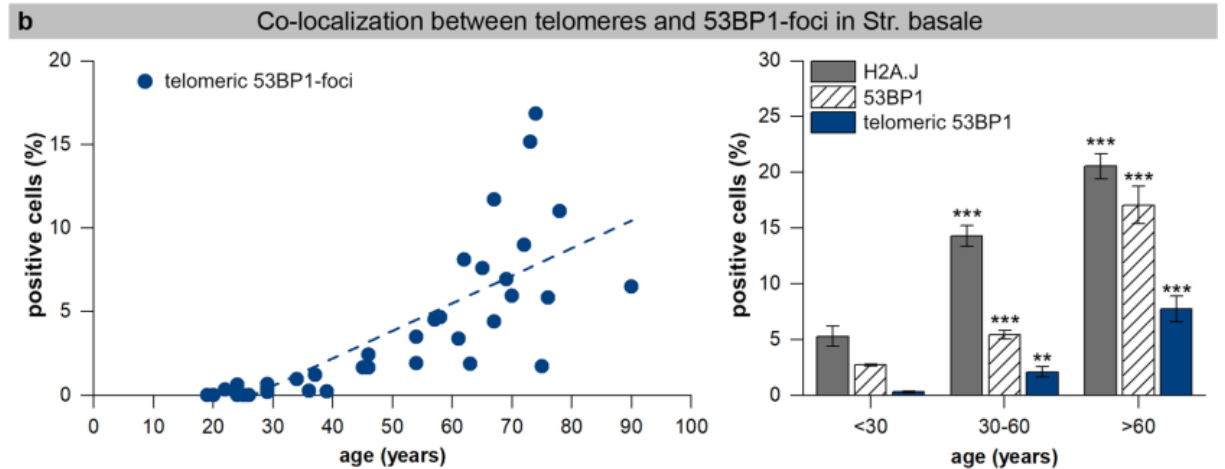
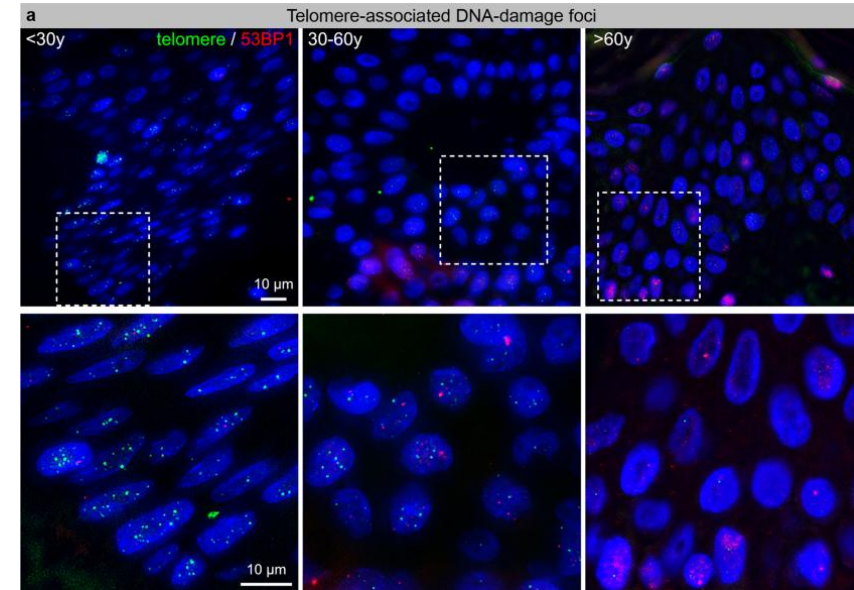
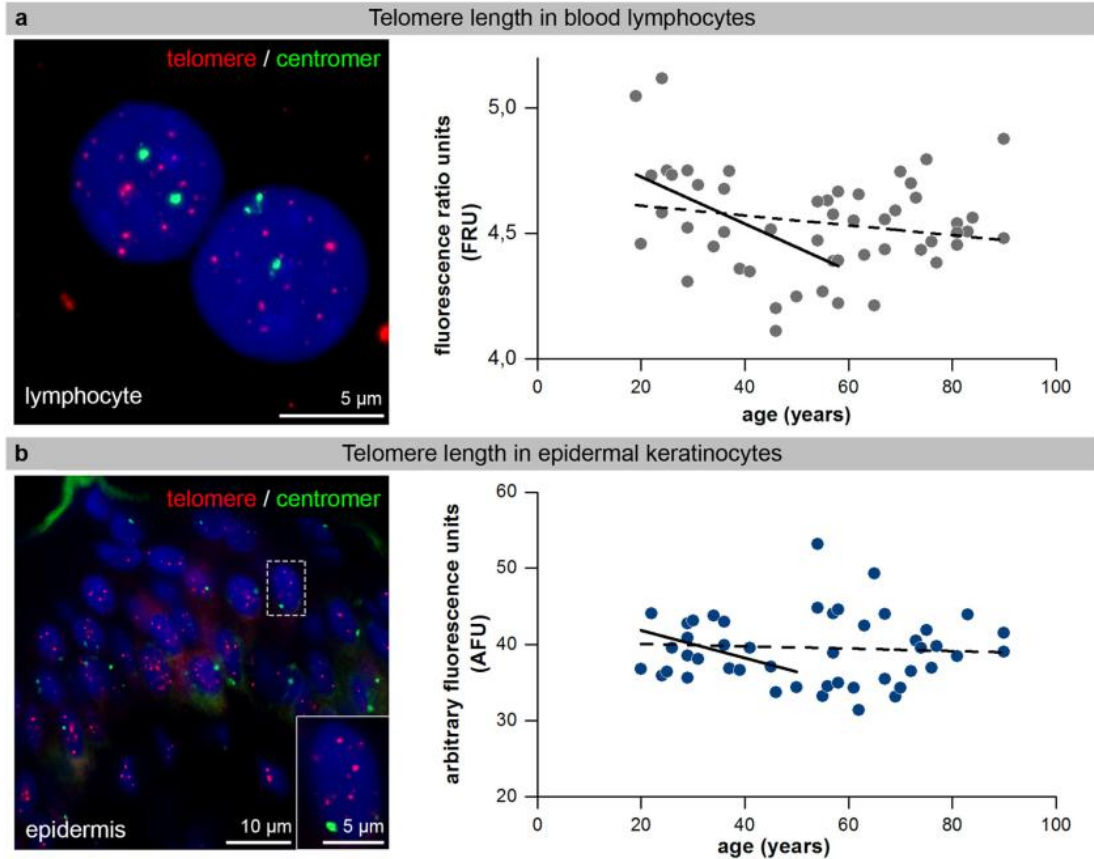
w/o *L. major*



Human Skin Aging (Dot Analysis)

npj | aging

Human skin aging is associated with increased expression of the histone variant H2A.J in the epidermis

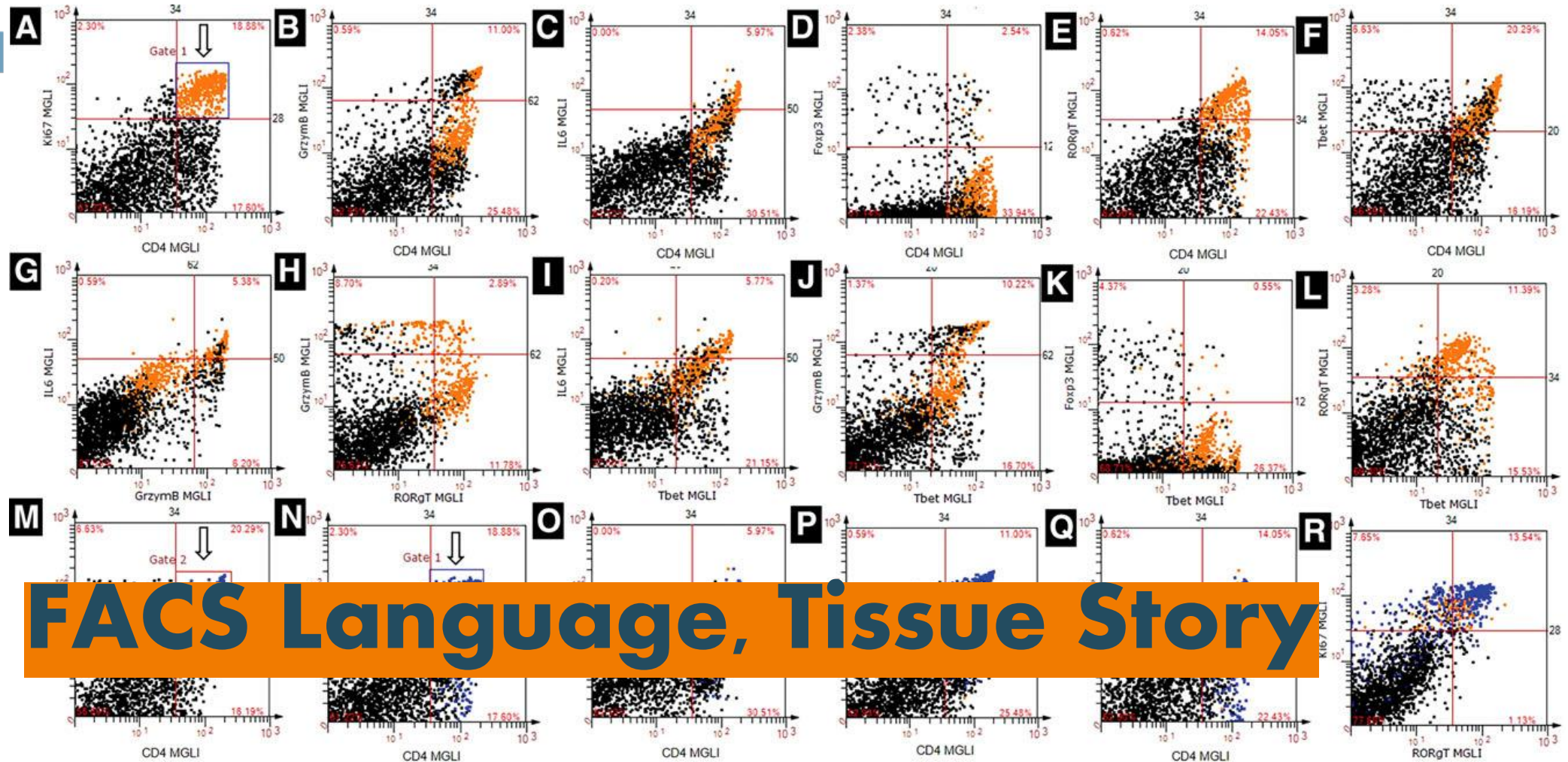


Phenotyping immune cells in situ

Cytometry
PART A



Journal of Quantitative
Cell Science



FACS Language, Tissue Story

USA installation sites (partial)



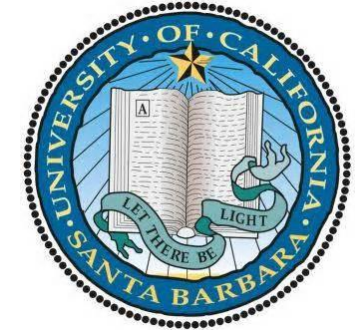
JOHNS HOPKINS
UNIVERSITY



HHMI
HOWARD HUGHES
MEDICAL INSTITUTE

FRED HUTCHINSON
CANCER RESEARCH CENTER

UCSF



DANA-FARBER
CANCER INSTITUTE

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University of Pittsburgh



BRIGHAM AND
WOMEN'S HOSPITAL



Memorial Sloan Kettering
Cancer Center

Massachusetts
Institute of
Technology

Asia Pacific installation sites (partial)



National University of Singapore



Singapore Institute for Clinical Sciences



National Cancer Center

国立研究開発法人国立がん研究センター



Juntendo University

順天堂大学



KOBE UNIVERSITY



國立臺灣大學
National Taiwan University



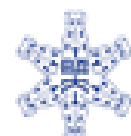
成功大學

National Cheng Kung University



中國醫藥大學附設醫院
China Medical University Hospital

KBSI KOREA BASIC SCIENCE INSTITUTE



昭和大学



臺北醫學大學
TAIPEI MEDICAL UNIVERSITY



長庚醫療財團法人
Chang Gung Medical Founder

SNUH SEUL NATIONAL UNIVERSITY HOSPITAL



東京大学
THE UNIVERSITY OF TOKYO



Sullivan Nicolaides
PATHOLOGY



高雄醫學大學
Kaohsiung Medical University

SUNGKYUNKWAN UNIVERSITY (SKKU)



ST JOHN OF GOD
Subiaco Hospital



中山醫學大學
Chung Shan Medical University

Industrial Installation sites (partial)



Increase Your Impact Factor!

3000+ papers,
400+ from Taiwan!!

Country	Total	%
USA	612	16.9%
China	596	16.5%
Austria	422	11.7%
Taiwan	402	11.1%
Germany	303	8.4%
The Netherlands	166	4.6%
UK	148	4.1%
Japan	127	3.5%
South Korea	106	2.9%
Italy	70	1.9%

until September, 2023

Cell
nature
Science



Highly Efficient TSA Multiplex
IHC Assay Kits
多色免疫組織染色分析套組

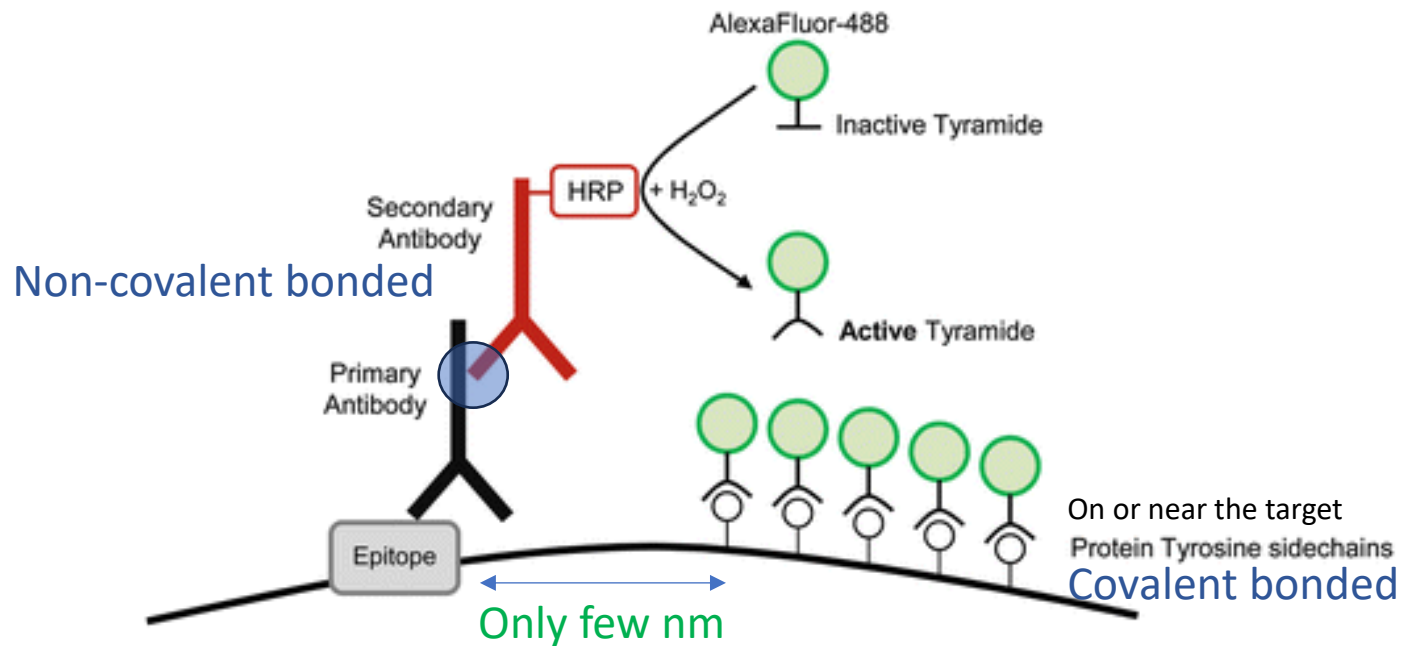


What is TSA?

TSA (Tyramide Signal Amplification) 染色技術是一種基於酵素催化反應的信號放大技術，可以大幅增強免疫螢光或免疫組織化學染色的訊號。它利用辣根過氧化物酶 (HRP) 對酪胺酸 (tyramide) 進行催化反應。

TSA相較於傳統螢光染色的優勢

TSA技術的主要優勢在於其極高的靈敏度和多重染色的能力。它特別適合用於檢測組織切片中低豐度的生物標誌物，或在有限的樣本上需要標記多個目標時。



TSA versus IF

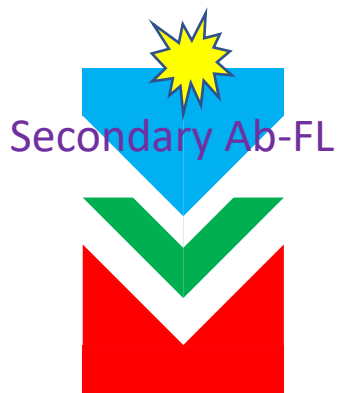
優勢	TSA染色技術	傳統螢光染色
信號強度	信號放大效果顯著，能檢測到低豐度的目標蛋白，靈敏度極高。	無信號放大機制，檢測低豐度蛋白的能力較差。
多重染色	由於酵素催化反應後，標記的螢光分子會與蛋白共價結合，因此可以透過移除抗體來進行多次循環染色 (Sequential Staining)，實現多達七個或更多的標記。	螢光抗體直接結合，無法移除。因此能同時標記的目標數量受限於光譜串擾 (spectral crosstalk)，通常只能標記3-4個。
抗體用量	由於信號放大，可以大幅減少一級抗體和二級抗體的使用量。	需要較高濃度的抗體才能產生足夠的信號。
背景雜訊	雜訊較低，因為標記只發生在目標蛋白附近，非特異性結合的螢光信號較少。	非特異性結合的抗體可能會產生較高的背景螢光。
樣本保存	染色後的樣本穩定性高，信號持久，不易褪色。	螢光分子容易光漂白，保存困難，信號會隨著時間減弱。

Increase Signal to 500-folds by Next Gen. TSA

Direct Labeling

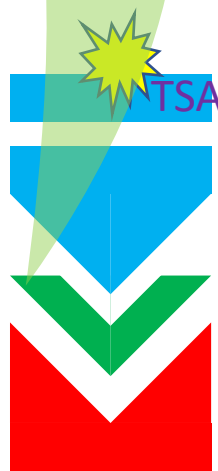


Indirect IHC/IF



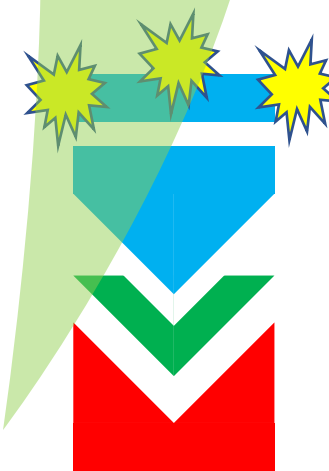
100-folds

TSA (Tyramide Signal Amplifier)

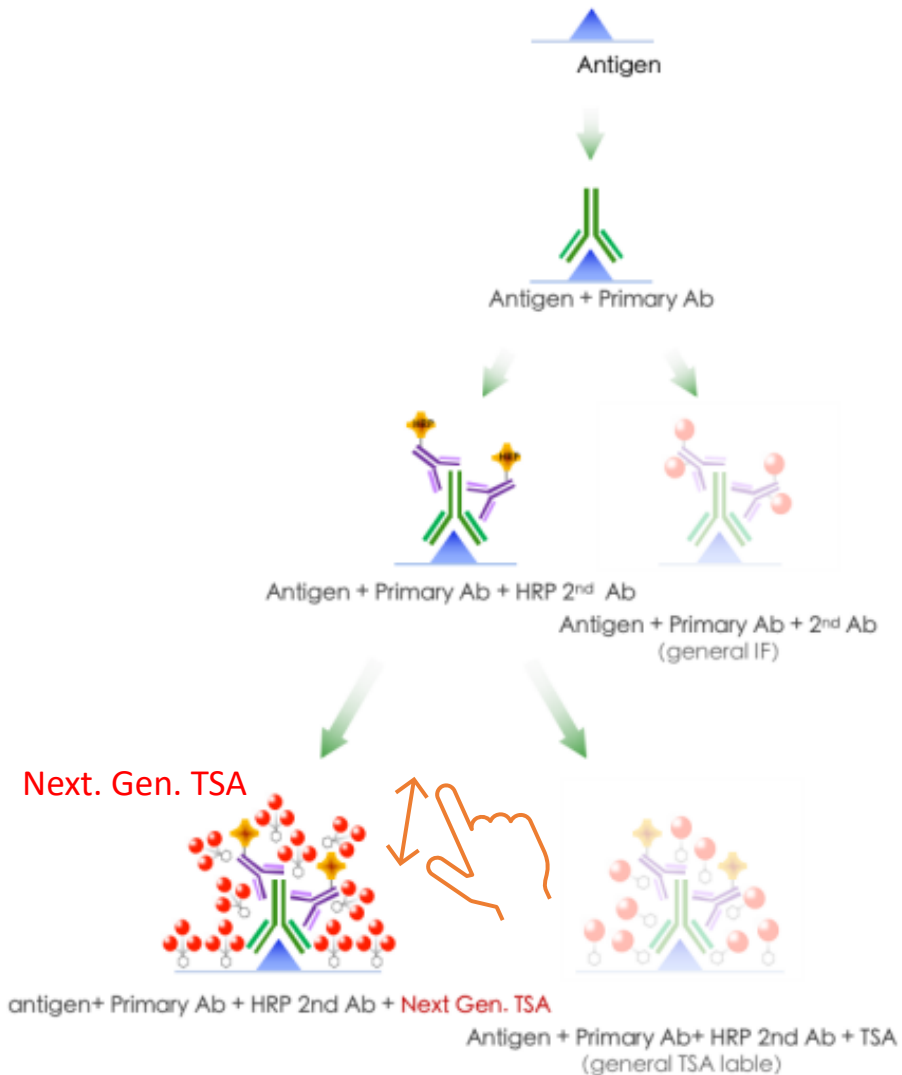


500-folds

TG Next Generation TSA

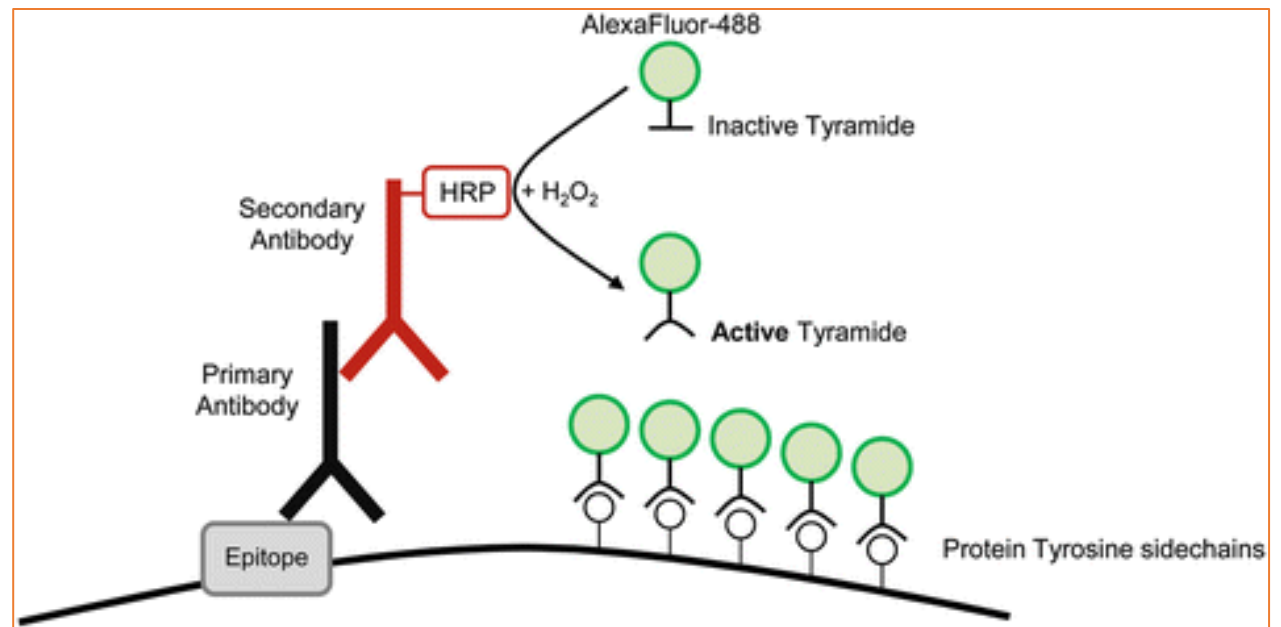
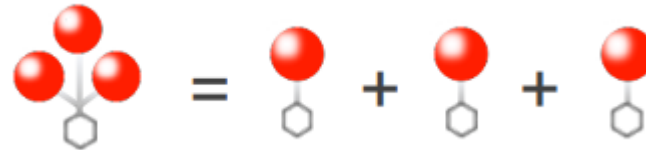


Next Gen. Tyramide Signal Amplification (TSA)



Next Gen. TSA

- binds more Fluor via Dendrimier molecule

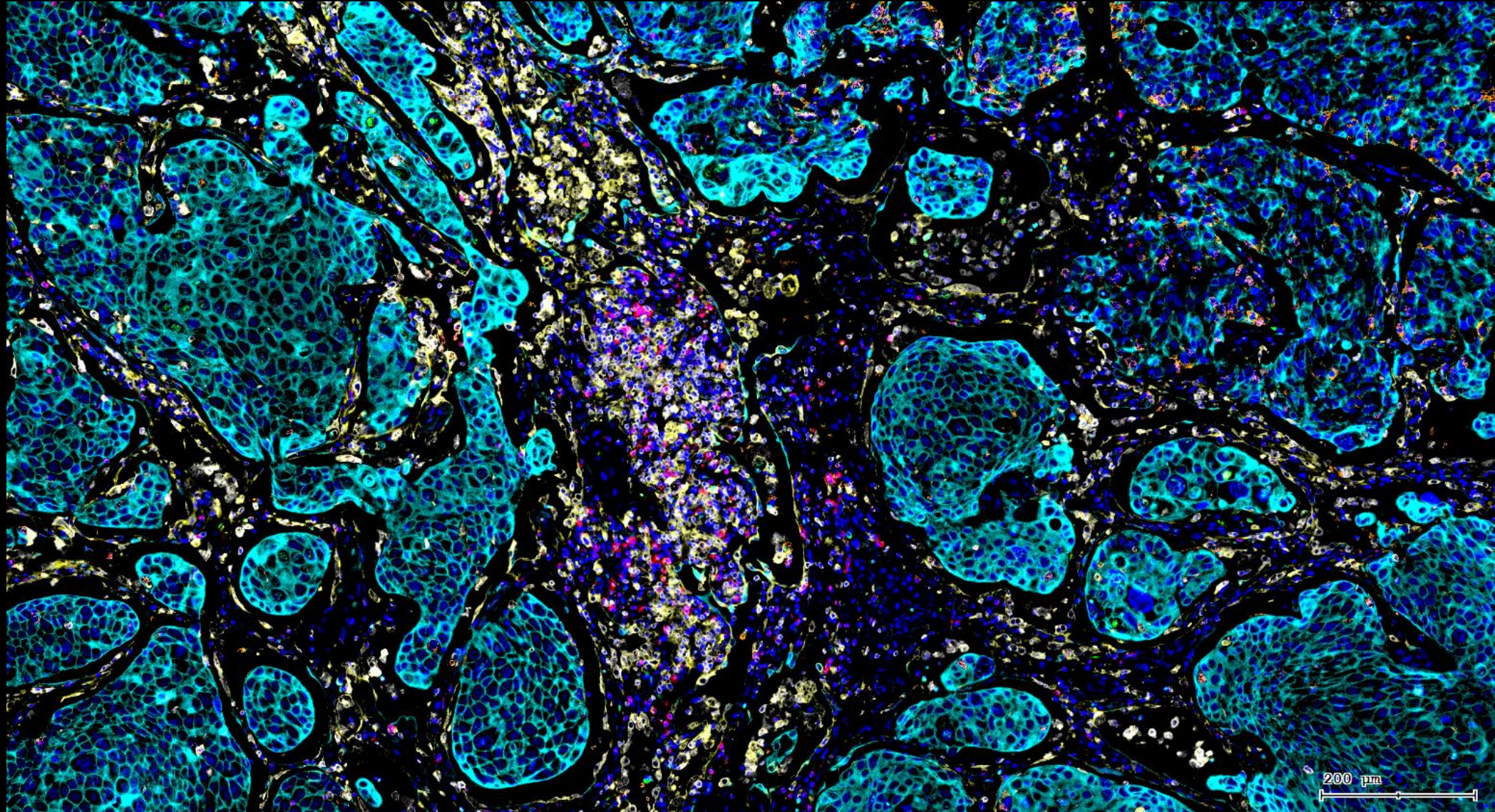


Features & Benefits of TSA IHC Assay Kits

- **Flexible panel design:**
 - ✓ Select antibodies from any commercial products
 - ✓ No overlap between primary and secondary
- **Higher Sensitivity**
 - ✓ 5-folds than other TSA
 - ✓ 500-folds than conventional
- **High Signal-to-noise ratio**
- **Cost effective**
 - ✓ Reduce the consumption of Primary Antibody
- **Up to 10 colors**

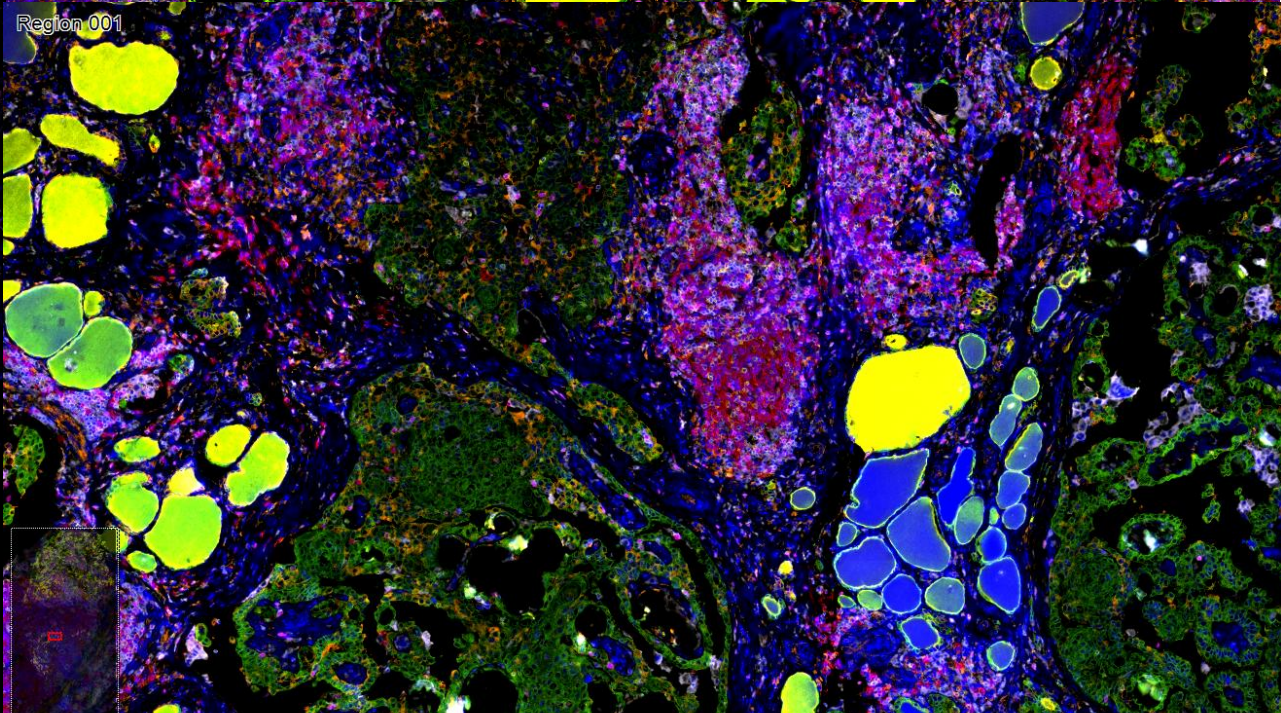
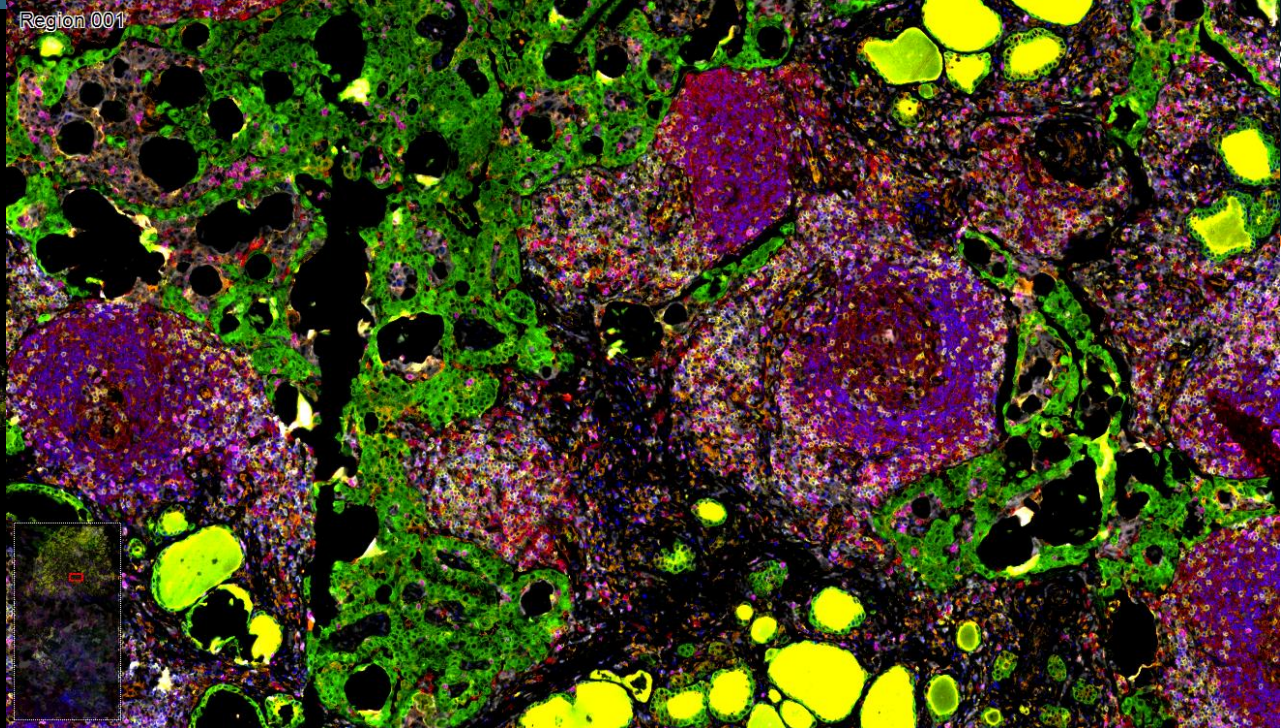
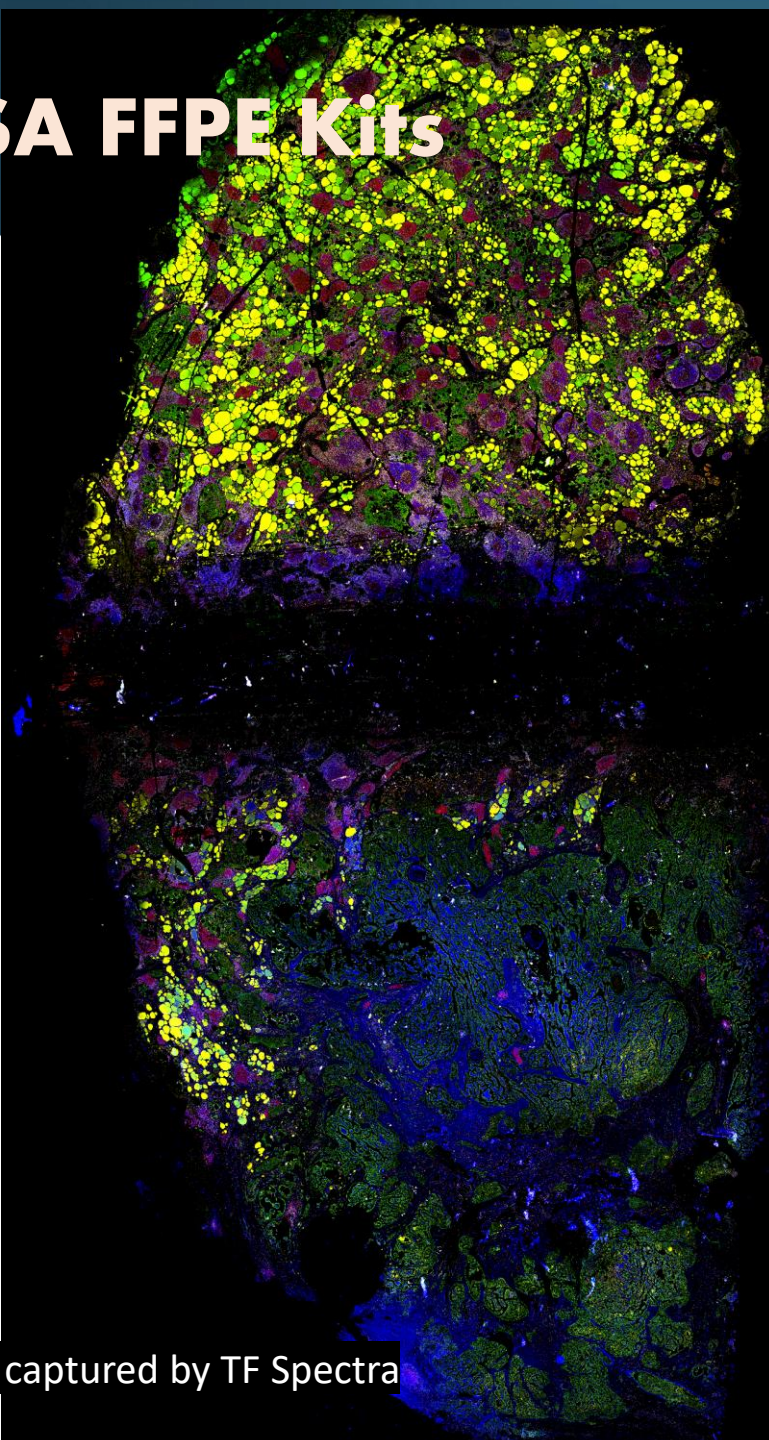


10-color TSA FFPE Kits



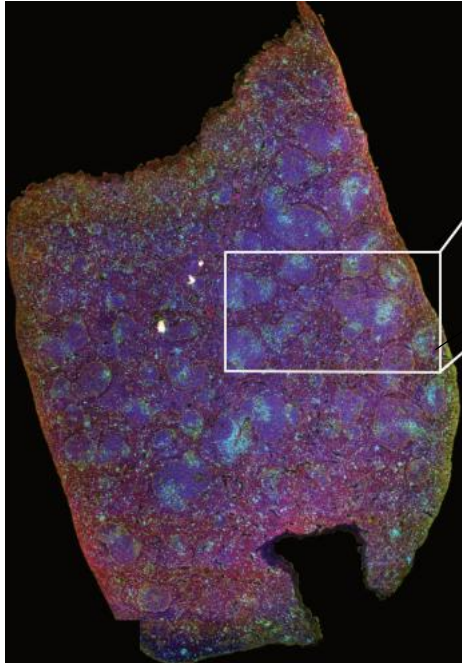
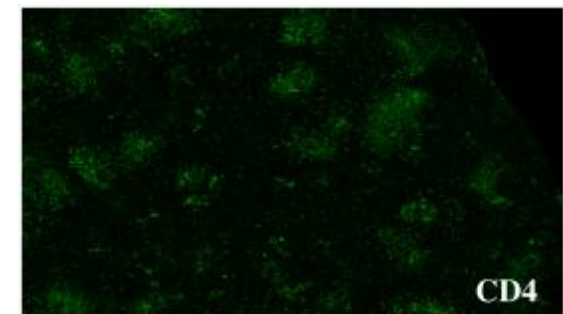
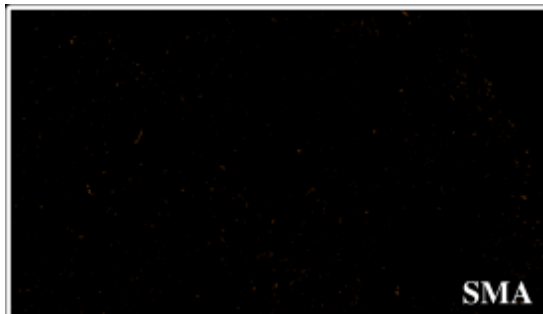
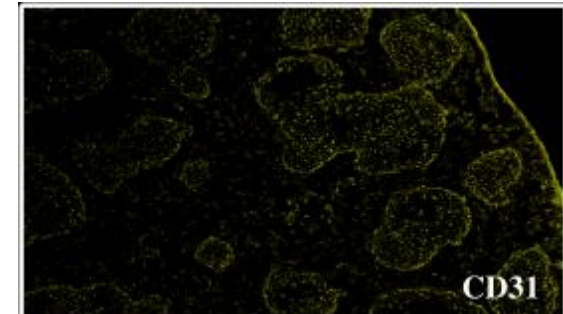
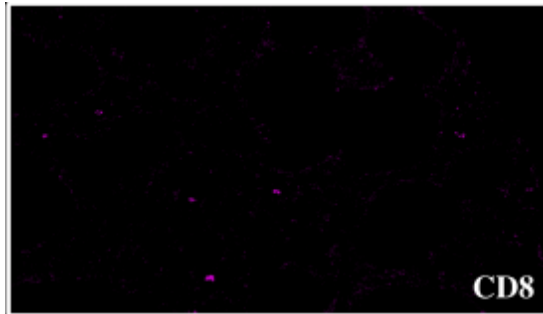
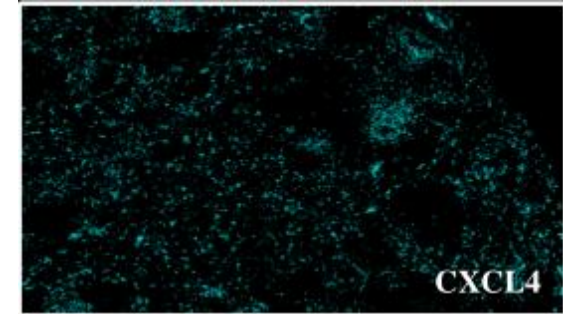
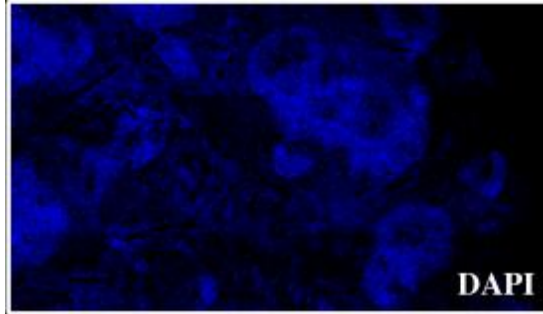
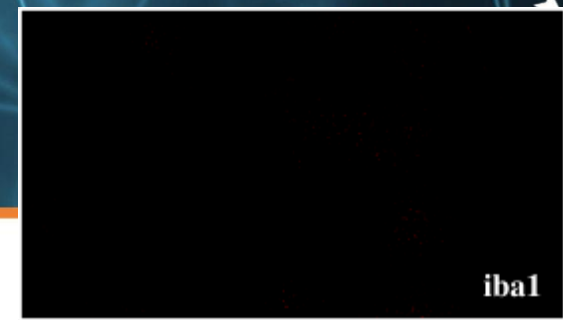
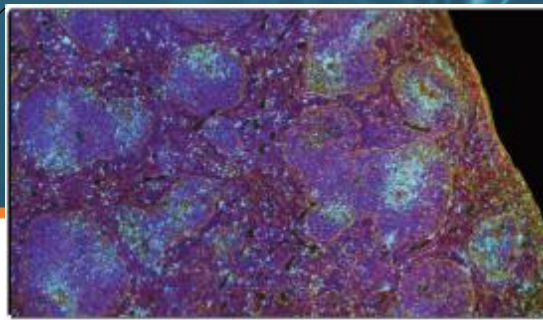
- CK19
- CD68
- FOXP3
- HLA-DR
- CD4
- CD163
- PD1
- CD8
- CD20

7-color TSA FFPE Kits



Human Thyroid Tumor captured by TF Spectra

6-color TSA FFPE Kits

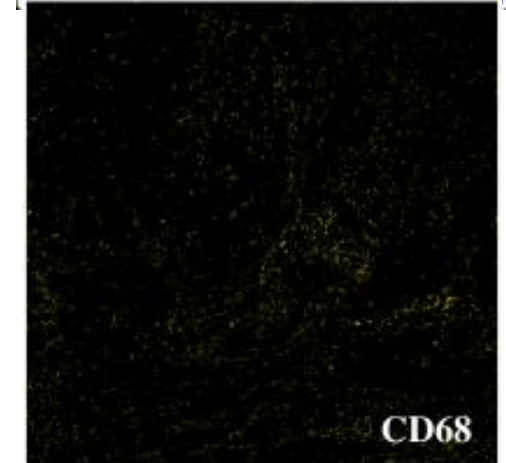
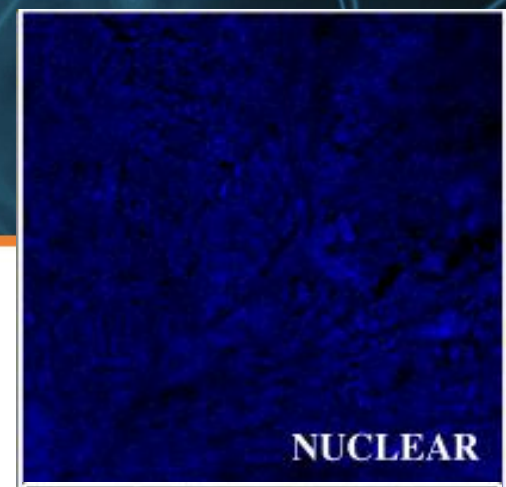
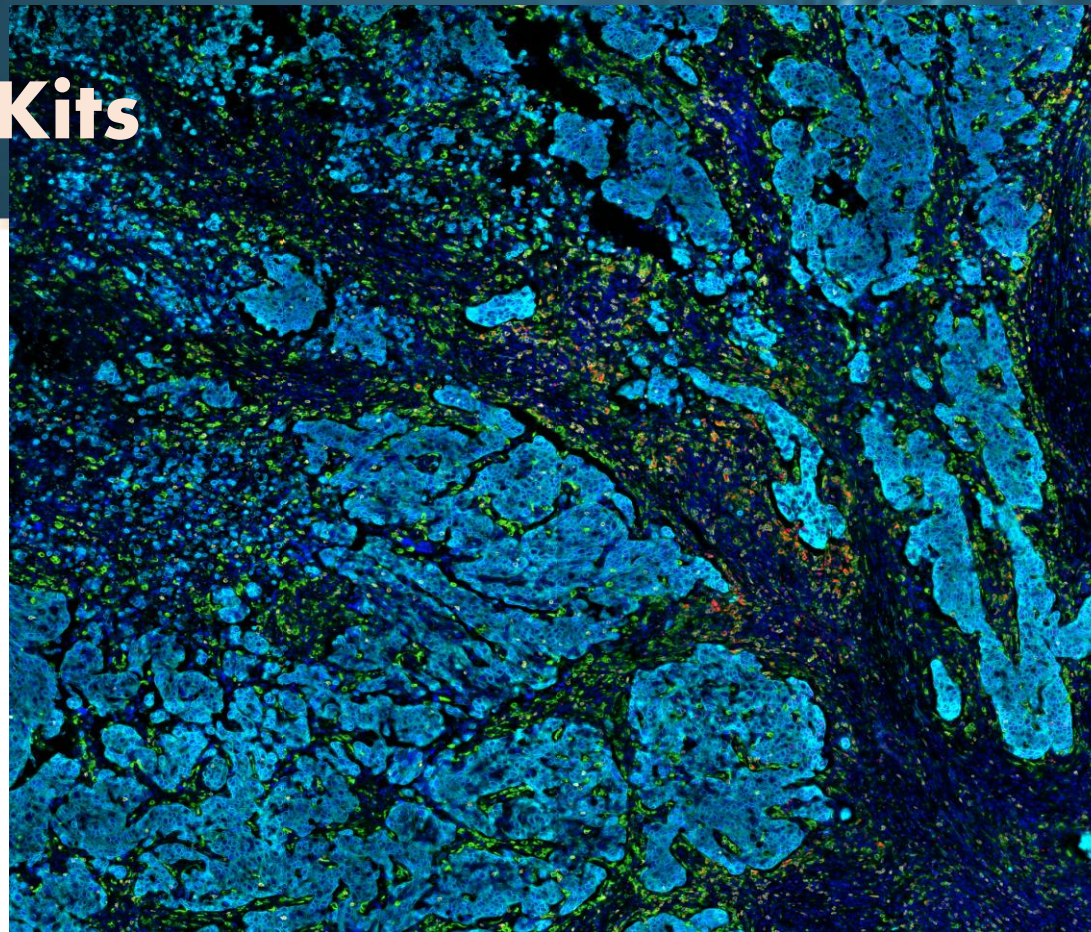


Mouse Spleen Tissue
Captured by TF Spectra

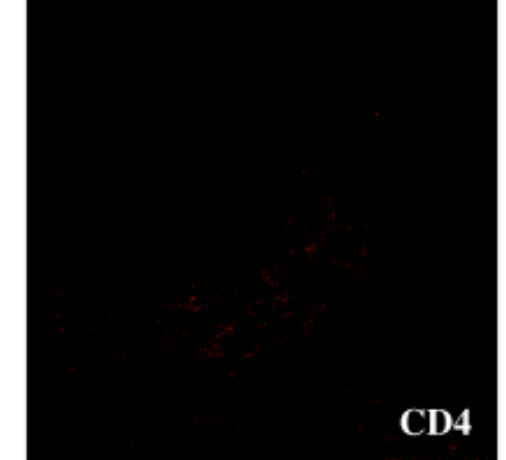
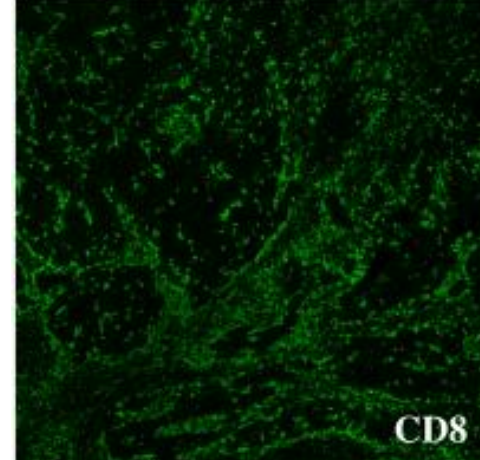
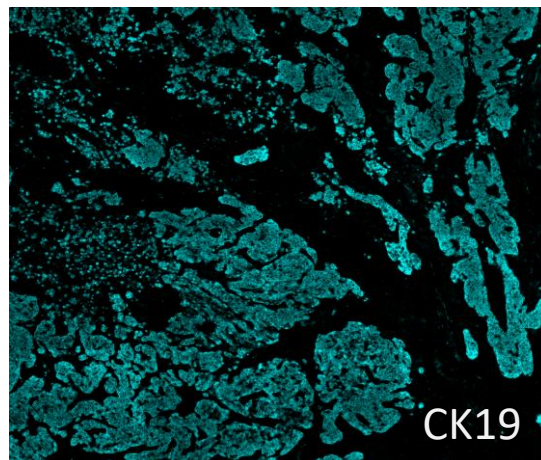
CD4 TG520N
CD31 TG620N
CXCL4 TG650N
SMA TG660S
CD8 TG570N
Iba1 TG700N
DAPI

5-color TSA FFPE Kits

Human Lung Tumor
Captured by TF Spectra



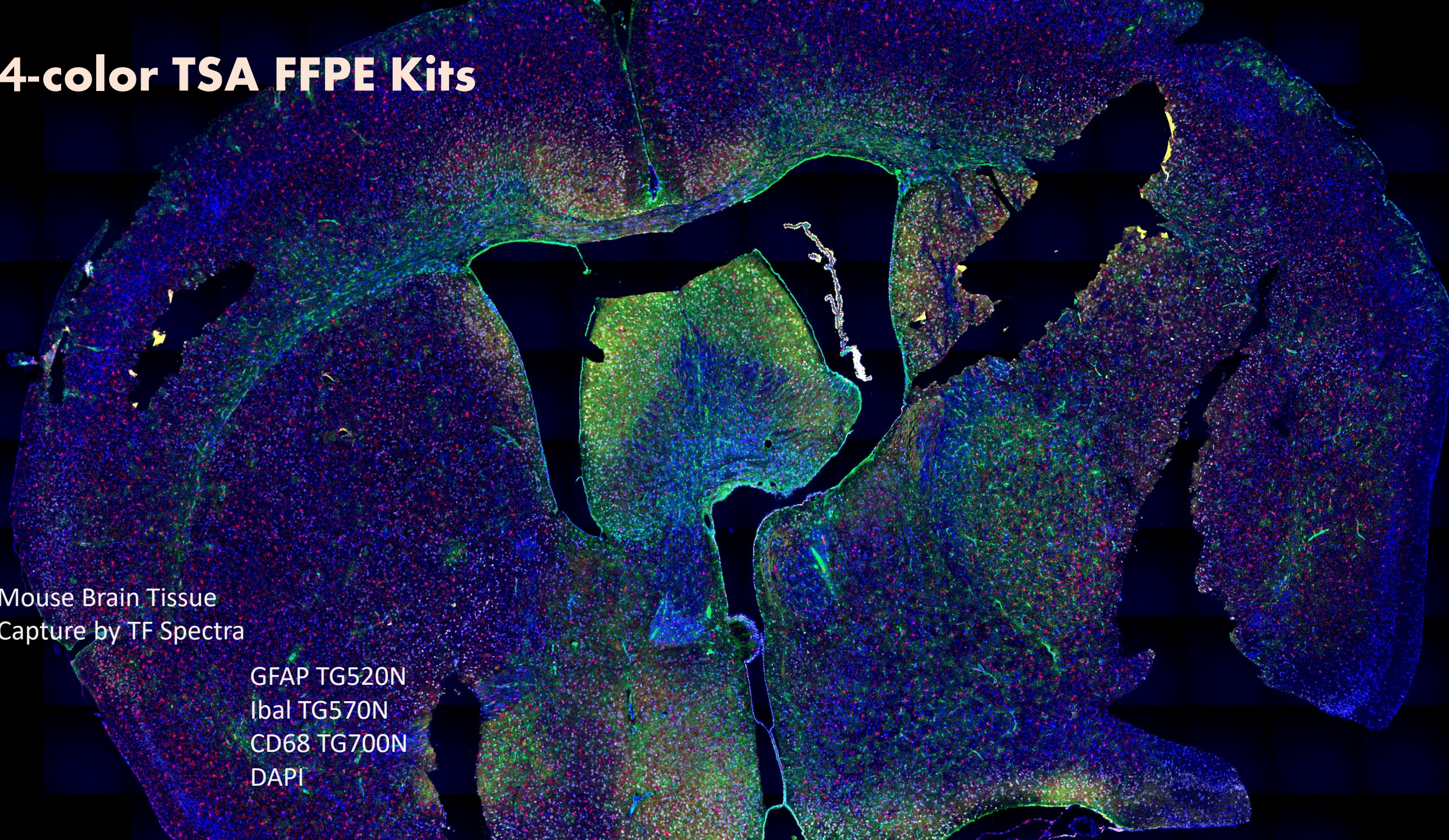
CK19 TG440N
CD8 TG5209N
CD4 TG570N
CD68 TG650N
Nuclei TG470SN



4-color TSA FFPE Kits

Mouse Brain Tissue
Capture by TF Spectra

GFAP TG520N
Ibal TG570N
CD68 TG700N
DAPI



TSA Dyes List

TSA Dyes	Brightness	Photo sensitivity	Heat Stability	pH Stability	Ex. (nm) Em.(nm)
TG420N	★★	★★	★★★	★★★	405/420
TG440N	★★★	★★★	★★★★	★★★★	362/436
TG520N	★★★★	★★★	★★★	★★★	488/519
TG540N	★★★★	★★★★	★★★★	★★★★	517/539
TG570N	★★★★	★★★★	★★★★	★★★★	555/570
TG620N	★★★★	★★★★	★★★★	★★★★	594/615
TG650N	★★★★	★★★★★	★★★★	★★★★	633/655
TG670N	★★★★	★★★★★	★★★★	★★★★	650/670
TG700N	★★★★	★★★★★	★★★★	★★★★	682/702
TG540S	★★	★★★★	★★★★	★★★★	405/540
TG660S	★★	★★★★	★★★★	★★★	500/660
Nuclear dyes					
DAPI	★★★★	★★★★★	★	★★★★	360/460
TG 470 SN	★★★	★★★★	★	★★★★	450/470

Product List

	Product Name	Contents	Size	Catalog no.
Standalone Dye	4-color dyes combination	TSA Amplification reagent: TG520N, TG570N, TG670N, DAPI, Amplification diluent buffer	50µL	TGT4C50
			100µL	TGT4C100
	5-color dyes combination	TSA Amplification reagent: TG520N, TG570N, TG620N, TG670N, DAPI Amplification diluent buffer	50 µL	TGT5C50
			100 µL	TGT4C100
	6-color dyes combination	TSA Amplification reagent: TG520N, TG570N, TG620N, TG650N, TG700N, DAPI Amplification diluent buffer	50µL	TGT6C50
			100µL	TGT6C100
	7-color dyes combination	TSA Amplification reagent: TG440N, TG520N, TG570N, TG650N, TG700N, TG660S, TG470SN Amplification diluent buffer	50µL	TGT7C50
			100µL	TGT7C100
	8-color dyes combination	TSA Amplification reagent: TG440N, TG520N, TG540N, TG570N, TG620N, TG650N, TG700N, TG470S, NAmplification diluent buffer	50µL	TGT8C50
			100µL	TGT8C100
	9-color dyes combination	TSA Amplification reagent: TG440N, TG520N, TG540N, TG570N, TG620N, TG650N, TG700N, TG540S, TG470SN, Amplification diluent buffer	50µL	TGT9C50
			100µL	TGT9C100
	10-color dyes combination	TSA Amplification reagent: TG440N, TG520N, TG540N, TG570N, TG620N, TG650N, TG700N, TG540S, TG660S, TG470SN, Amplification diluent buffer	50µL	TGT10C50
			100µL	TGT10C100

FFPE slide (or frozen/ cell slide)

FFPE slide (or frozen/ cell slide)	FFPE 4-color TSA mIHC Kits	TSA Amplification reagent: TG520N, TG570N, TG670N, DAPI, Amplification diluent buffer, Antigen retrieval buffer (or Stripping Buffer for frozen/ cell slide), Mounting medium, Peroxidase blocking solution, Primary Antibody Dilutions, Blocking Buffer	50 µL	TGFP450
	FFPE 5-color TSA mIHC Kits	TSA Amplification reagent: TG520N, TG570N, TG620N, TG670N, DAPI , Amplification diluent buffer, Antigen retrieval buffer (or Stripping Buffer for frozen/ cell slide), Mounting medium, Peroxidase blocking solution, Primary Antibody Dilutions, Blocking Buffer	100 µL	TGFP4100
			50µL	TGFP550
	FFPE 6-color TSA mIHC Kits	TSA Amplification reagent: TG520N, TG570N, TG620N, TG650N, TG700N, DAPI , Amplification diluent buffer, Antigen retrieval buffer (or Stripping Buffer for frozen/ cell slide), Mounting medium, Peroxidase blocking solution, Primary Antibody Dilutions, Blocking Buffer	100µL	TGFP5100
			50µL	TGFP650
	FFPE 7-color TSA mIHC Kits	TSA Amplification reagentTG440N, TG520N, TG660S, TG470SN , Amplification diluent buffer (or Stripping Buffer for frozen/ cell slide), Mounting medium, Peroxidase blocking solution, Primary Antibody Dilutions, Blocking Buffer	100µL	TGFP8100
	FFPE 9-color TSA mIHC Kits	TSA Amplification reagent: TG440N, TG520N, TG540N, TG570N, TG620N, TG650N, TG700N, TG540S, TG470SN, , Amplification diluent buffer, Antigen retrieval buffer (or Stripping Buffer for frozen/ cell slide), Mounting medium, Peroxidase blocking solution, Primary Antibody Dilutions, Blocking Buffer	50µL	TGFP950
			100µL	TGFP9100
	FFPE 10-color TSA mIHC Kits	TSA Amplification reagentTG440N, TG520N, TG540N, TG570N, TG620N, TG650N, TG700N, TG540S, TG660S, TG470SN, Amplification diluent buffer, Antigen retrieval buffer (or Stripping Buffer for frozen/ cell slide), Mounting medium, Peroxidase blocking solution, Primary Antibody Dilutions, Blocking Buffer	50µL	TGFP1050
100µL			TGFP10100	

Antigen retrieval buffer
 Mounting medium
 Peroxidase blocking solution
 Primary antibody dilution
 Blocking buffer