Next Generation Digital Pathology:

TissueFAXS™ Cytometer
類流式組織細胞定量分析儀



Chris Huang
Cellbio Biotechnology
chris.cellbio@gmail.com

尚博生物科技有限公司
Cell-Bio Biotechnology Co., Ltd.

TISSUE GNOSTICS
MEDICAL & BIOTECH SOLUTIONS

„Conventional“ Digital Pathology

Digital Pathology = Scanning
+ Viewing
+ Managing
+ Sharing
of VIRTUAL SLIDES.

„Next Generation“ Digital Pathology

Digital Pathology = Scanning
+ Viewing
+ Managing
+ Sharing
of VIRTUAL SLIDES.

Now, as multitude of techniques and technologies for creating virtual slides have been established within the community, we are entering the next stage of Digital Pathology, which focuses on digital image analysis!

“**Combine the strengths of the human brain**
(learning, interpolation, experience, error-handling)
with the strengths of computers!
(speed, endurance, reproducibility, large-scale data handling)”

Scanning
+
Analysis

Principles of TissueFAXS

Image



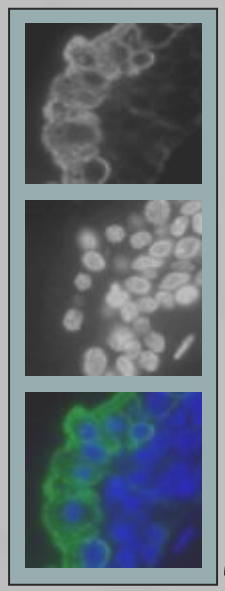
樣本種類:
各類組織切片
組織微陣列TMA
細胞抹片



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

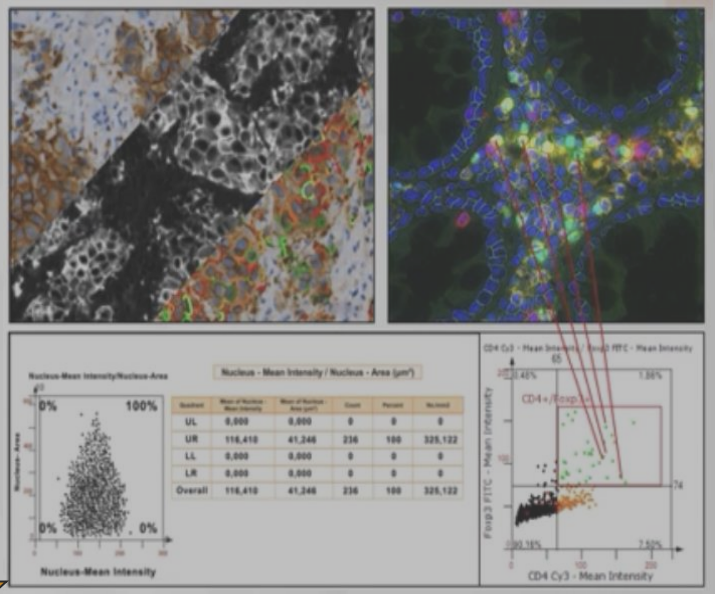
Analysis

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

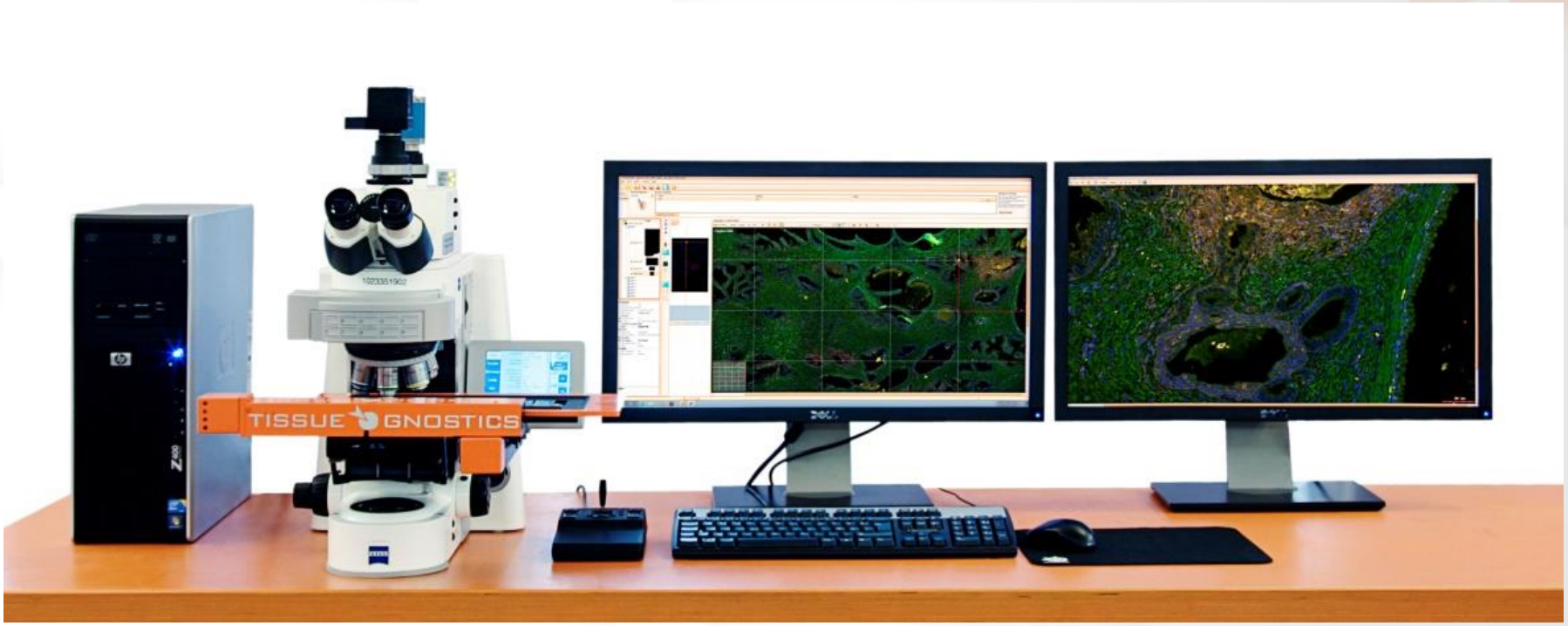


流式數據報告產出:
功能性二維散點圖
數據 ⟷ 細胞聯動
ROI分析自動報告輸出

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The *TissueFAXS*™ product family

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

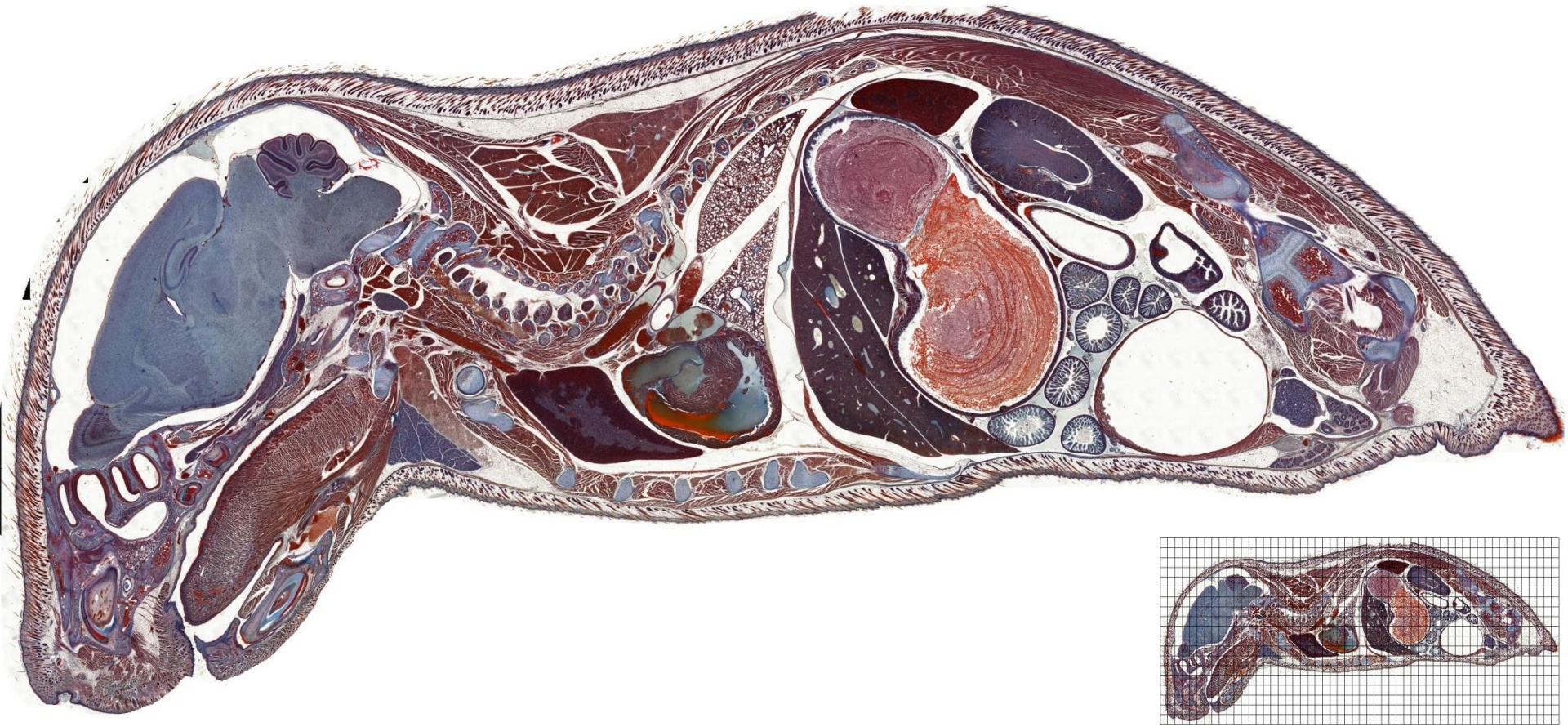


Tissuegnostics High Throughput Scanning Solution for Immunohistochemistry sample



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The Virtual Slide in IHC

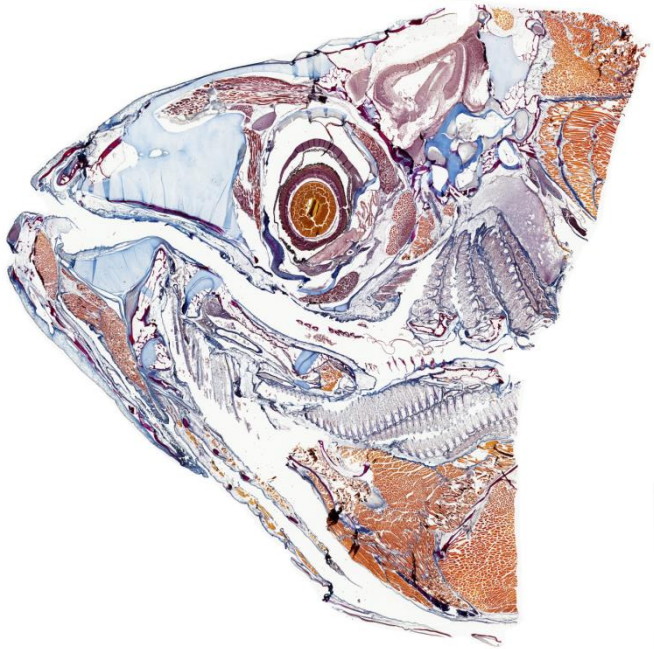


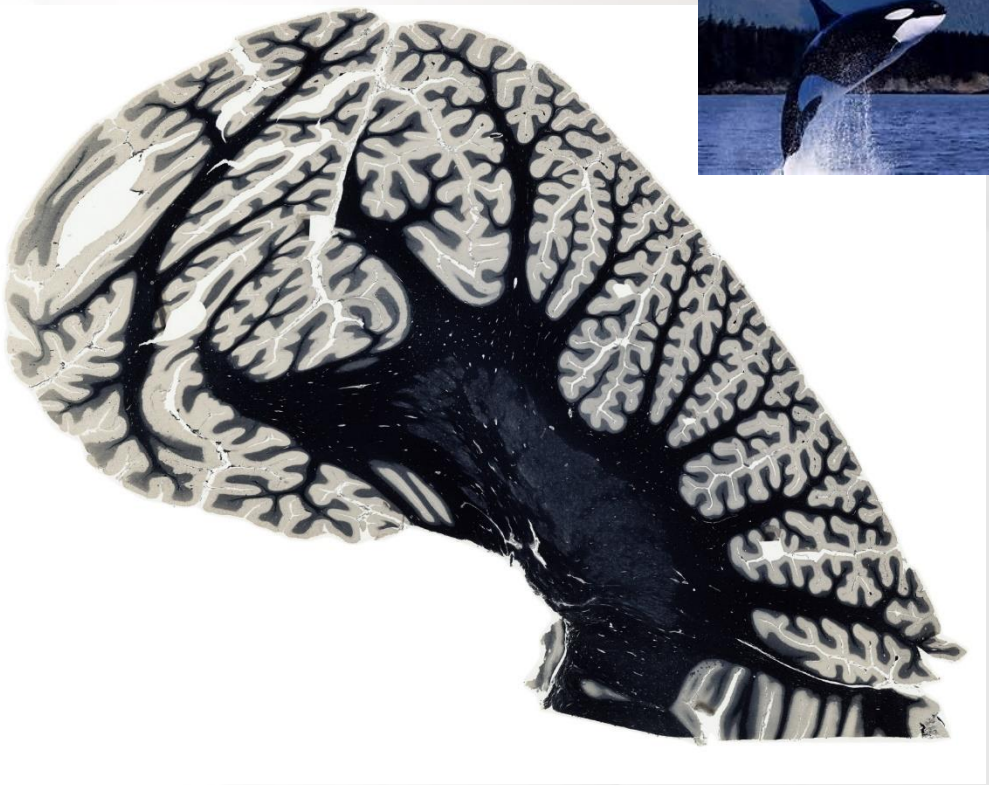
The *digital sample / virtual slide* might consist of thousands of individual fields of view!

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The Virtual Slide in IHC

Over-sized slide



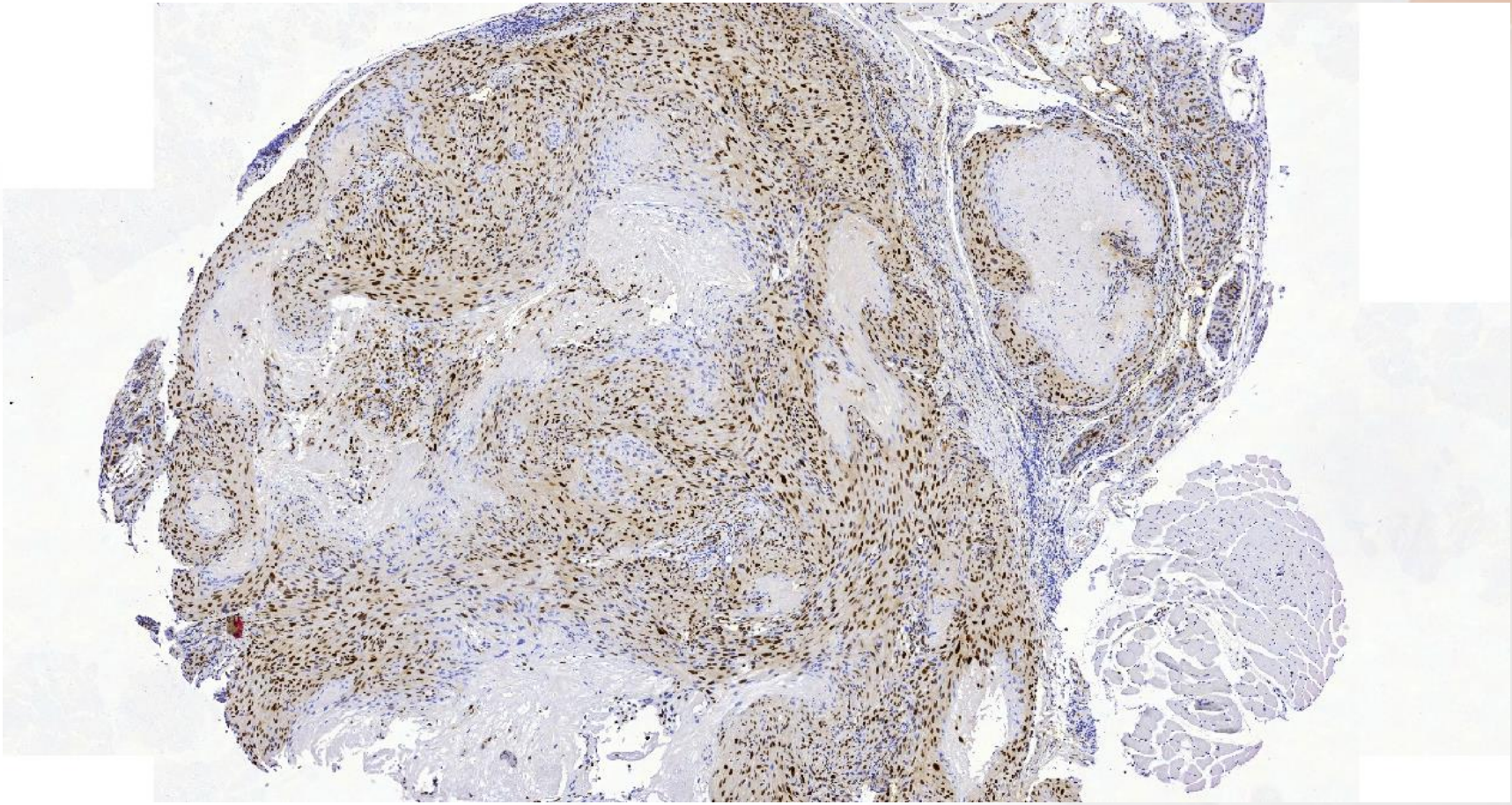


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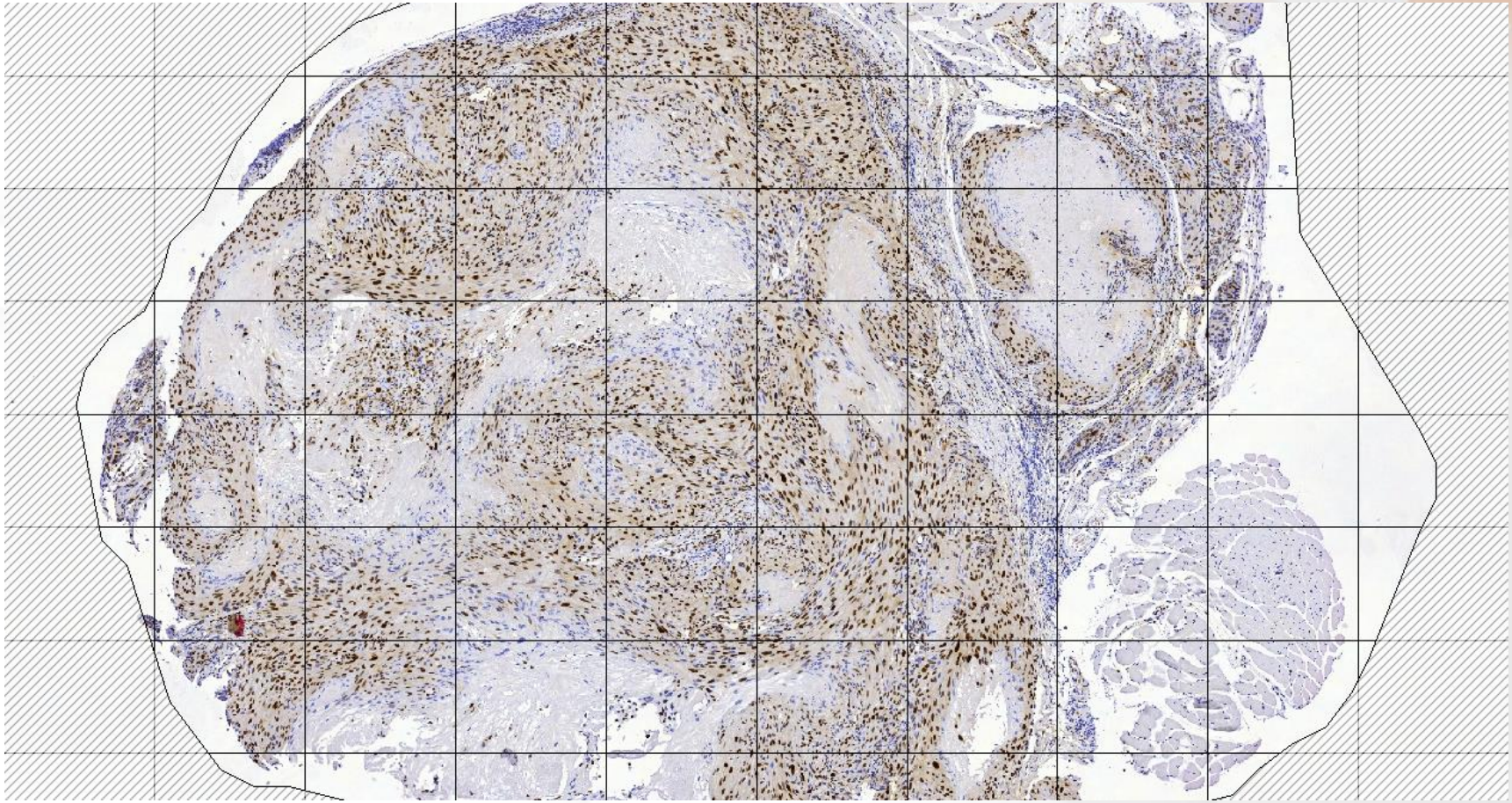
The Virtual Slide in IHC

Slide overview



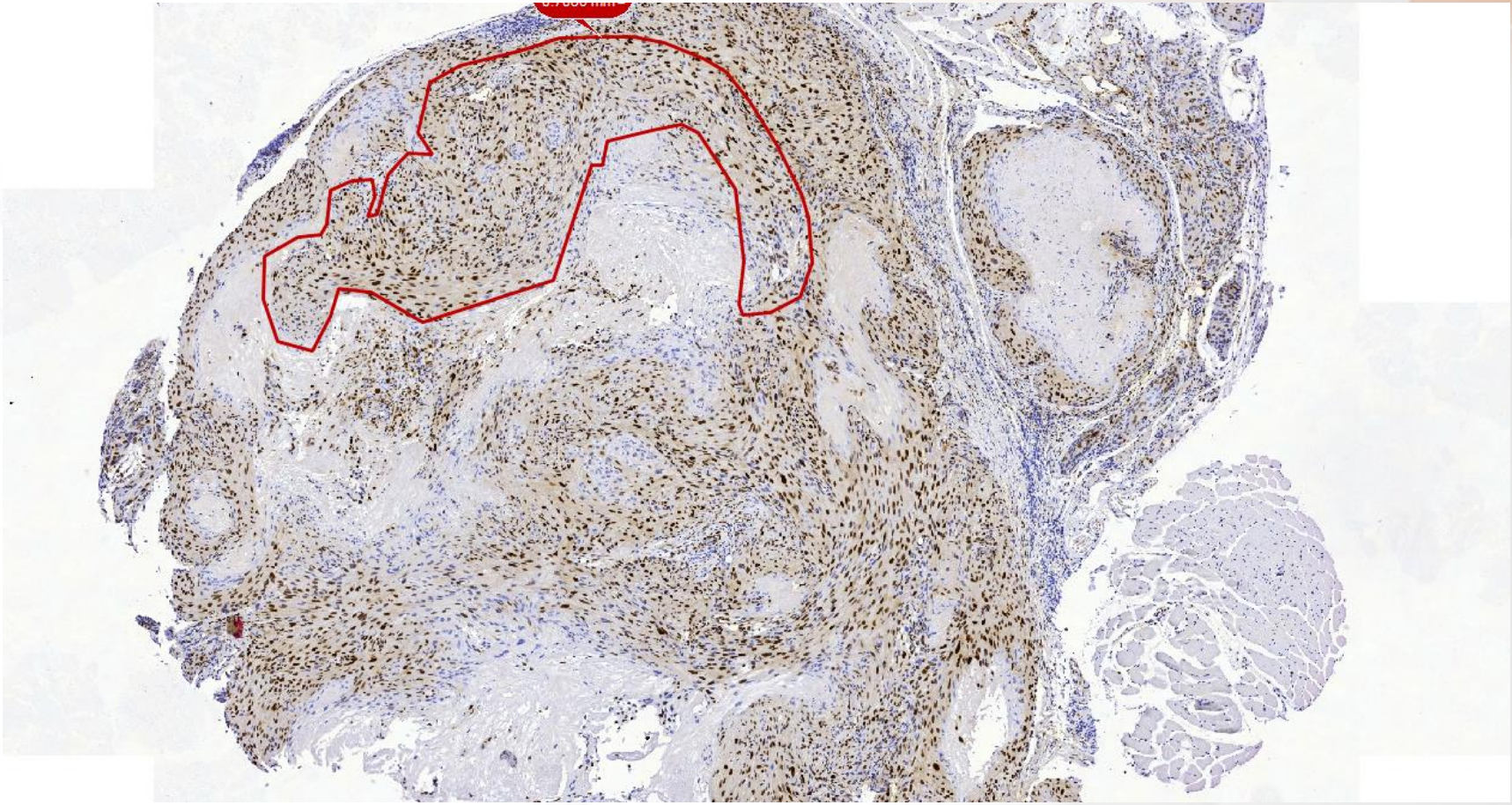
The Virtual Slide in IHC

Corp and FOV line



The Virtual Slide in IHC

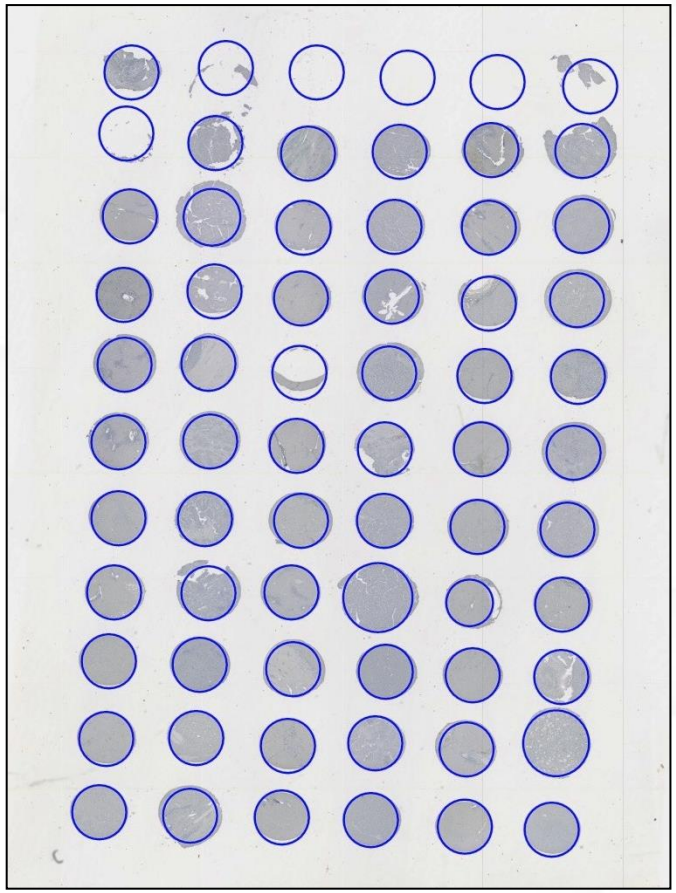
Category in virtual slide



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The Virtual Slide in IHC

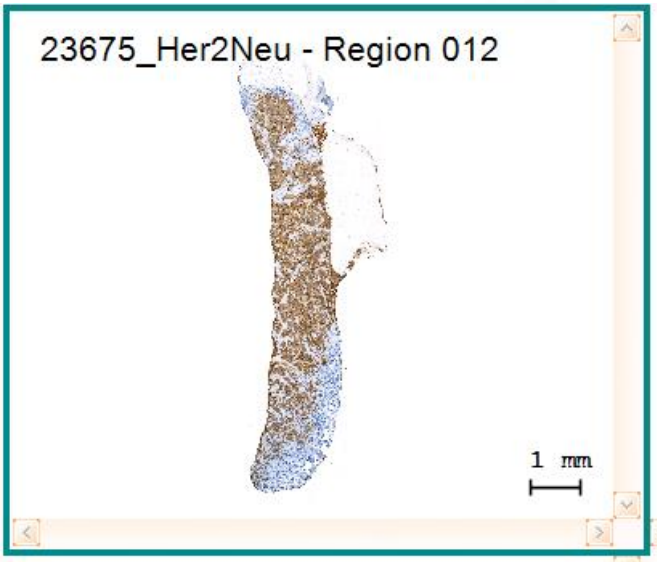
TMA acquisition and manage

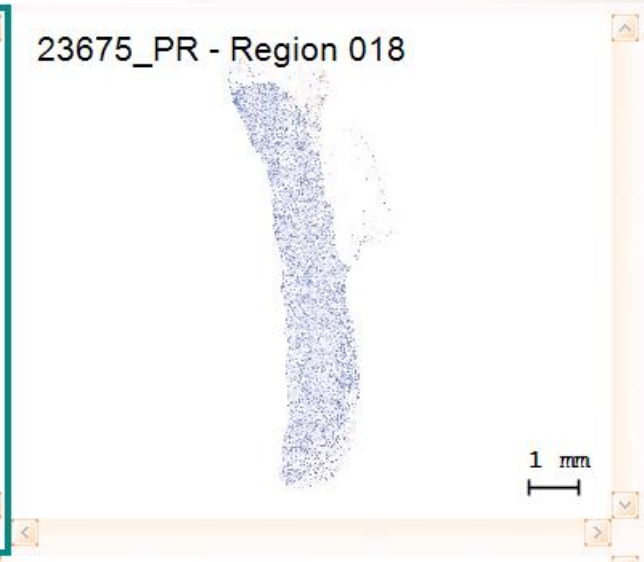


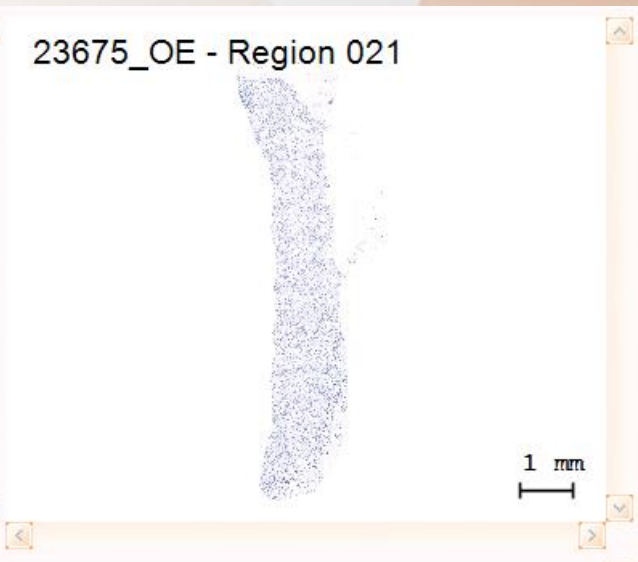
- Auto detect each core
- Missing core identify
- Block grouping
- Each core size and shape can be manually correct
- Block angle and cores distance can be manually correct
- Only scan identified core
- Focus once on each core

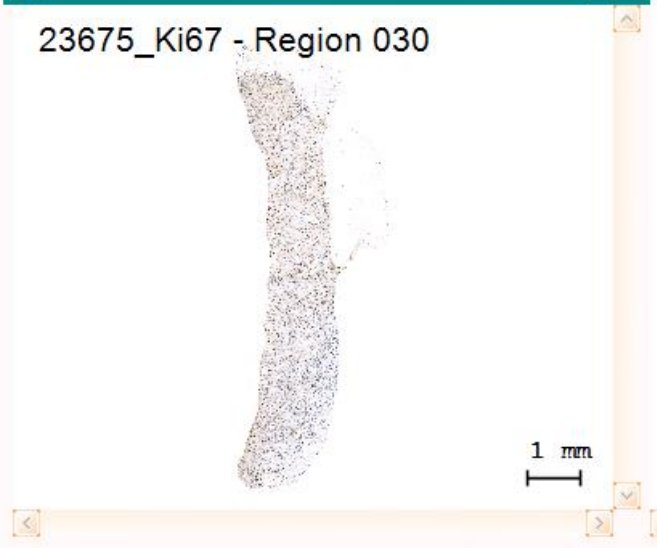
The Virtual Slide in IHC

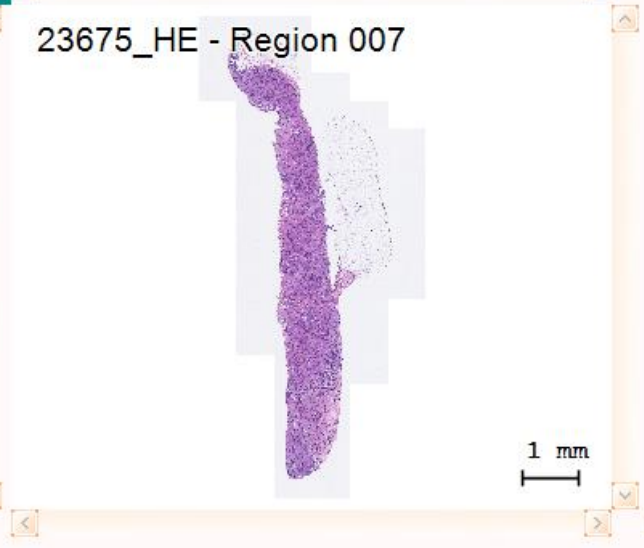
Consecutive sections





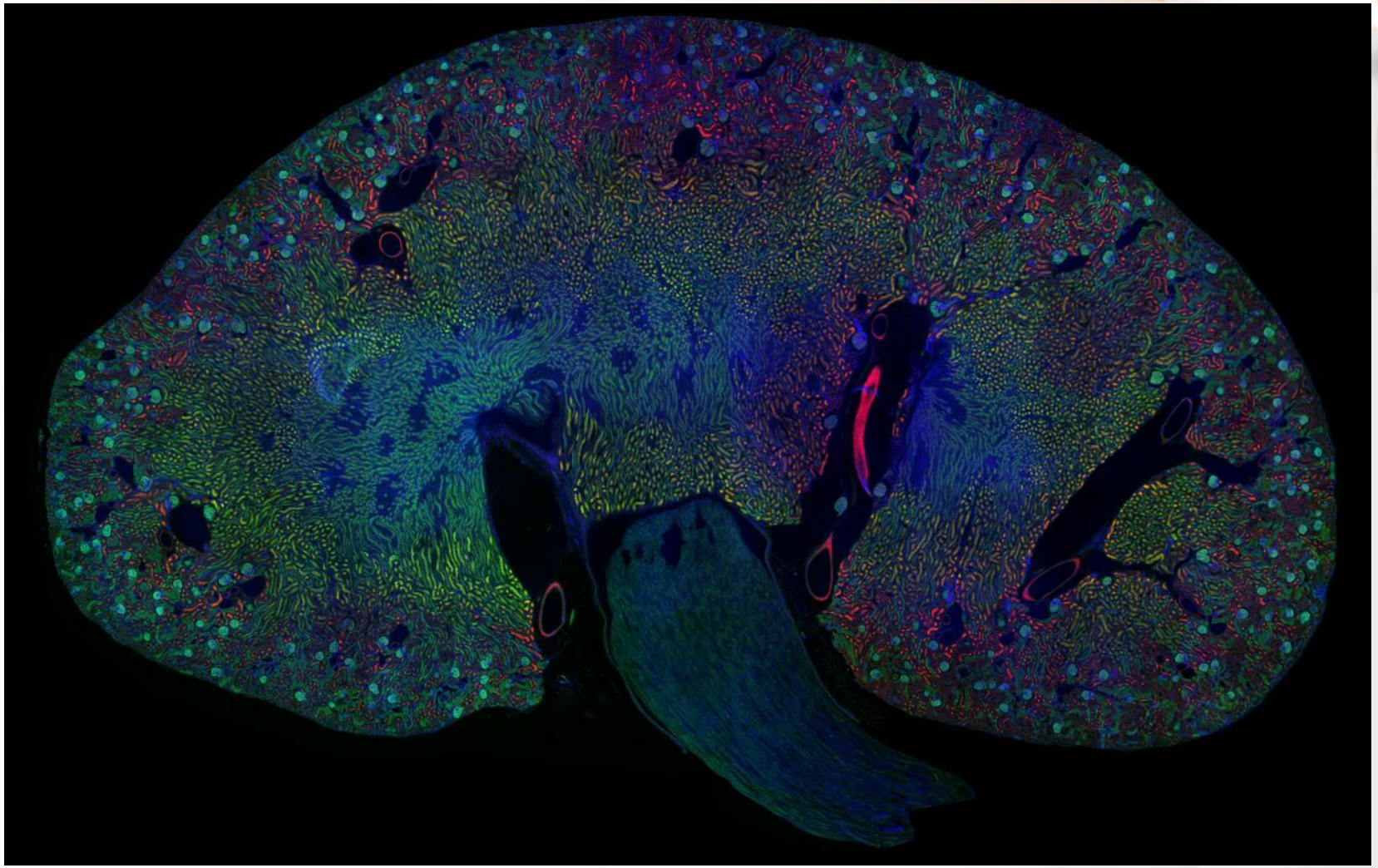








The Virtual Slide in IF



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

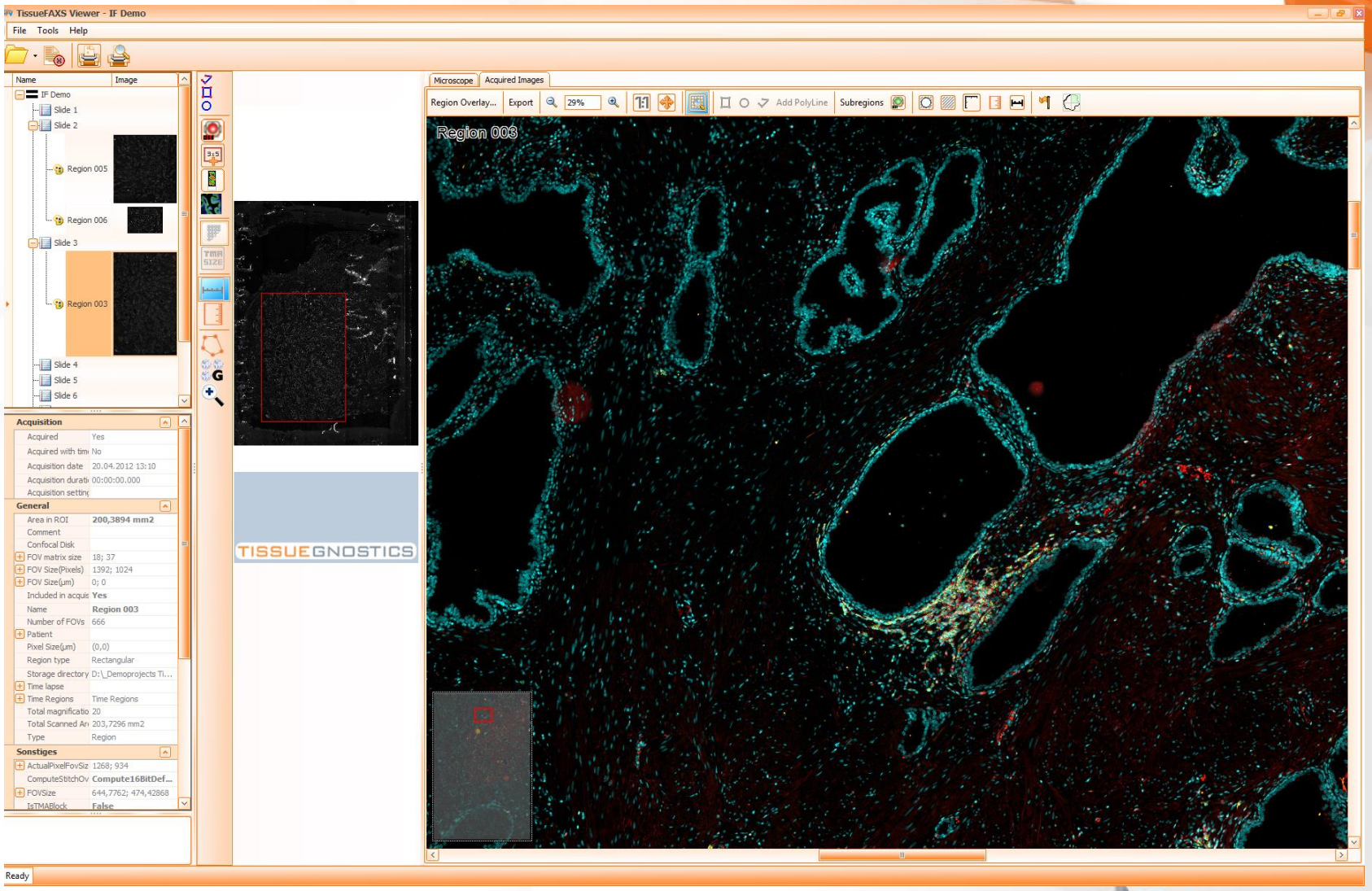
The Virtual Slide in IF



Scanning of 50 µm thick retina

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

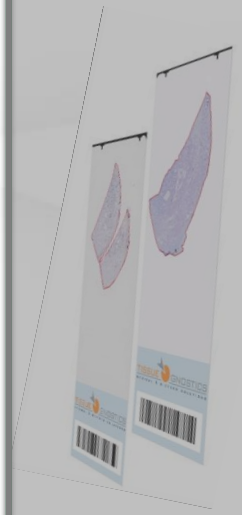
TissueFAXS ™ VIEWER – Freeware!



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Principles of TissueFAXS

Image



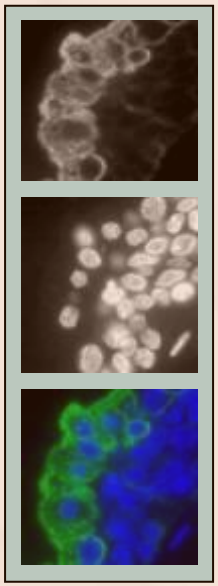


樣本種類:
各類組織切片
組織微陣列TMA
細胞抹片

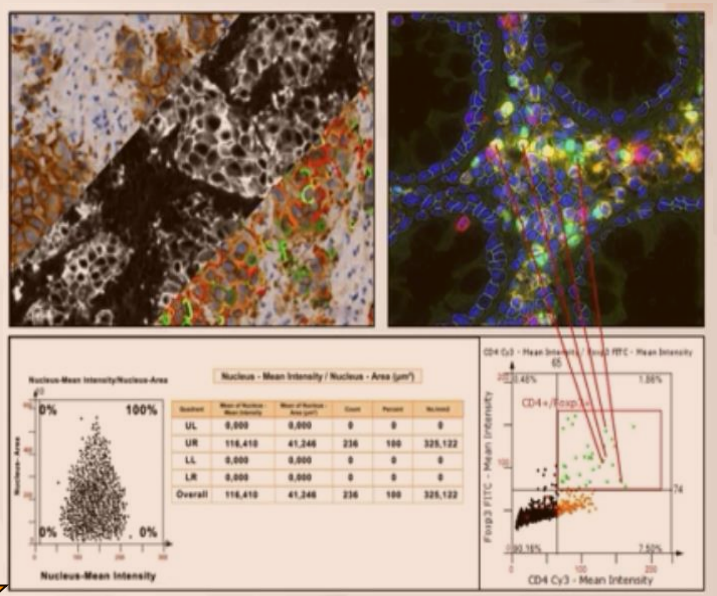
全自動高速拍攝:
可見光/螢光樣本
自動對焦拍攝
全景無縫拼接

Analysis

Result I



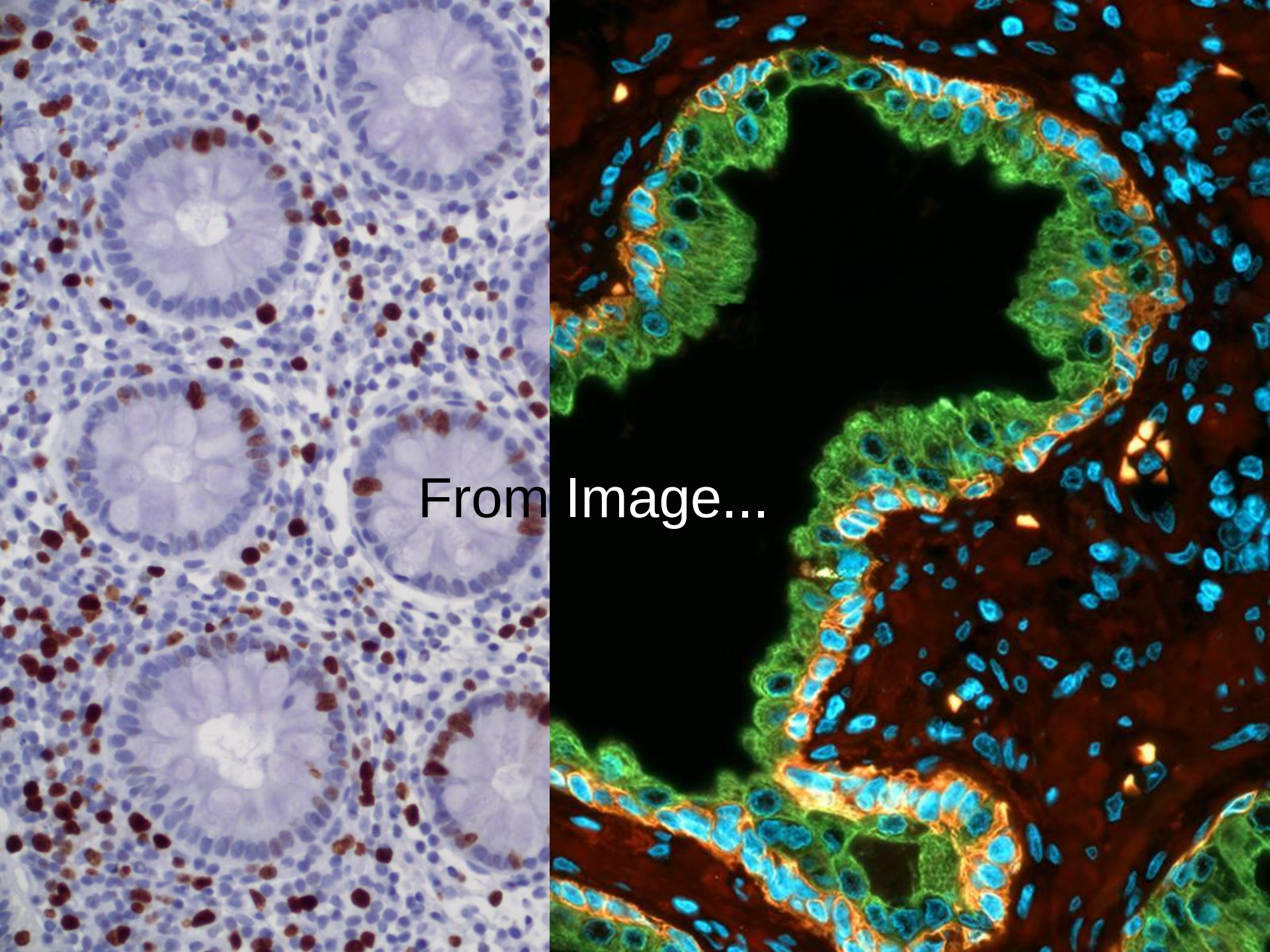
Result II



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

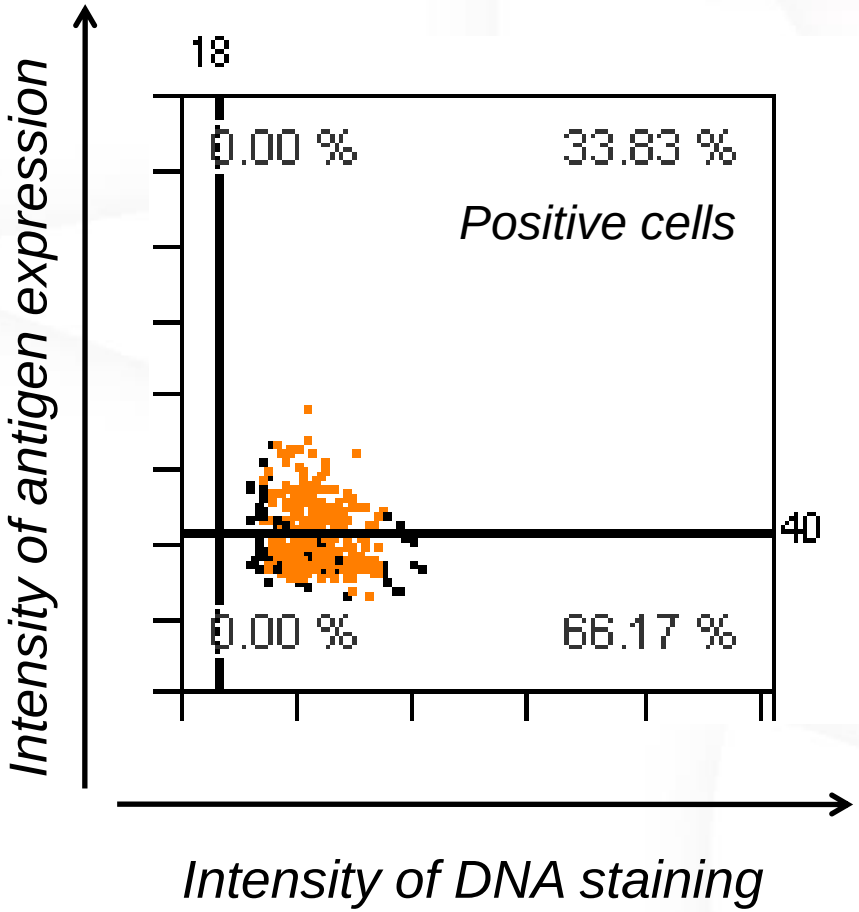
流式數據報告產出:
功能性二維散點圖
數據 ←→ 細胞聯動
ROI分析自動報告輸出

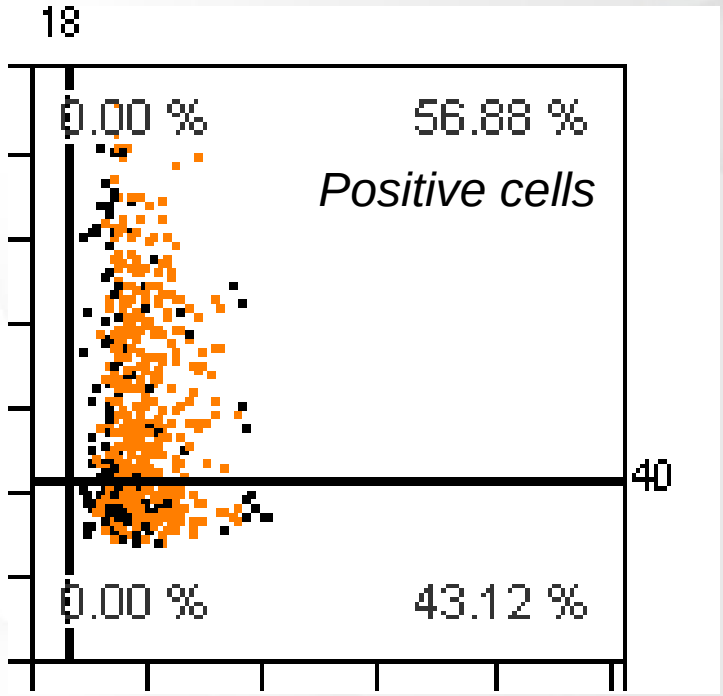
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From Image...

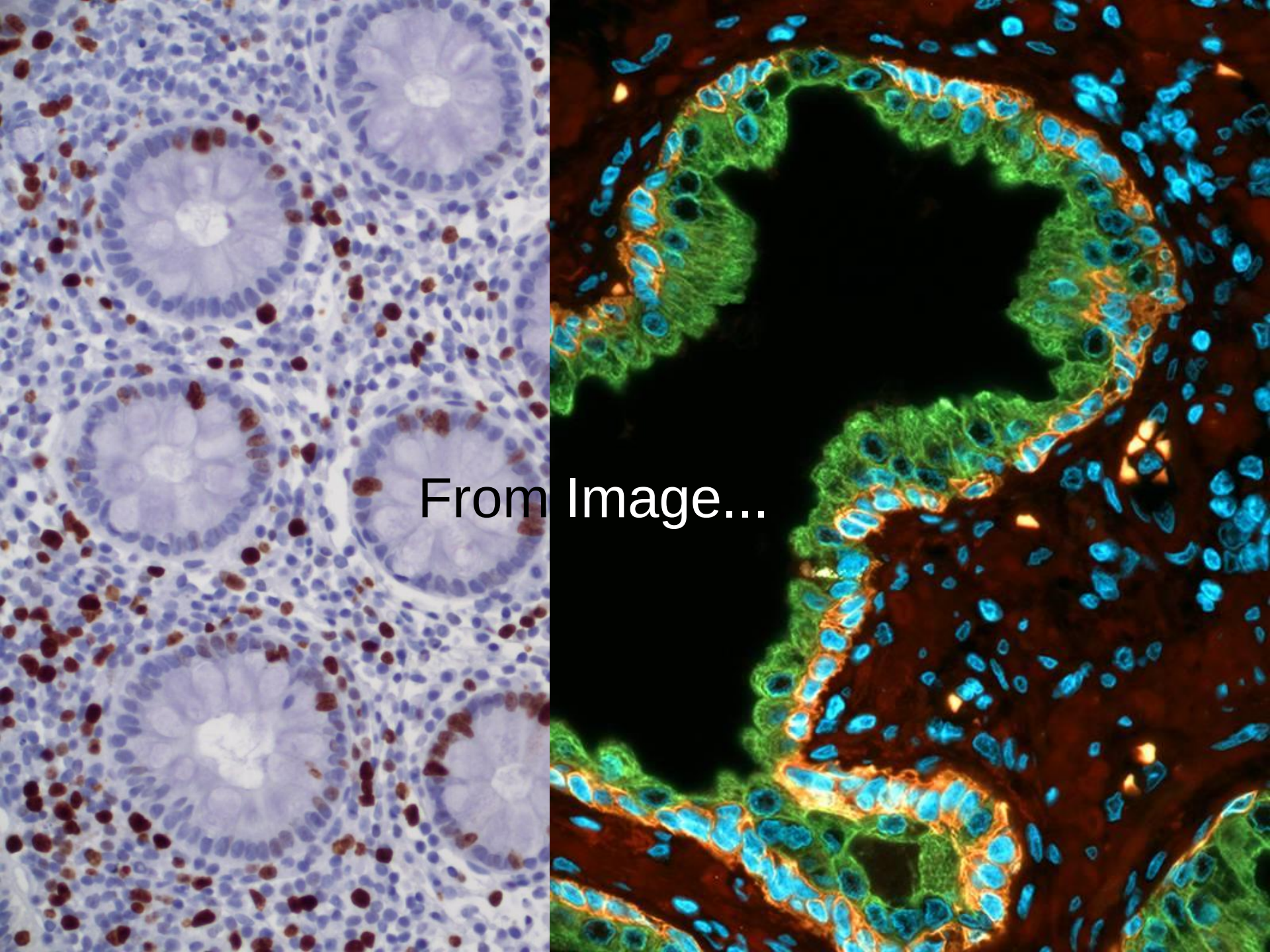
…to Analysis

Each cell is indicated as one dot
The reactivity of two channels is plotted on the x- and y-axes



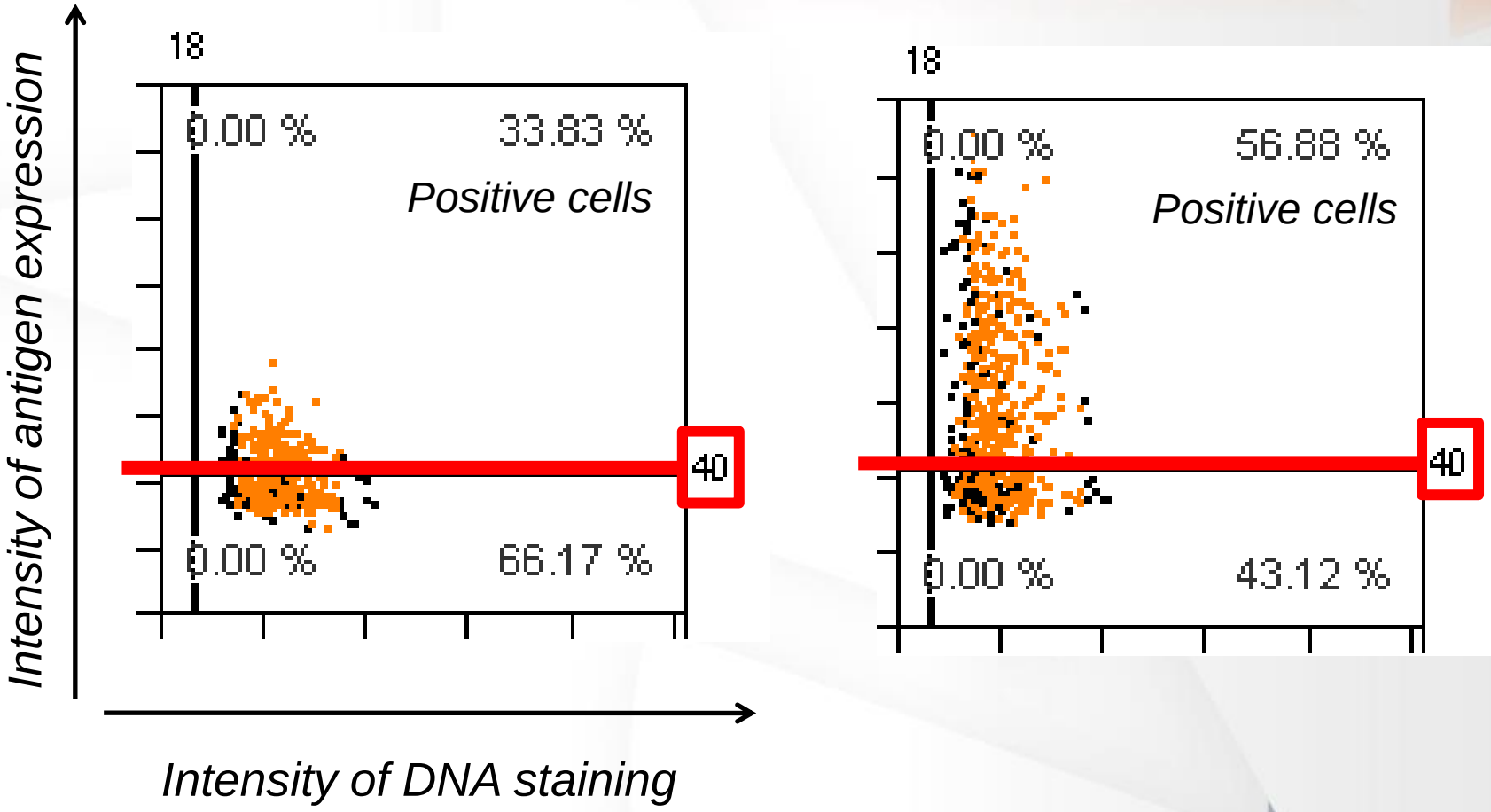


TISSUE GNOSTICS
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From Image...

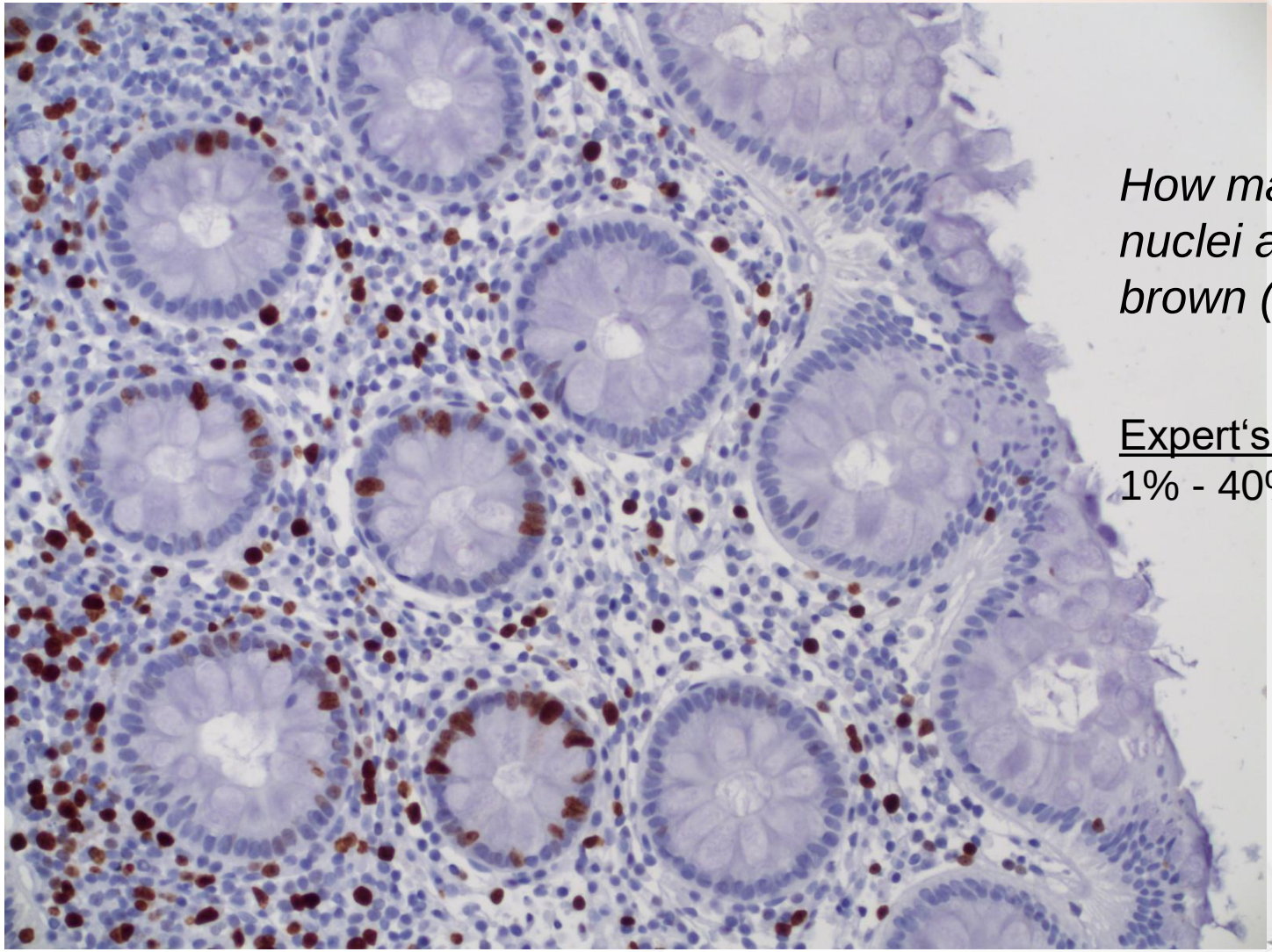
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TISSUE GNOSTICS
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TissueFAXS™ Cytometry

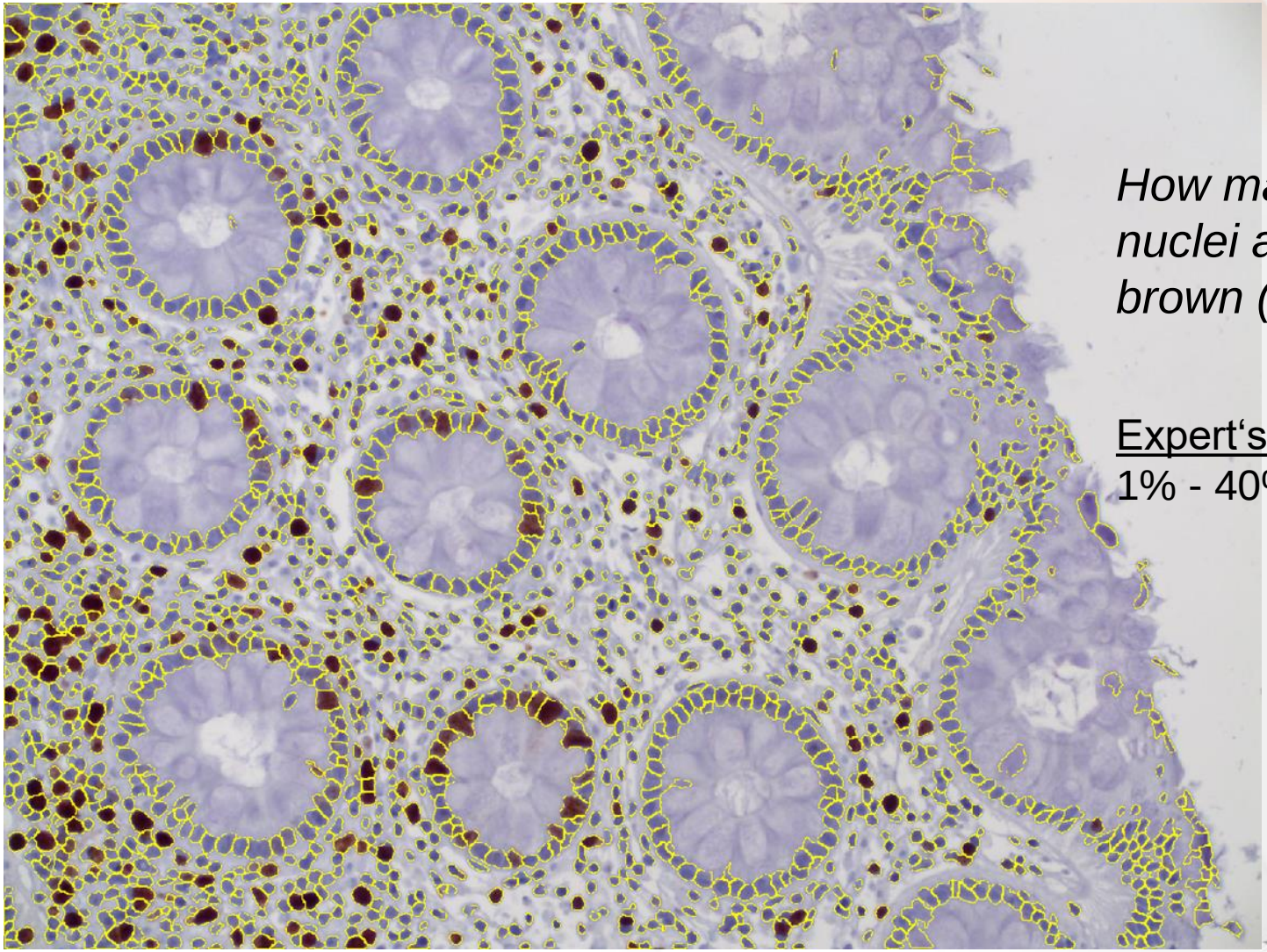


How many of the blue nuclei are also stained in brown (in %)?

<u>Expert‘s estimations:</u>
1% - 40%

TISSUE GNOSTICS
MEDICAL & BIOTECH SOLUTIONS

TissueFAXS™ Cytometry

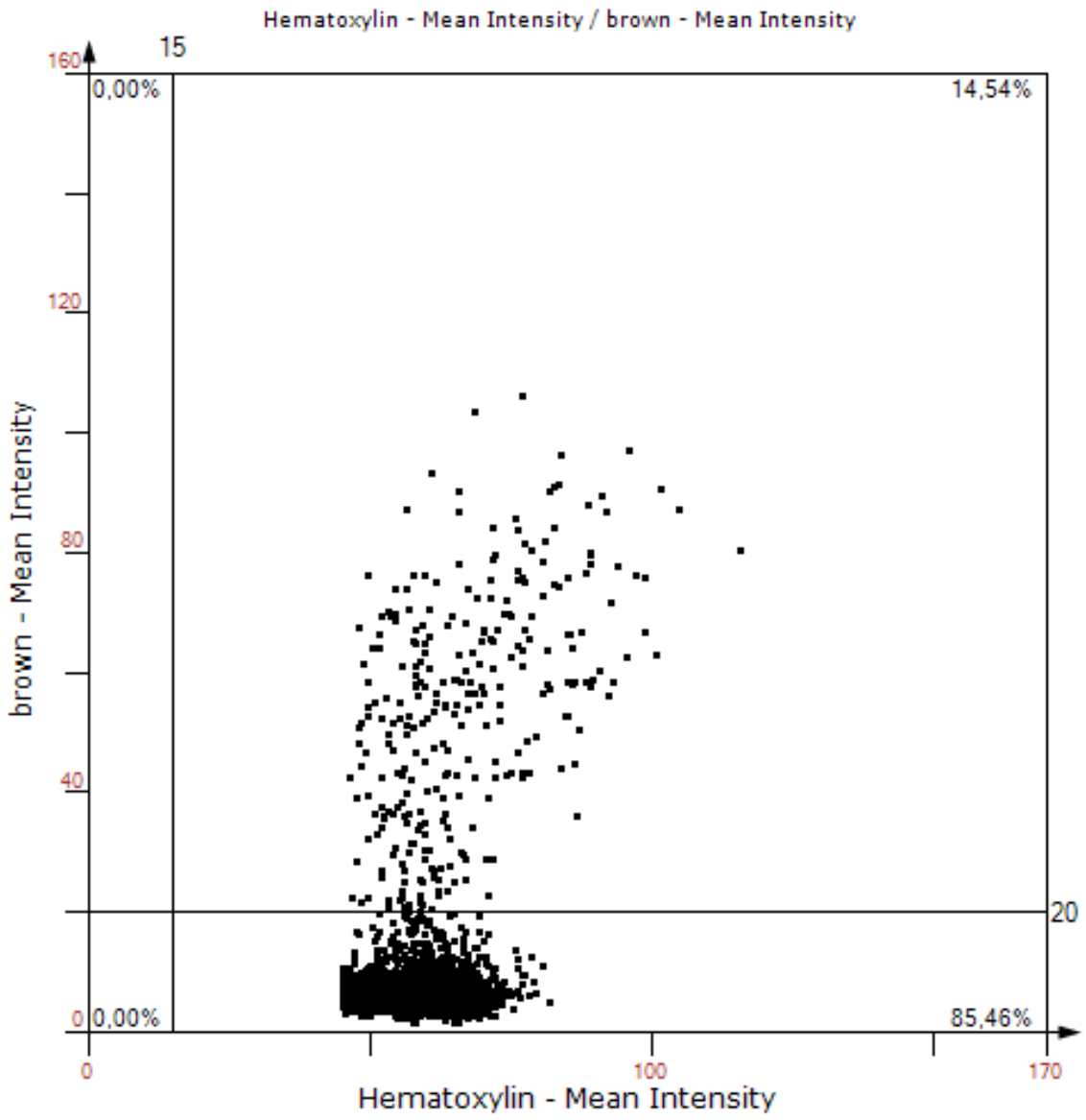


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TISSUE GNOSTICS
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TissueFAXS™ Cytometry



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

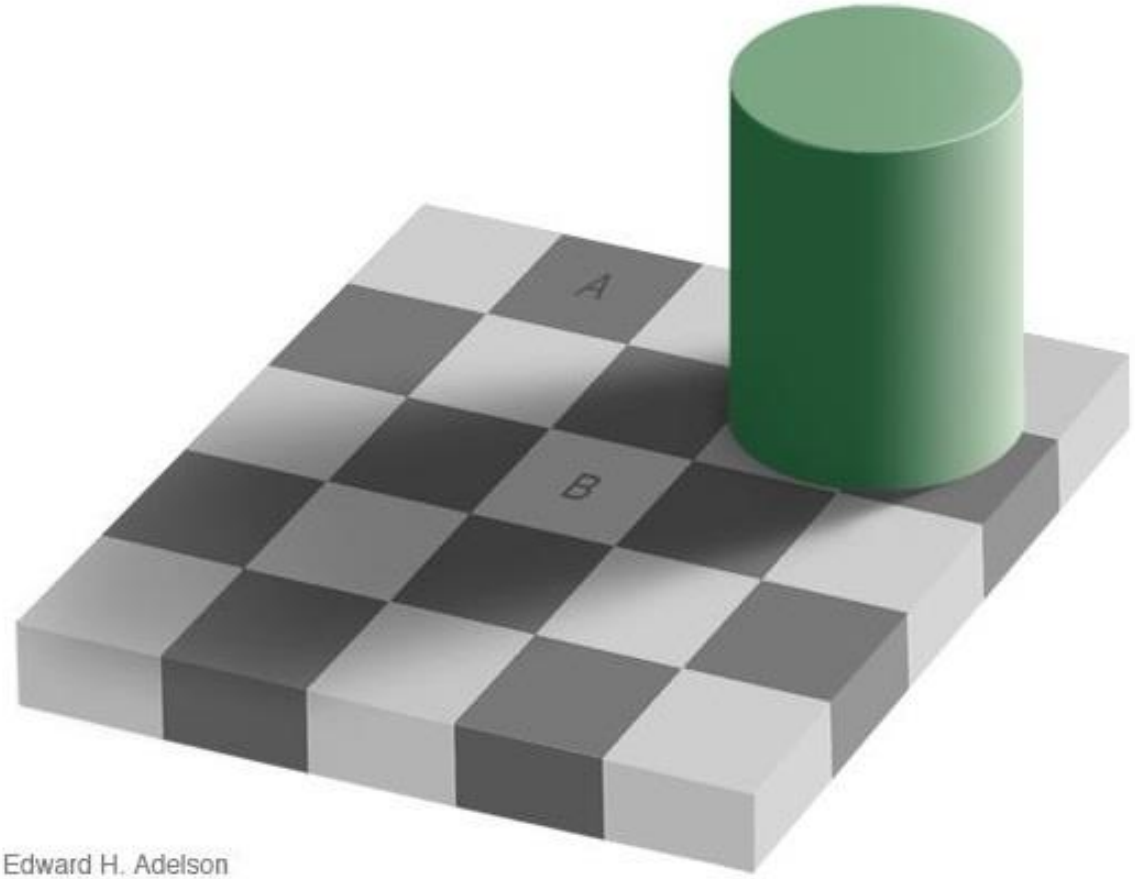
Expert‘s estimations:
1% - 40%

Observer independent measurement: 14,54%

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Estimation versus Measurement

Relative Staining Intensity = gray value(GV)



Edward H. Adelson

GV = 0: BLACK

GV = 255: WHITE

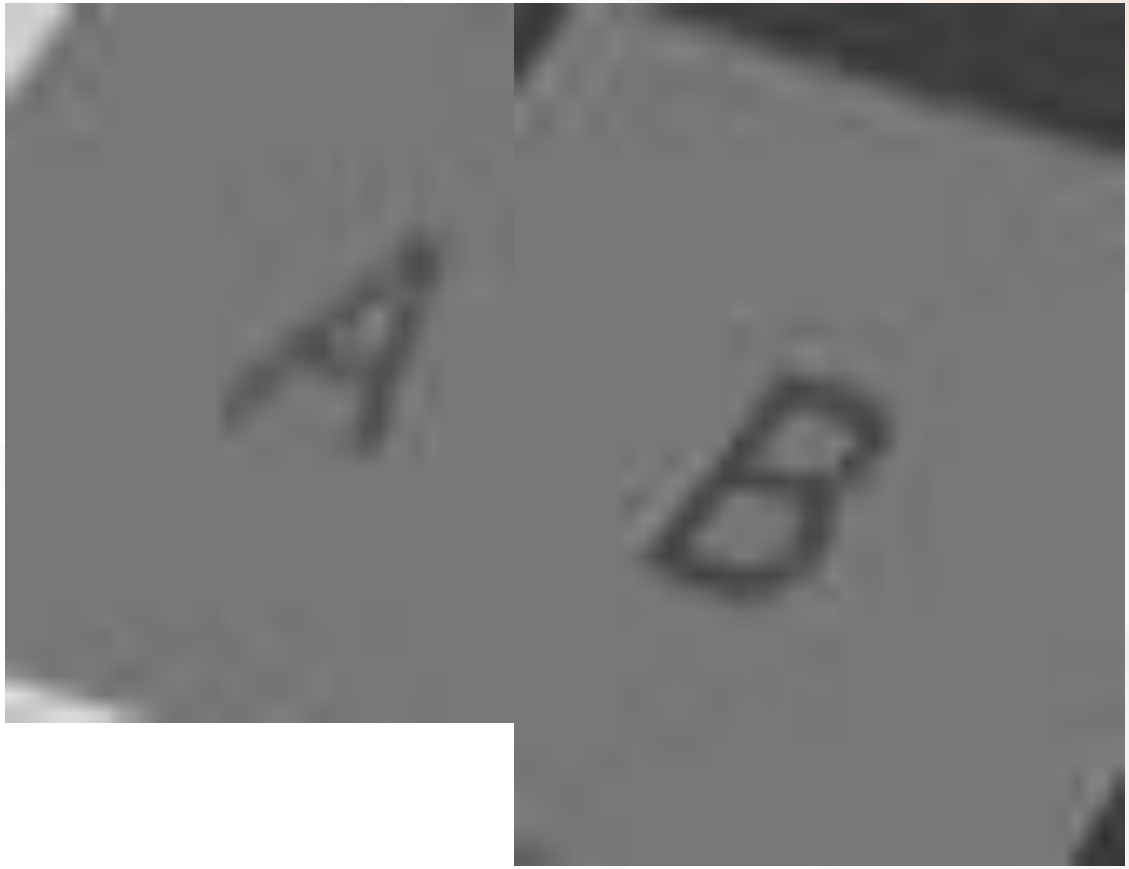
What is the difference in GV between A and B?

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)?

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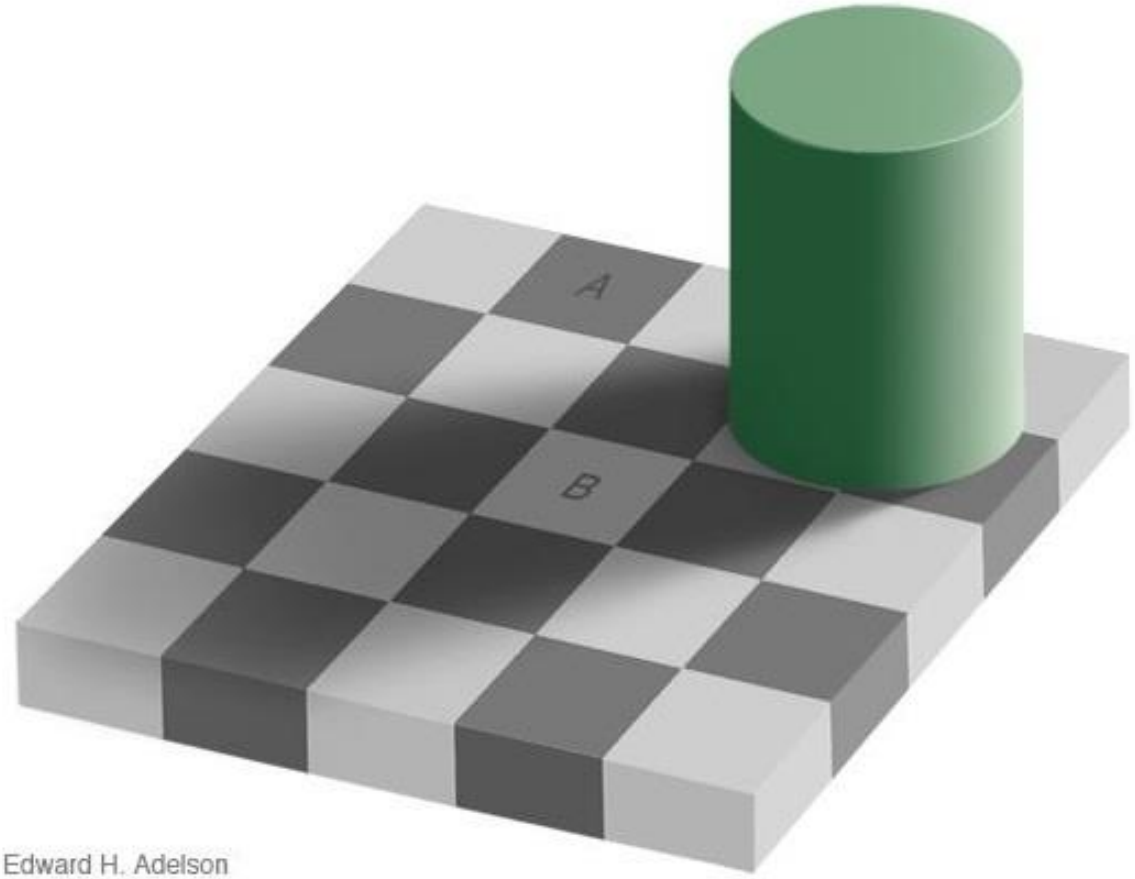
Estimation versus Measurement



$\Delta = 0$!!!!

Estimation versus Measurement

Relative Staining Intensity = gray value(GV)

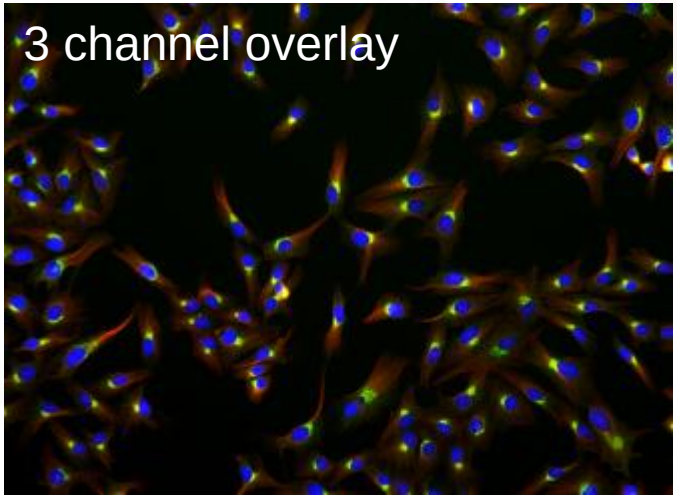


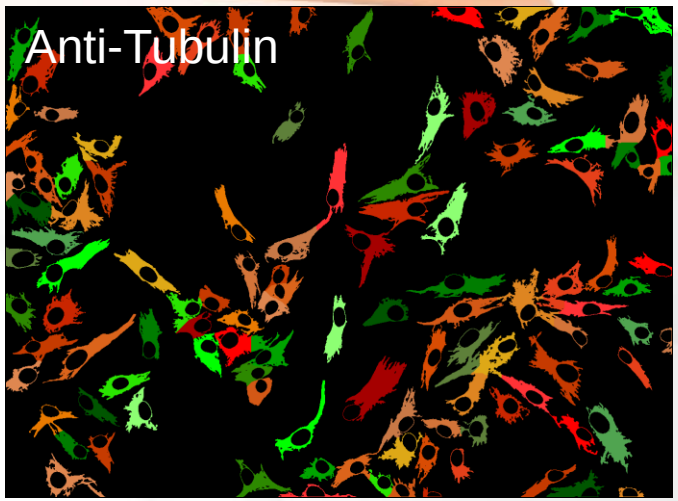
Edward H. Adelson

Assume A and B are 2 samples stained for:

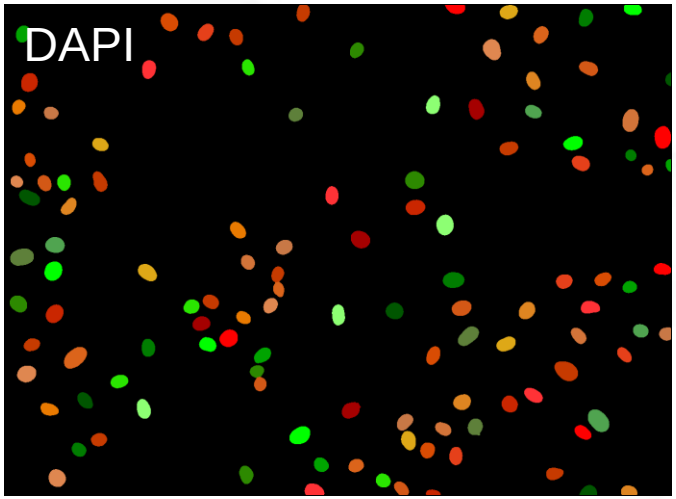
- Tumor marker
- Cytokine expression
- Apoptosis
- Proliferation
- …..

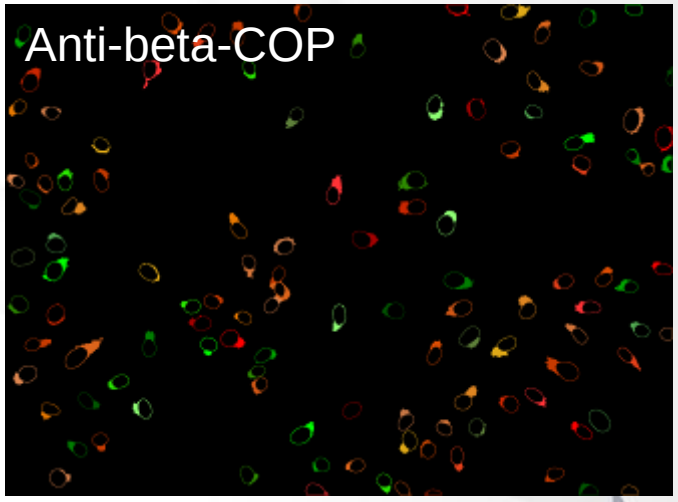
Distinguish between Nucleus and Cytoplasm!





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

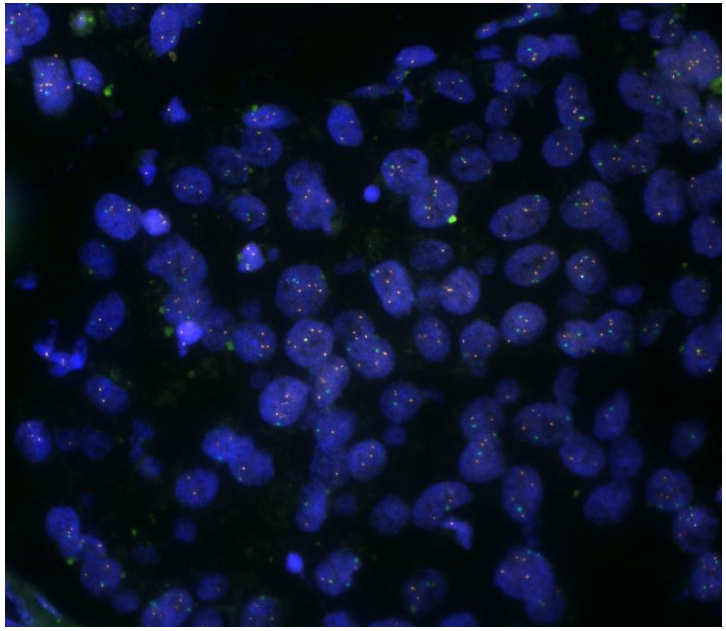


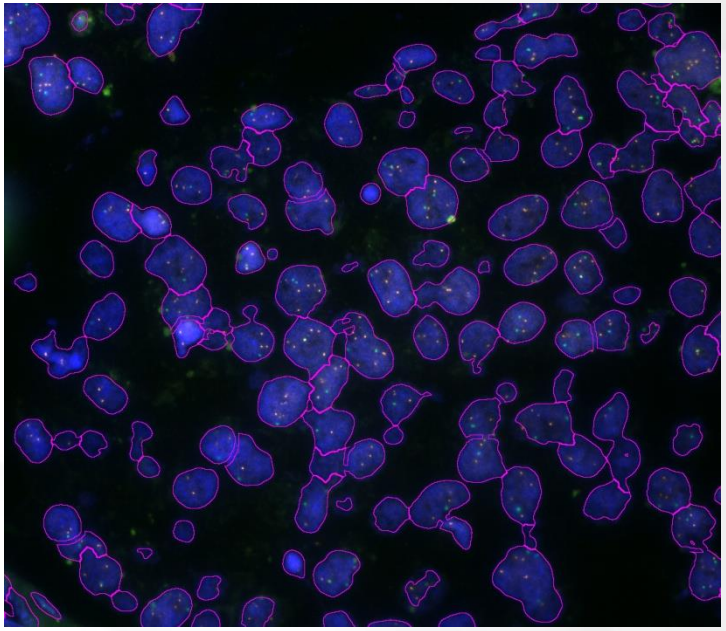


TISSUE GNOSTICS
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Courtesy Dr. R. Pepperkok, Advanced Light Microscopy Facility, EMBL Heidelberg

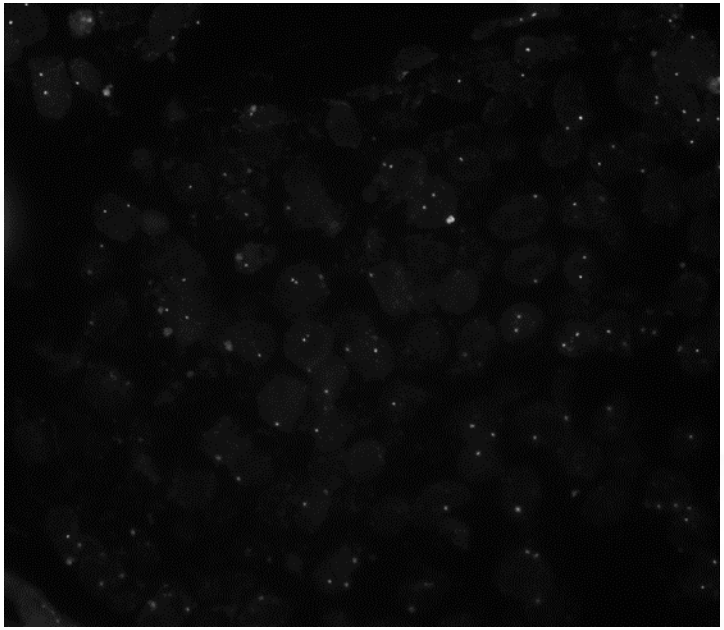
- Nuclear Identification by DAPI

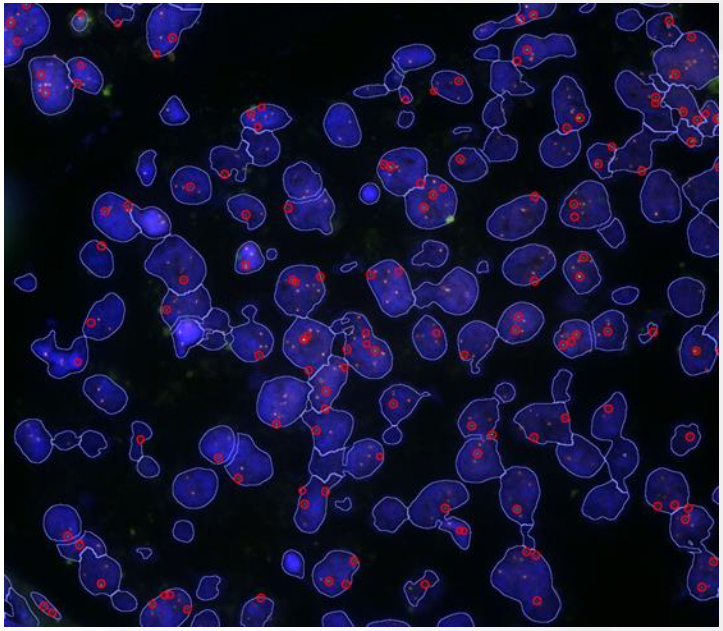




Identifies and quantifies dots optionally in the nucleus or in the cytoplasm

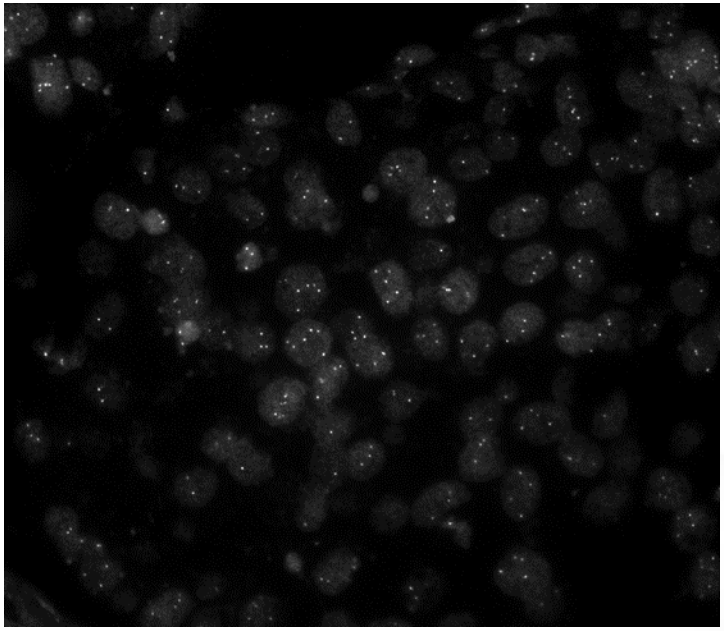
- Green Dots Count

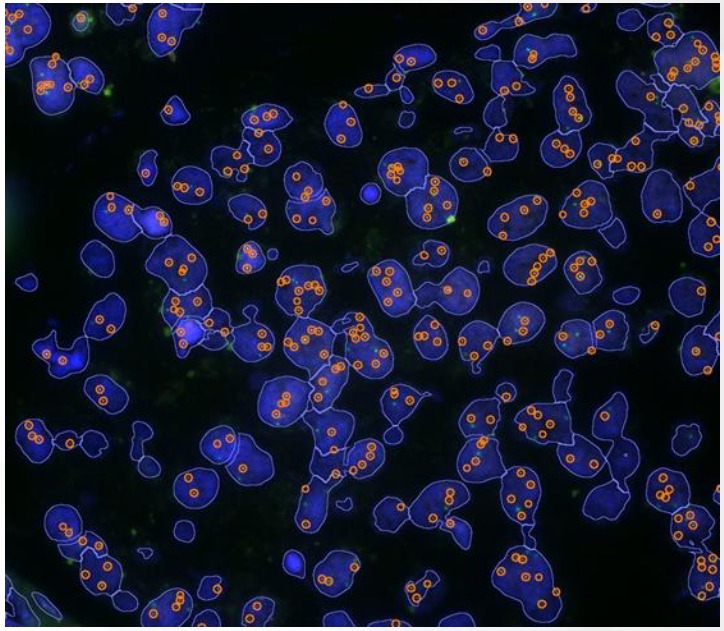




Identifies and quantifies dots optionally in the nucleus or in the cytoplasm

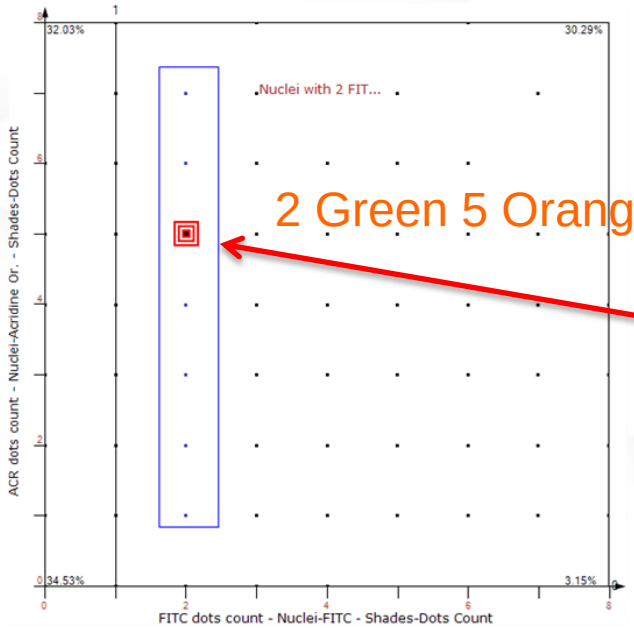
- Orange Dots Count

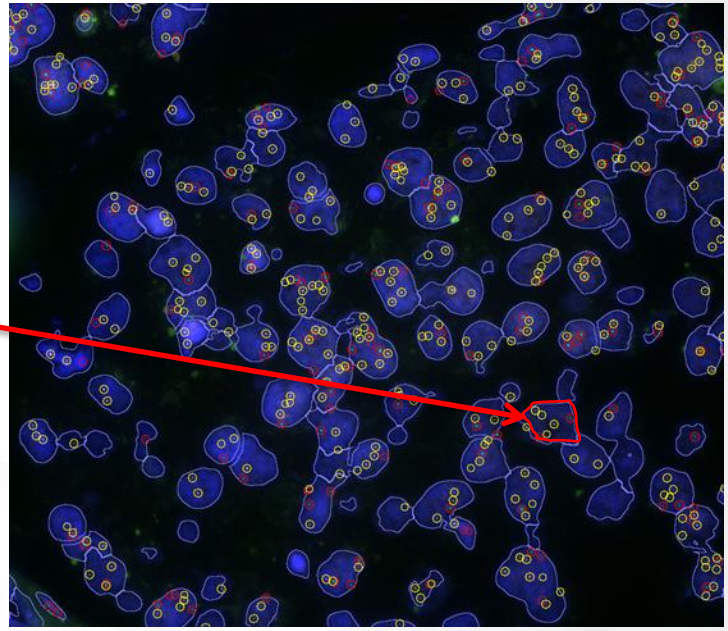




Identifies and quantifies dots optionally in the nucleus or in the cytoplasm

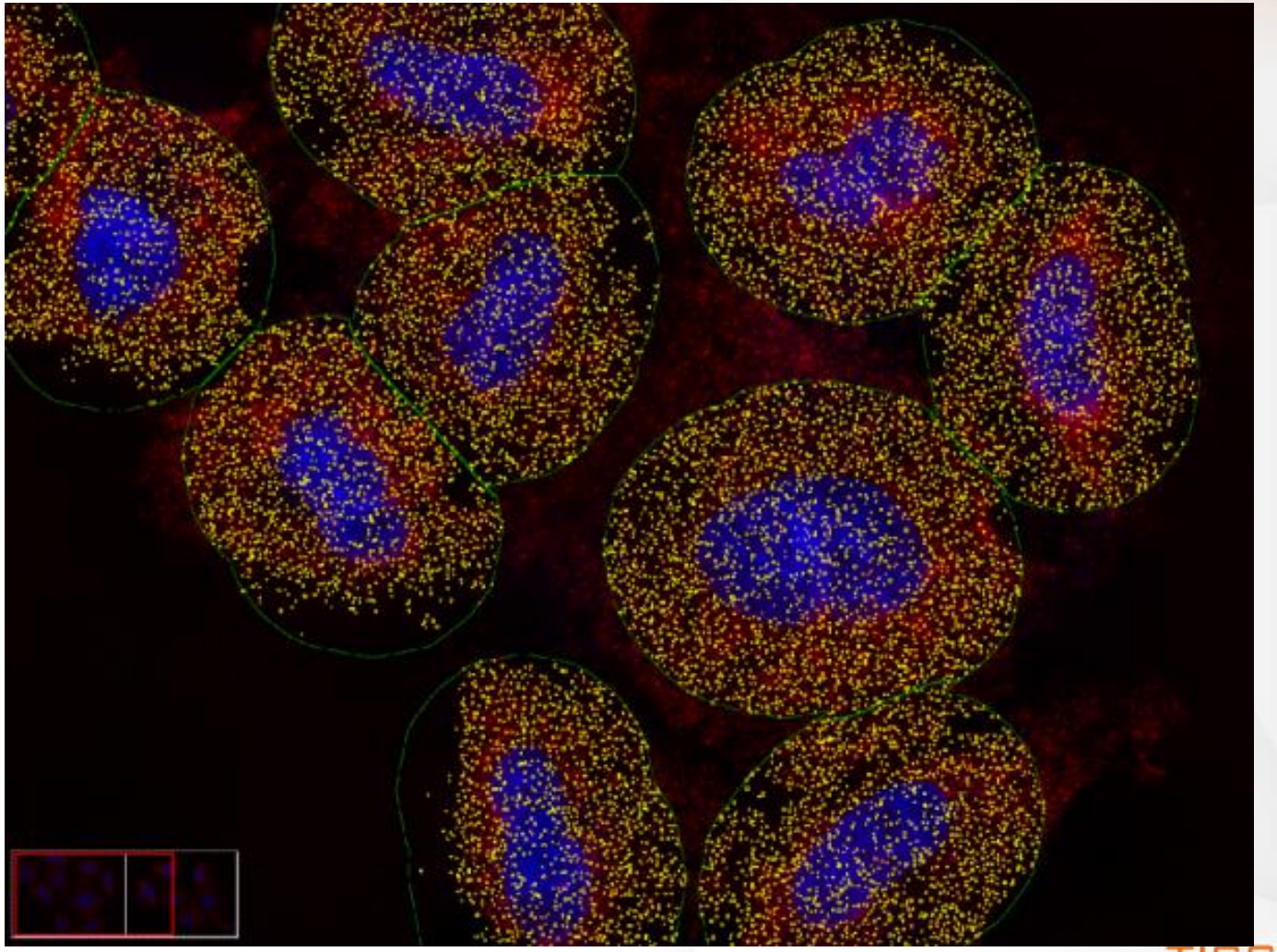
- Green dots and Orange dots finding results





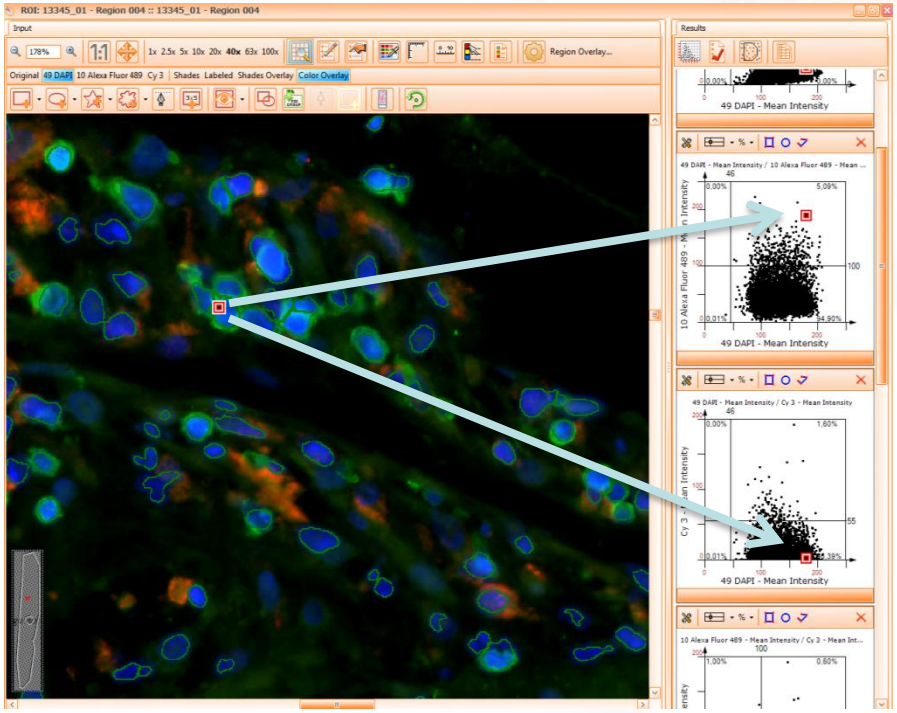
Identifies and quantifies dots optionally in the nucleus or in the cytoplasm

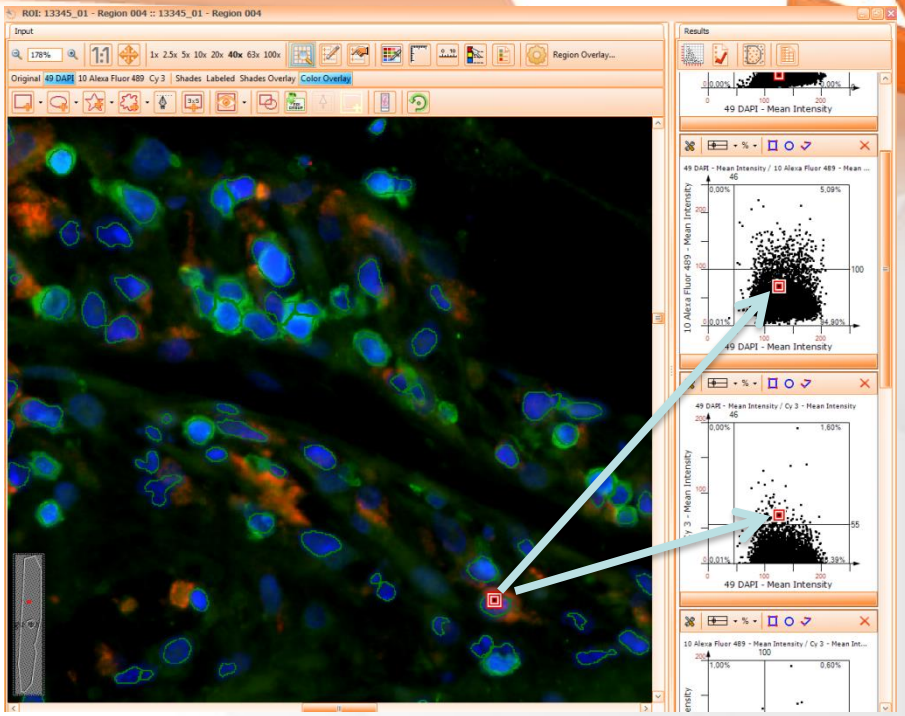
Example of detection of all dots from the image with all z-levels merged, within a given range (“ring mask”) from the automatically detected nuclear border



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

Forward Connection



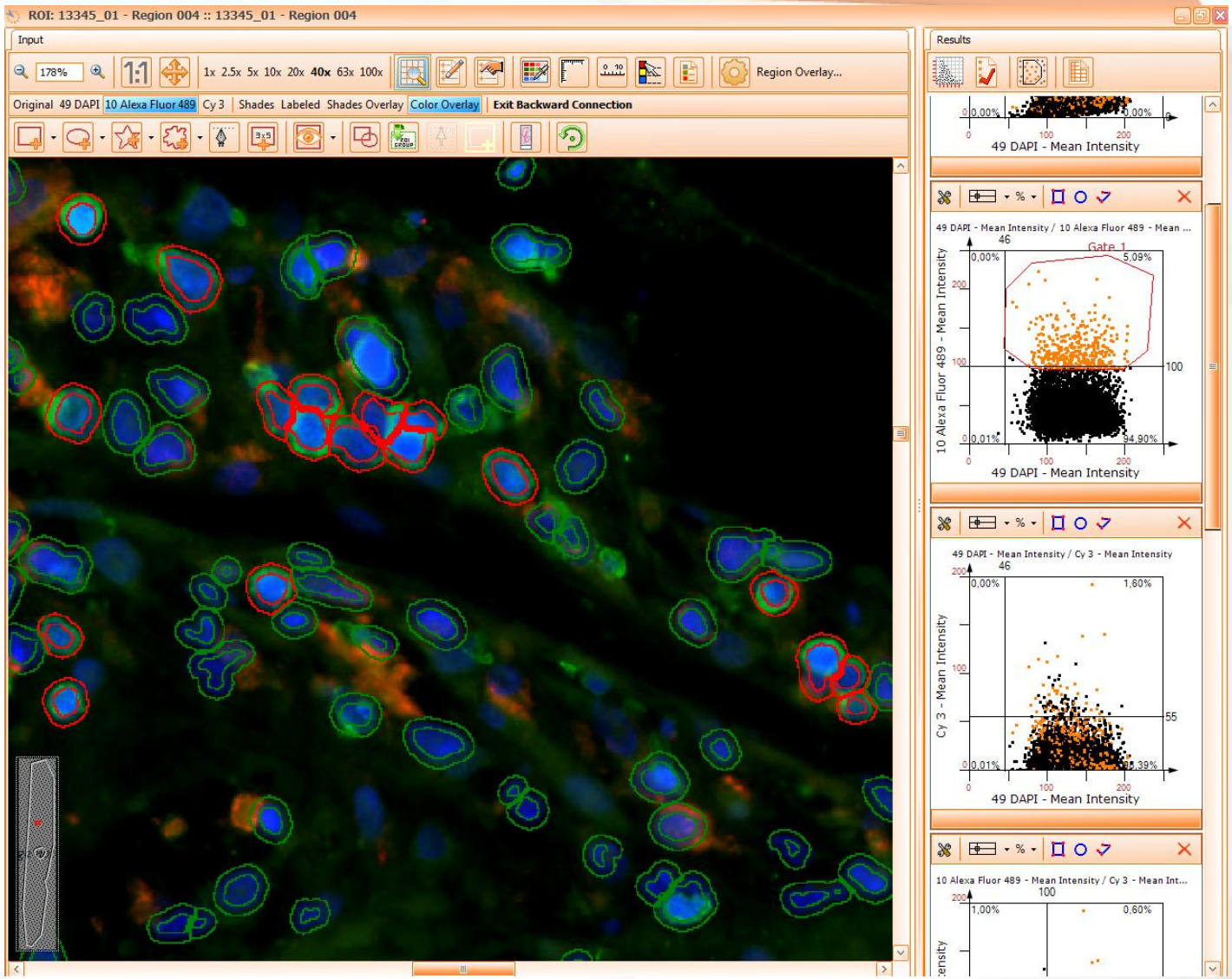


GREEN positive
RED negative

GREEN negative
RED positive

TISSUE GNOSTICS
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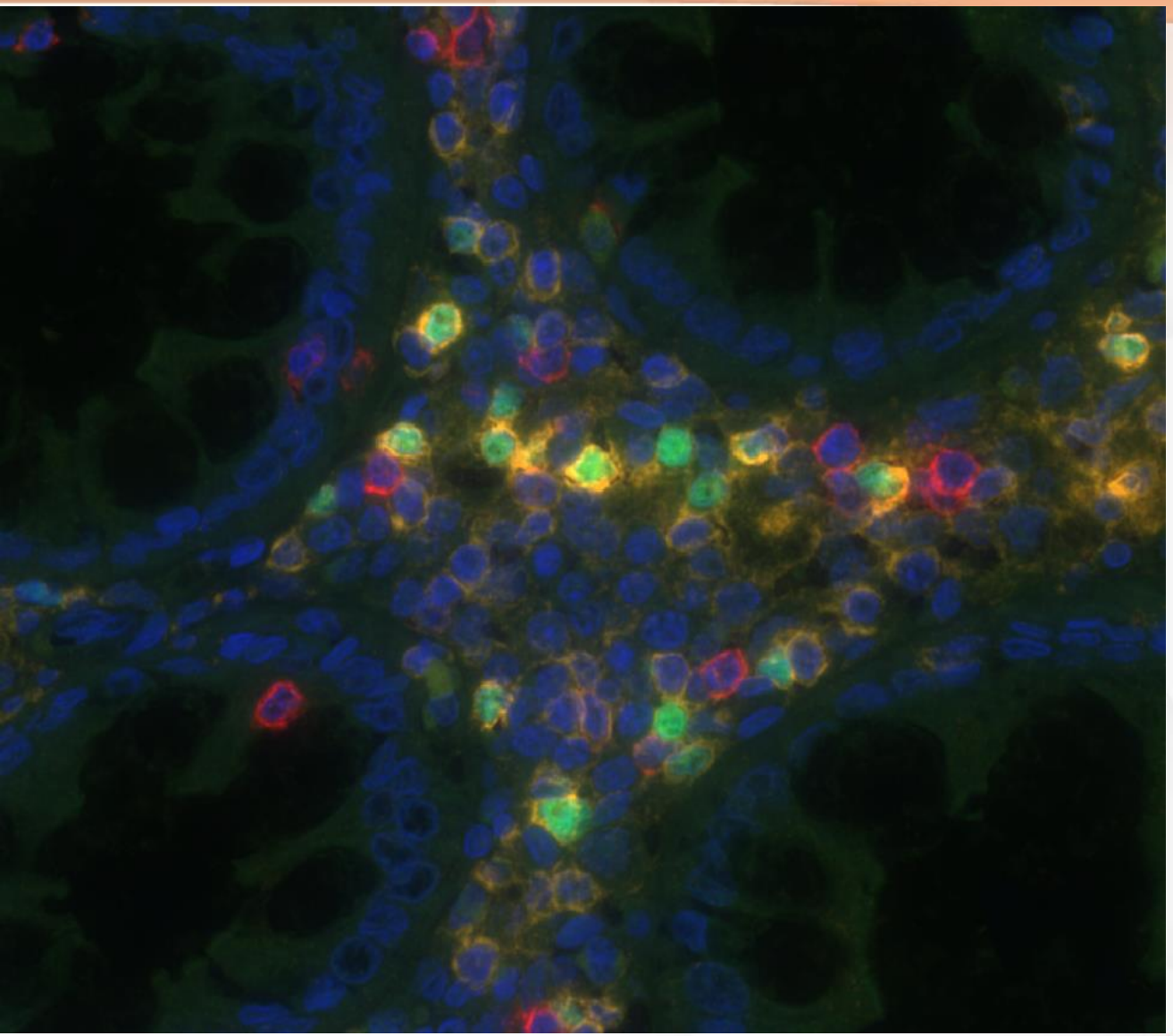
Backward Connection



Cells with red contours belong to the highlighted Gate.

TISSUE GNOSTICS
MEDICAL & BIOTECH SOLUTIONS

Phenotype of Tissue Infiltrating Leukocytes



DAPI

CD4

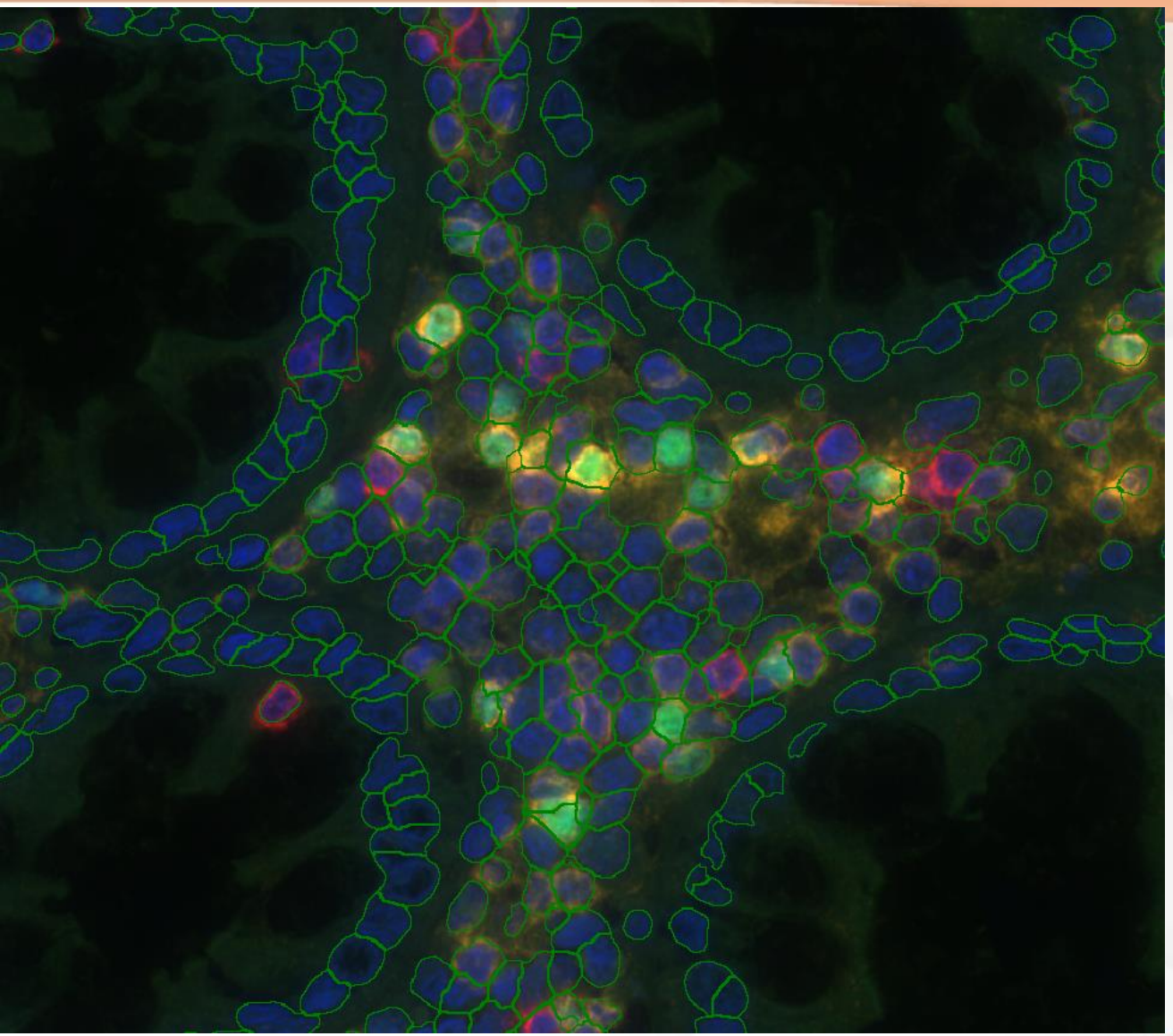
CD8

Foxp3

TISSUE GNOSTICS
MEDICAL & BIOTECH SOLUTIONS

Courtesy Dr. Melanie McCoy, University of Western Australia, Perth

Phenotype of Tissue Infiltrating Leukocytes



DAPI

CD4

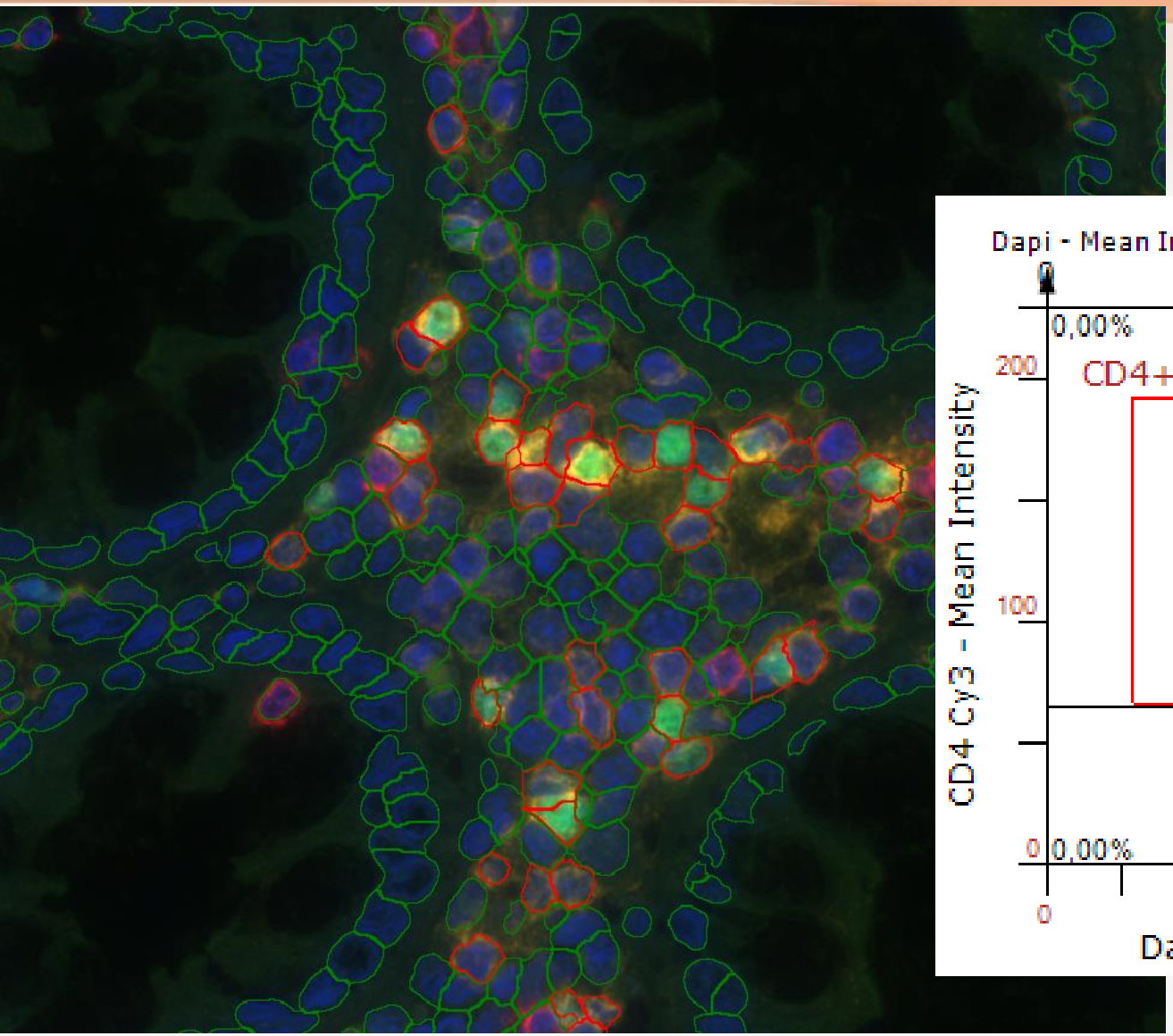
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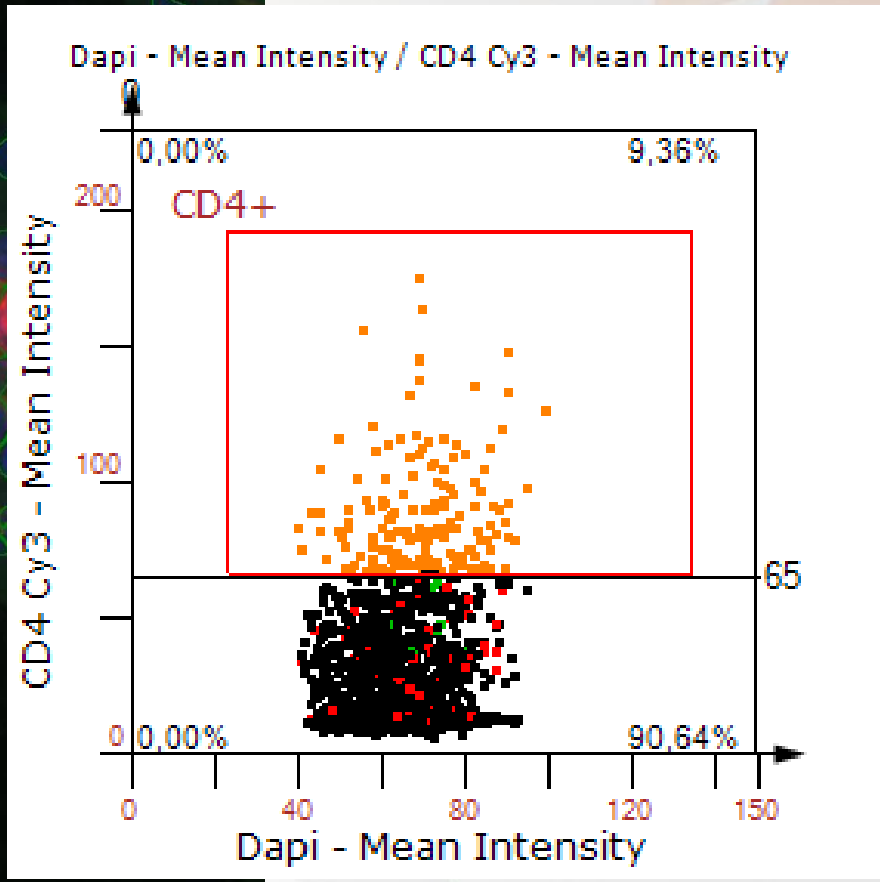
Foxp3

TISSUE GNOSTICS
MEDICAL & BIOTECH SOLUTIONS

Courtesy Dr. Melanie McCoy, University of Western Australia, Perth

Phenotype of Tissue Infiltrating Leukocytes

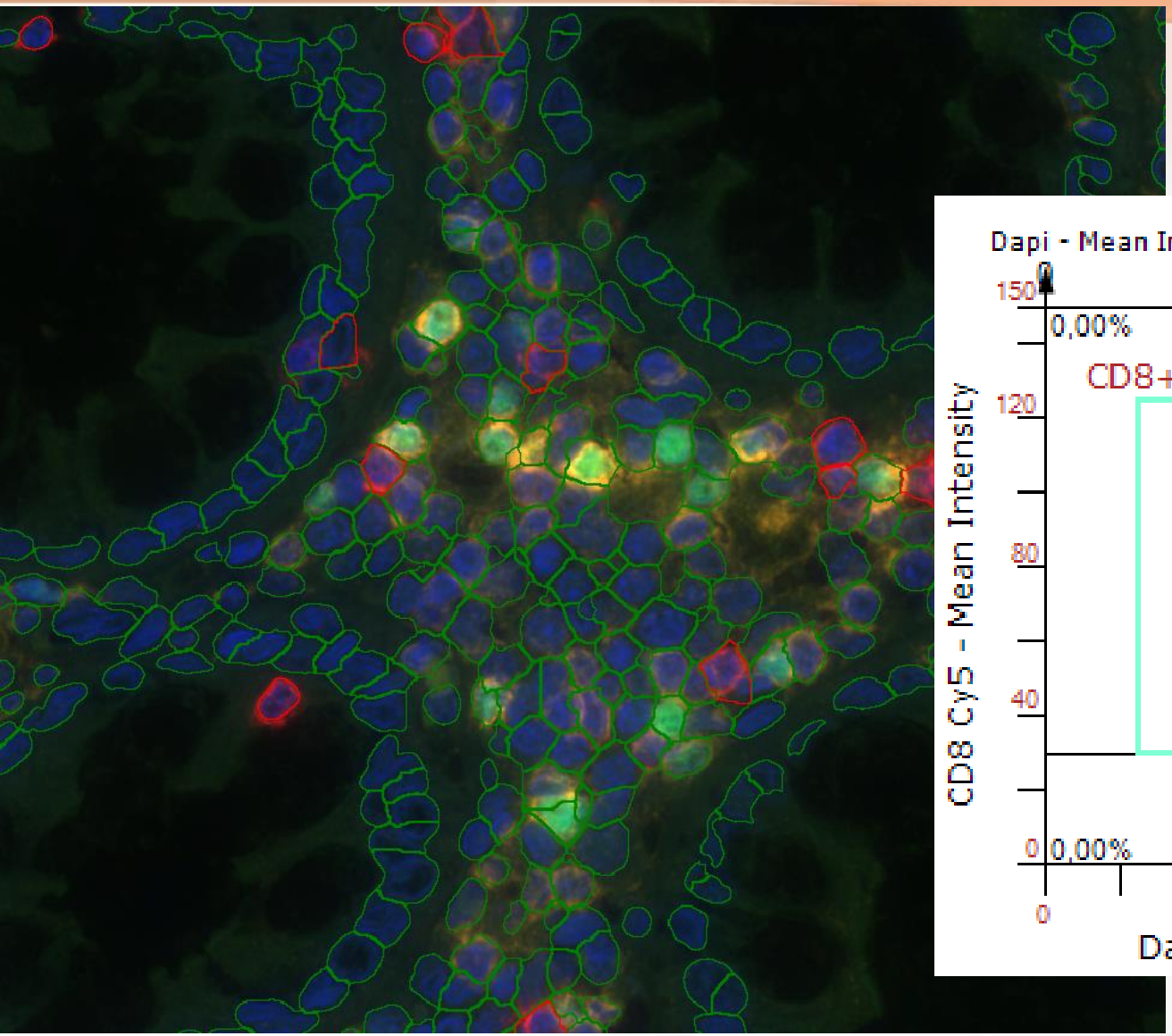


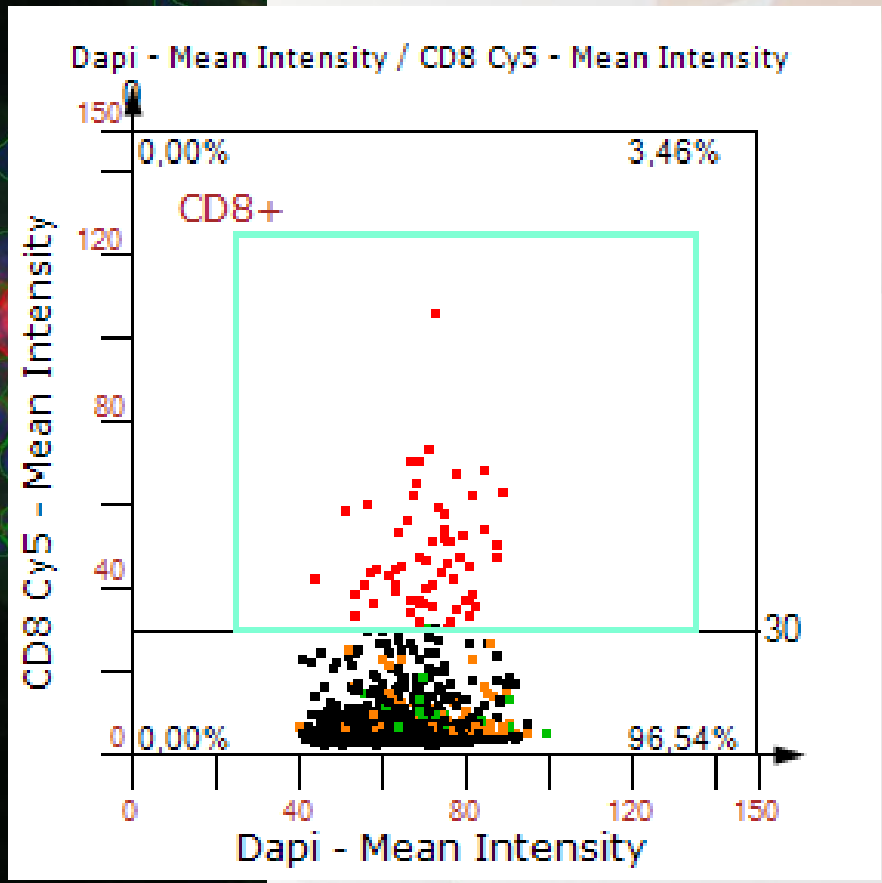


TISSUE GNOSTICS
MEDICAL & BIOTECH SOLUTIONS

Courtesy Dr. Melanie McCoy, University of Western Australia, Perth

Phenotype of Tissue Infiltrating Leukocytes

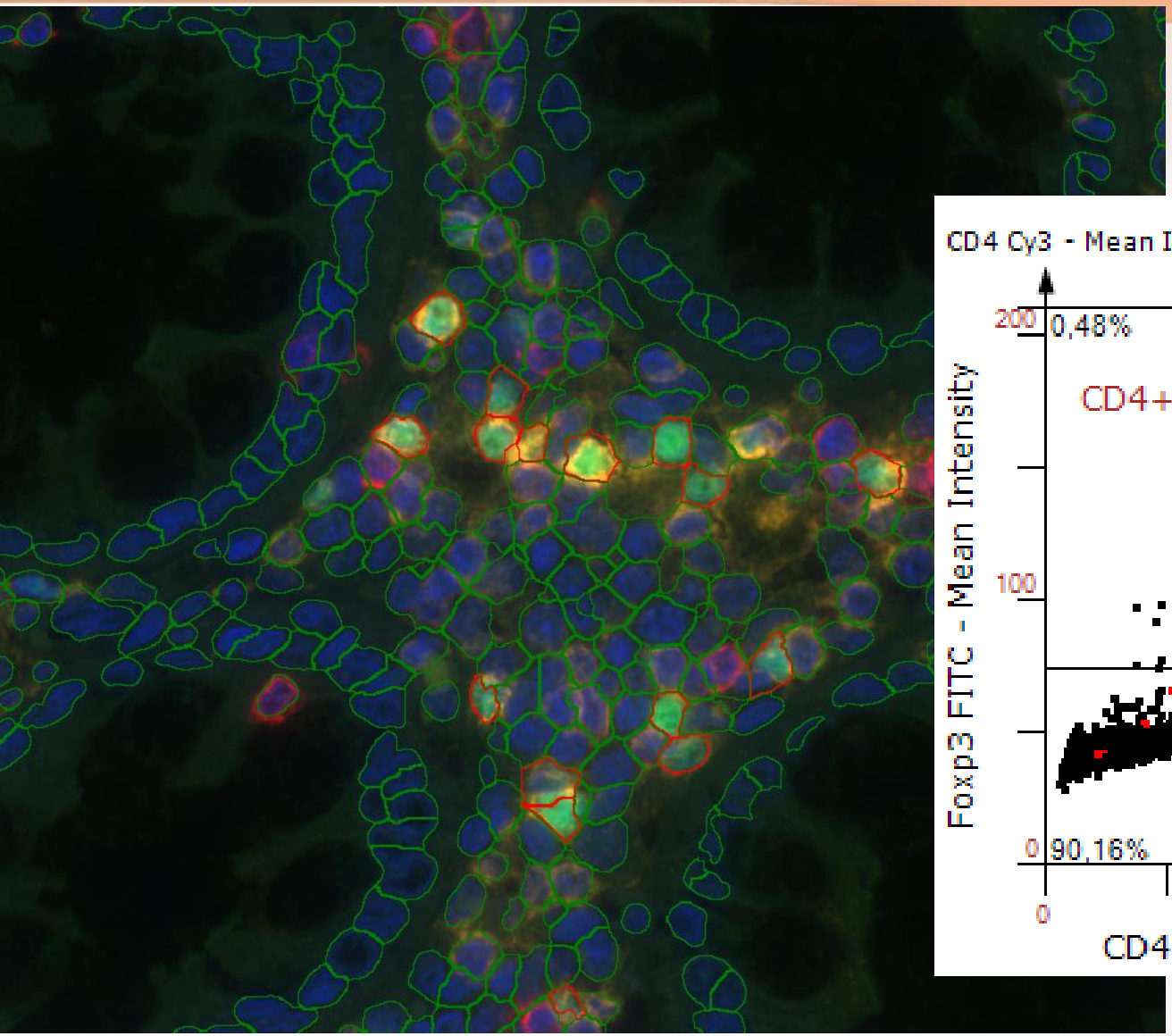


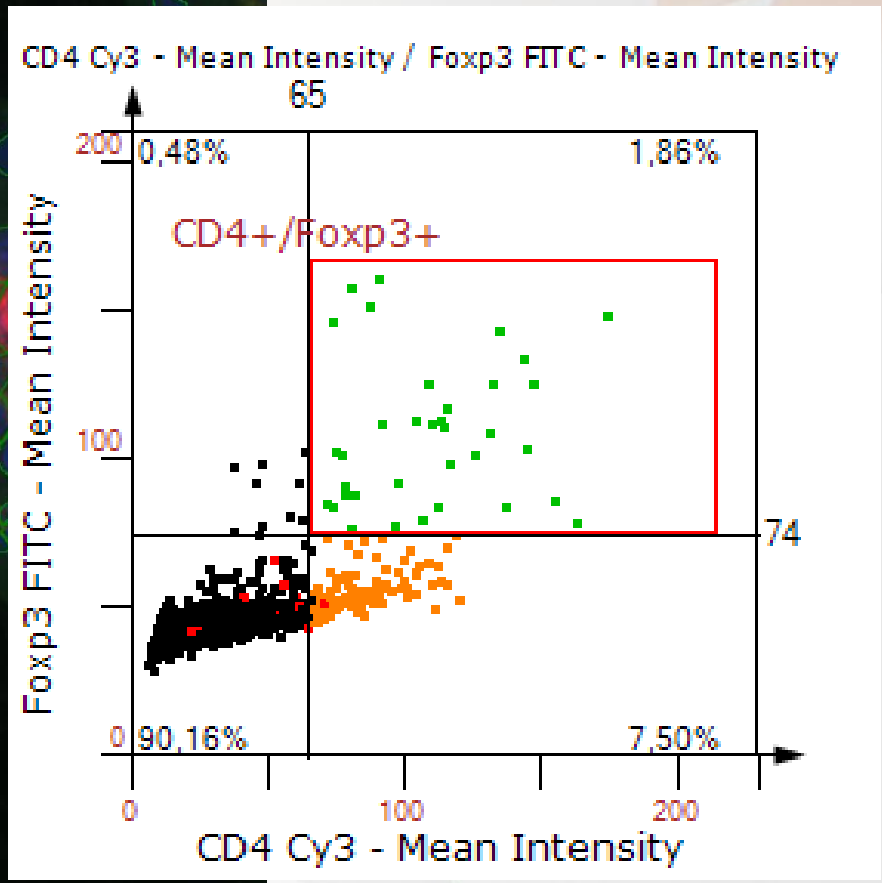


TISSUE GNOSTICS
MEDICAL & BIOTECH SOLUTIONS

Courtesy Dr. Melanie McCoy, University of Western Australia, Perth

Phenotype of Tissue Infiltrating Leukocytes



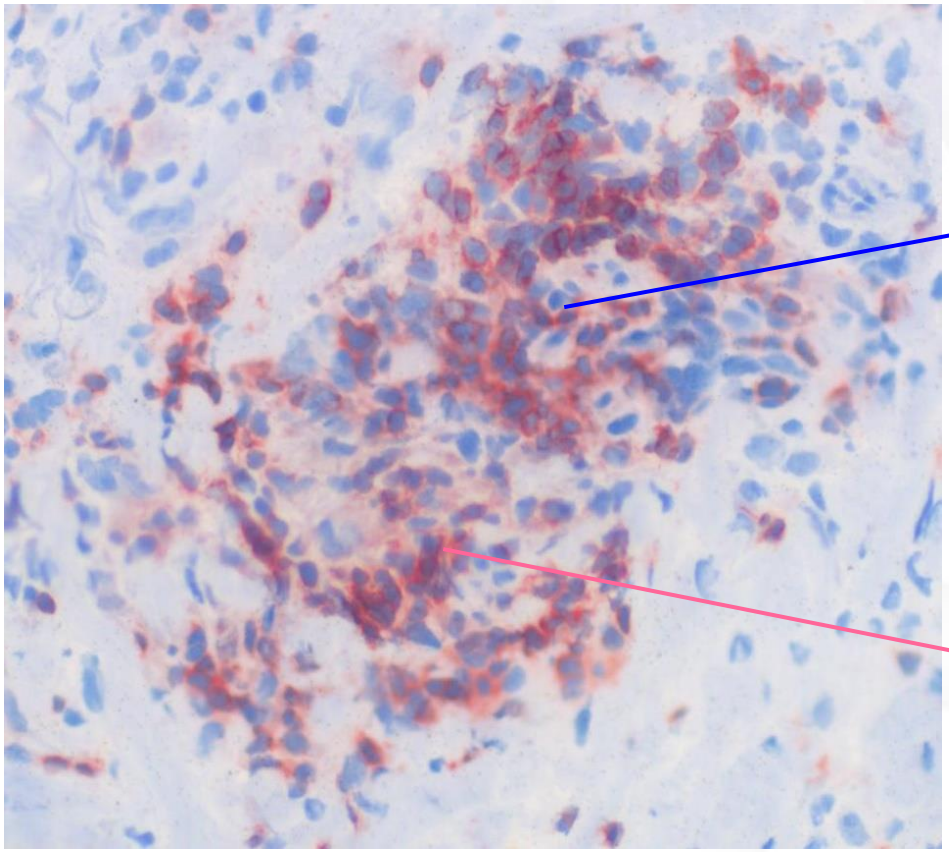


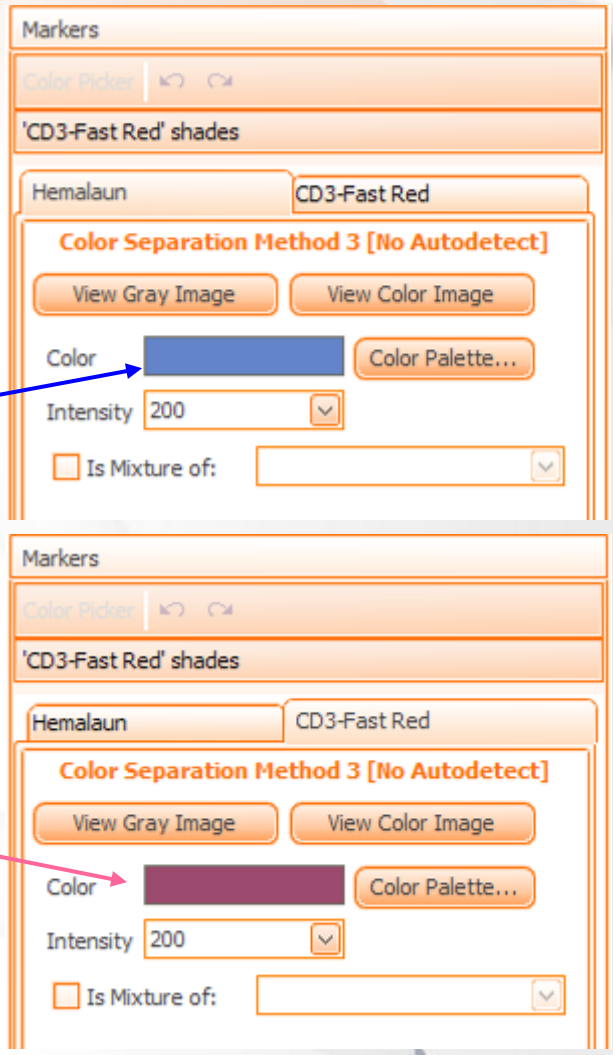
TISSUE GNOSTICS
MEDICAL & BIOTECH SOLUTIONS

Courtesy Dr. Melanie McCoy, University of Western Australia, Perth

HistoFAXS: the system for immunohistochemistry

Original image

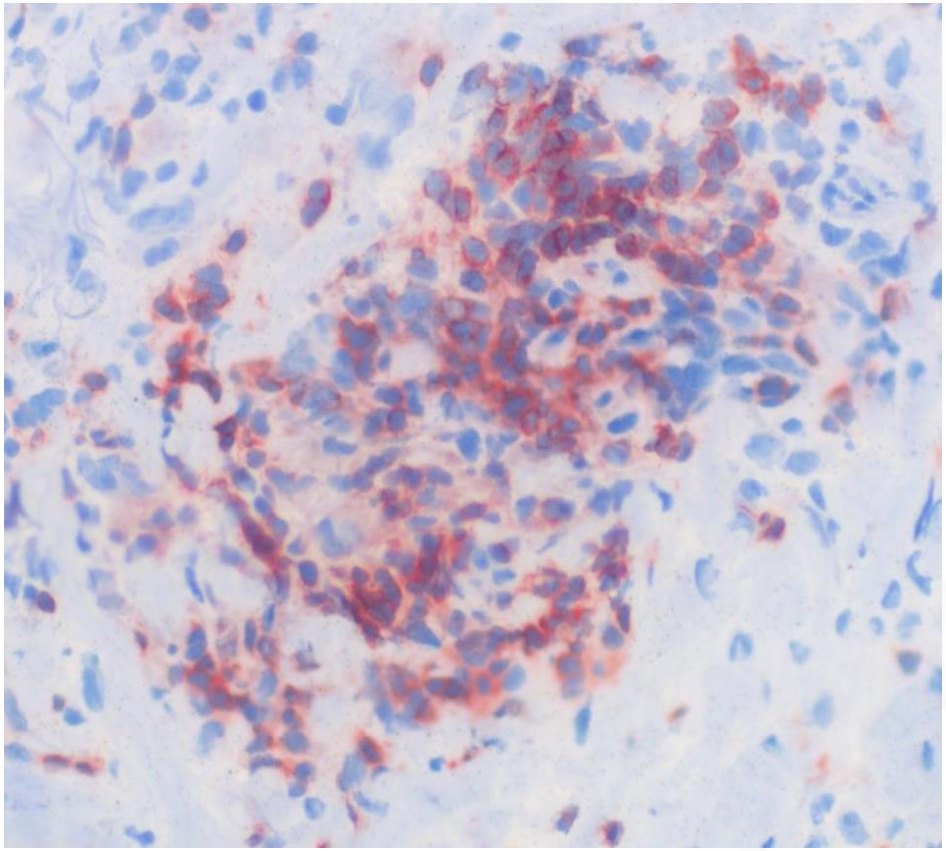




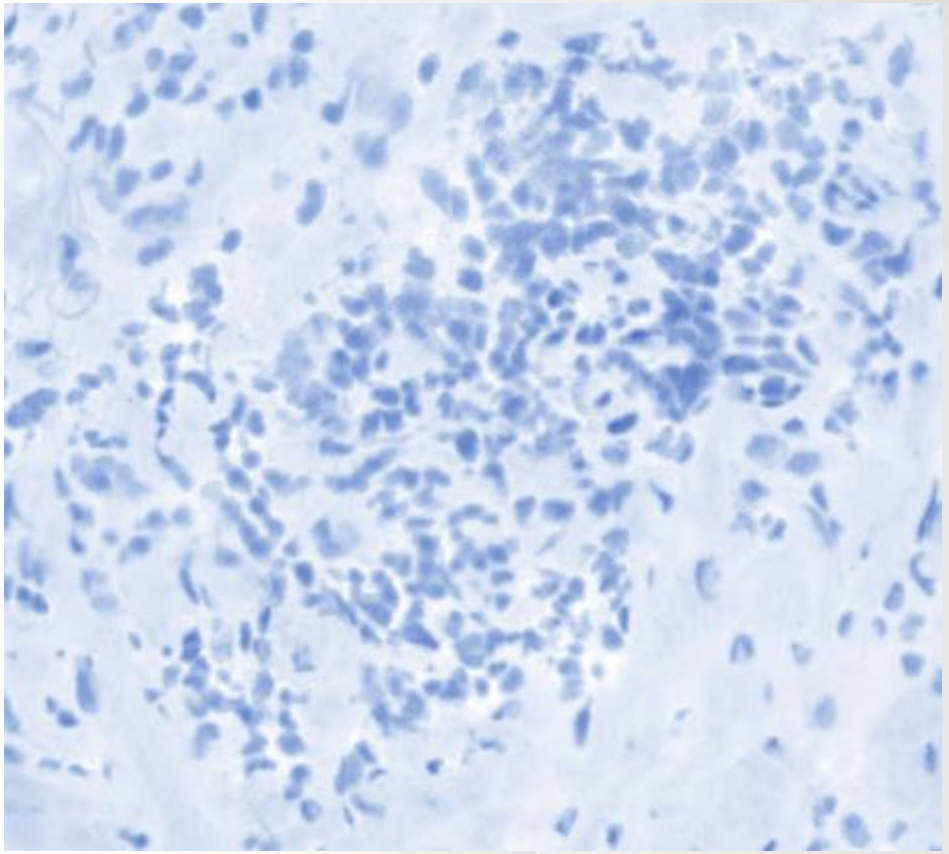
TISSUE GNOSTICS
MEDICAL & BIOTECH SOLUTIONS

HistoFAXS: the system for immunohistochemistry

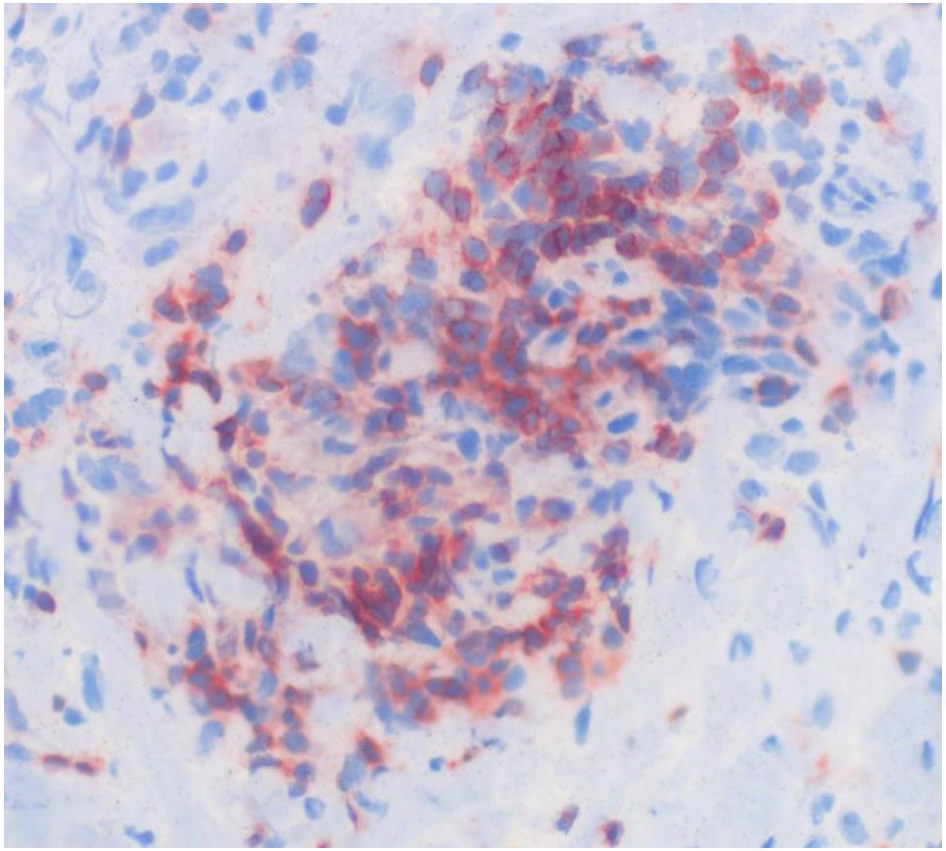
Original image



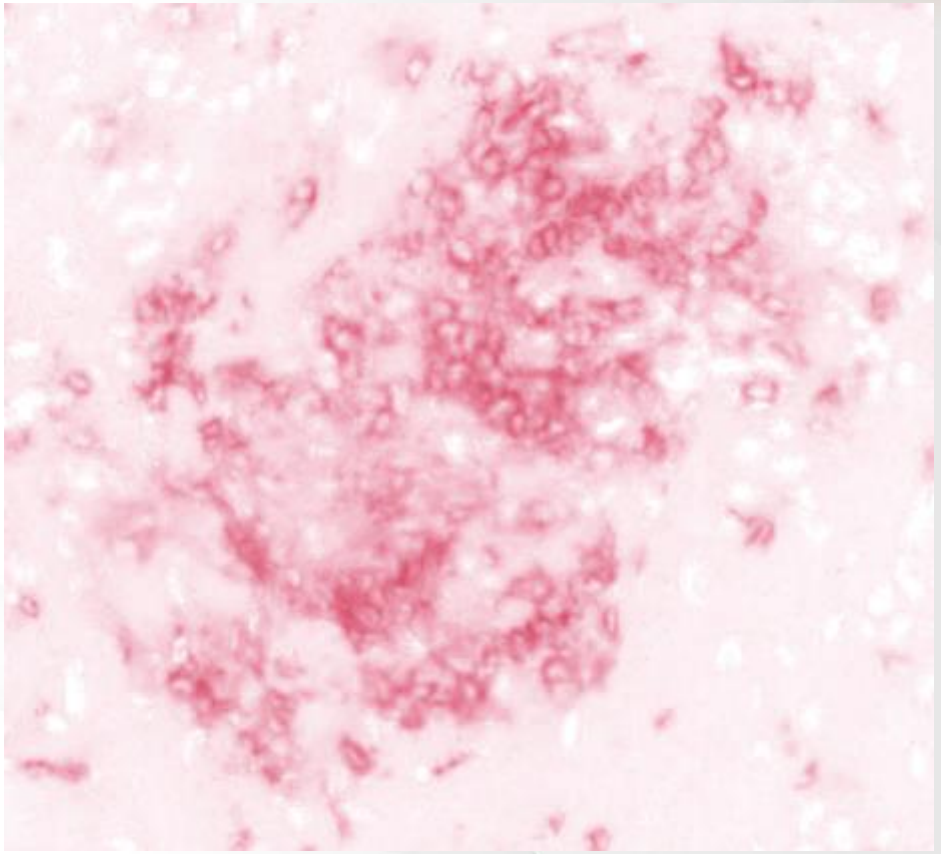
The blue channel generated by color separation algorithm



Original image

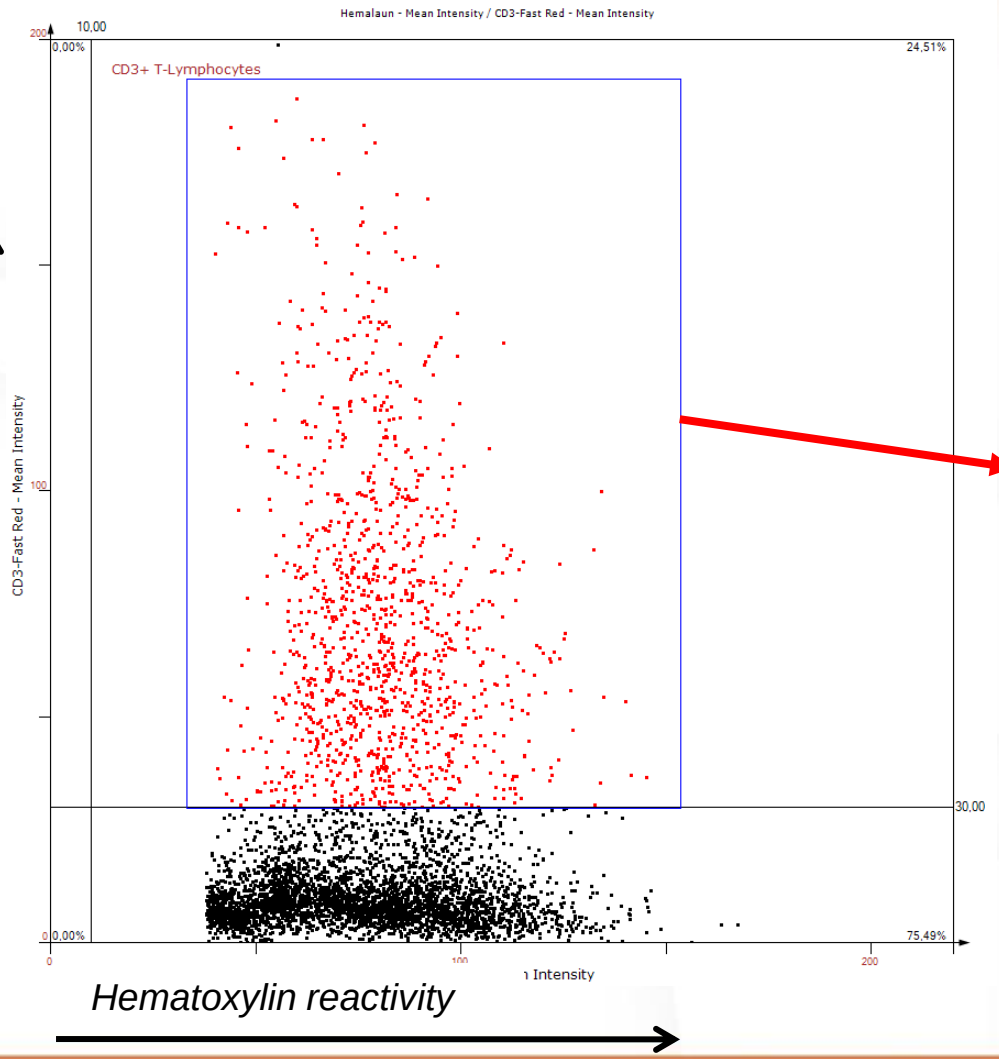


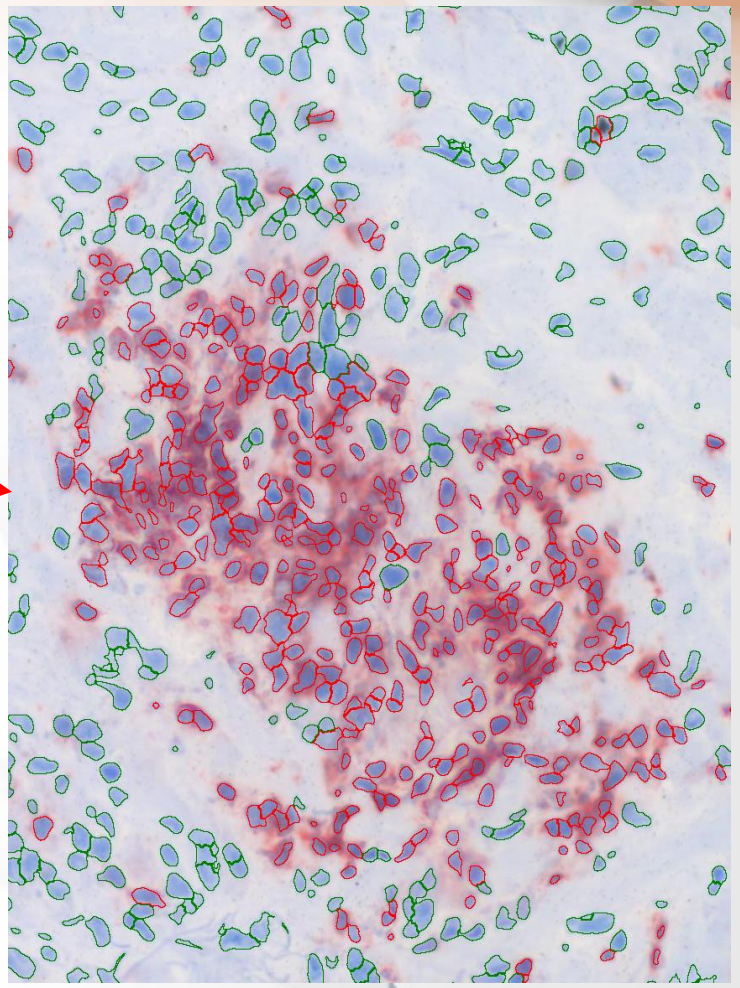
The red channel generated by color separation algorithm



HistoFAXS: Backward Connection

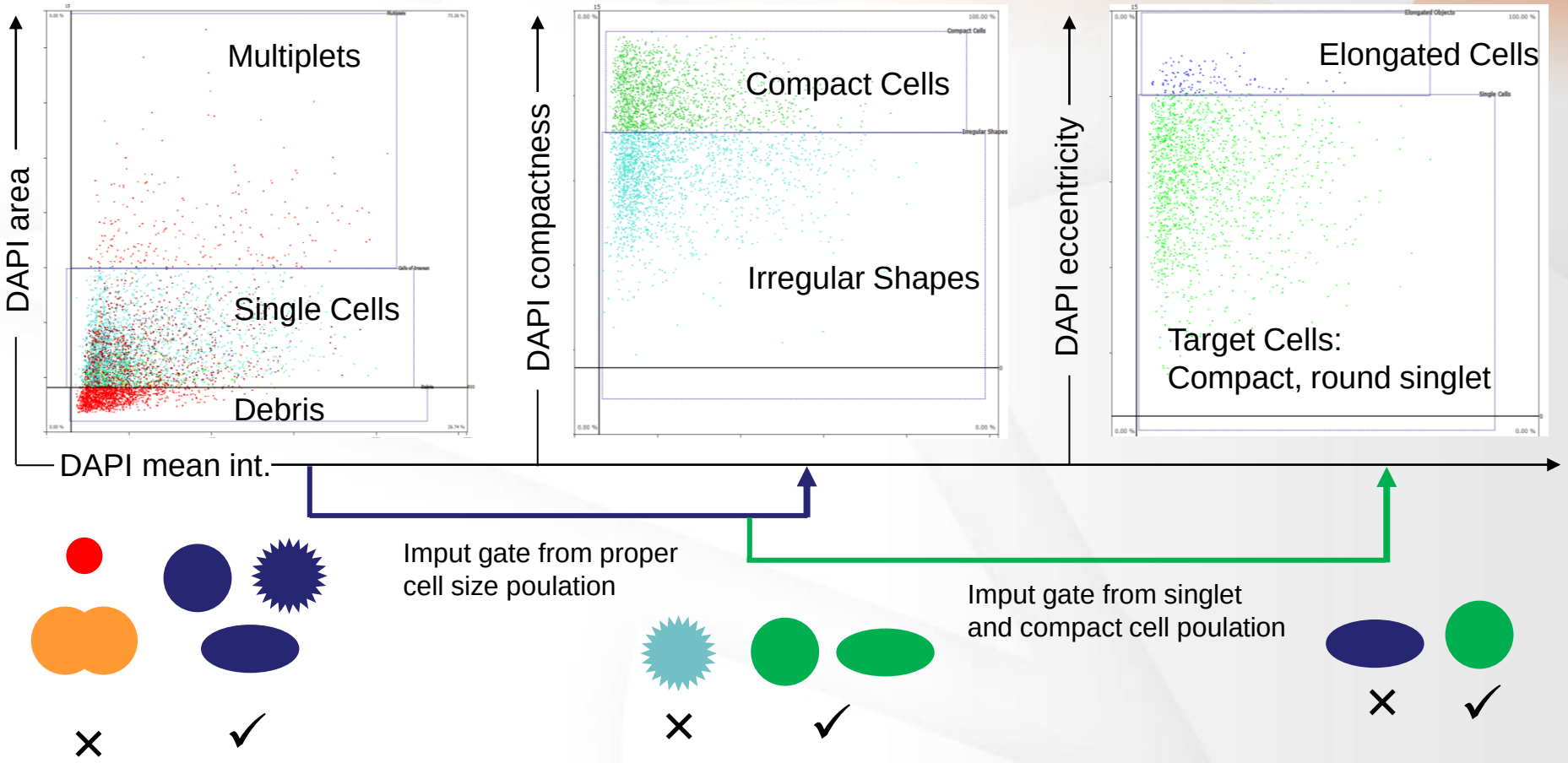
All dots within the Gate have a red contour in the source images





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TissueGnostics provide a solution for image analysis equivalent to Flow cytometry !



TISSUE GNOSTICS
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Courtesy Prof. Arvydas Laurinavicius, National Center of Pathology, Lithuania

Measurement of Extracellular Matrix

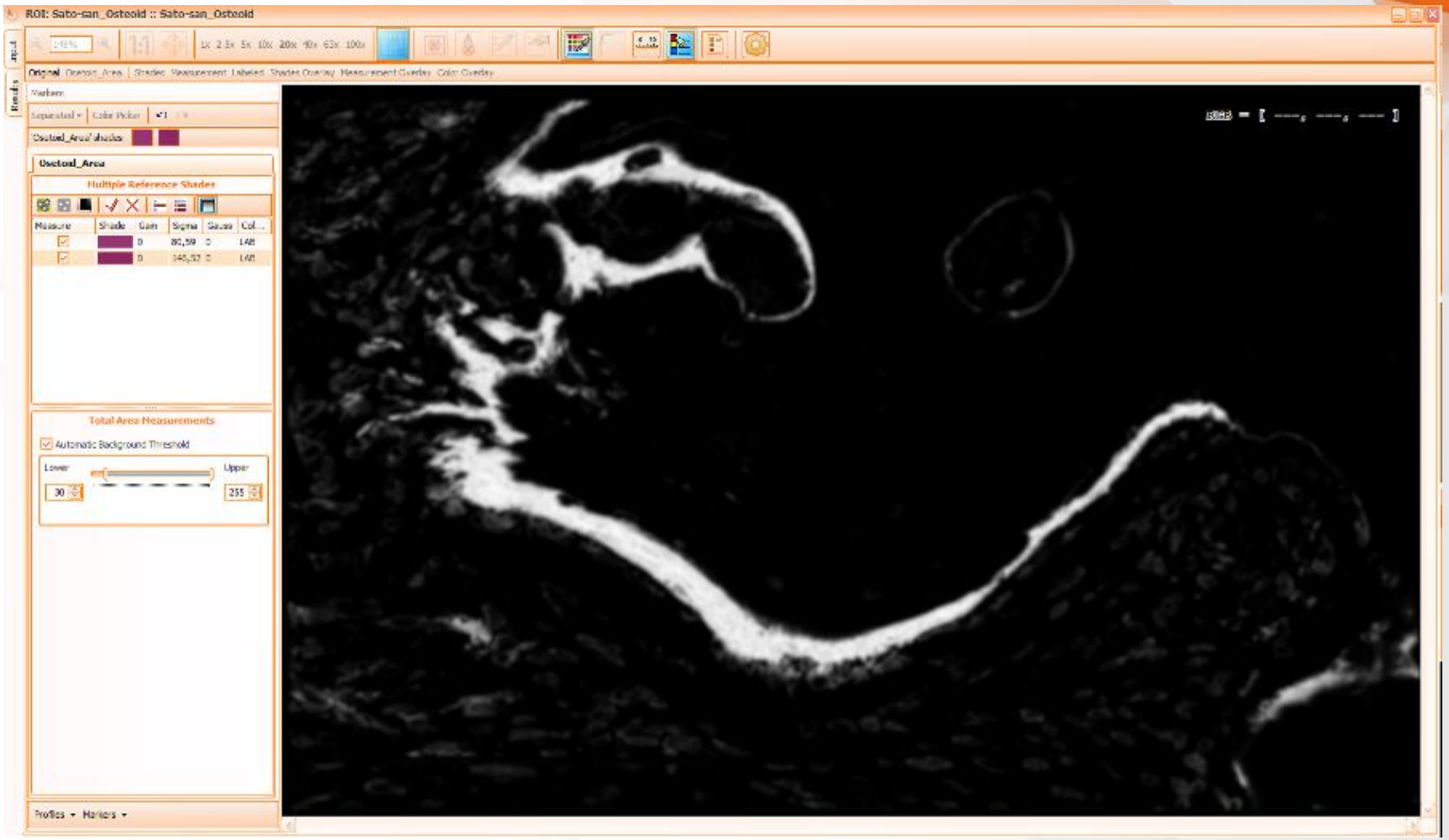
Total Area Measurement



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Measurement of Extracellular Matrix

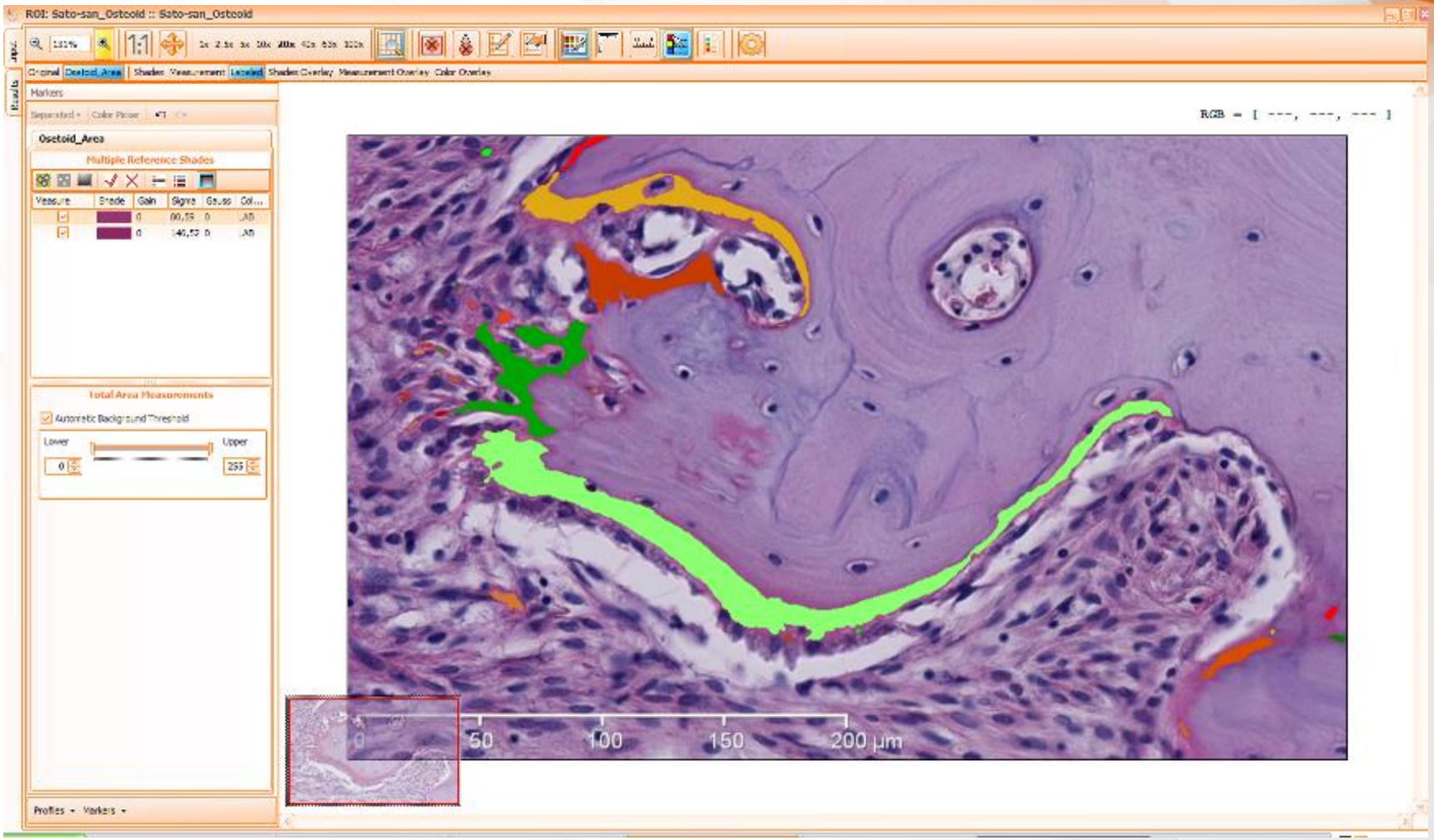
Total Area Measurement



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Measurement of Extracellular Matrix

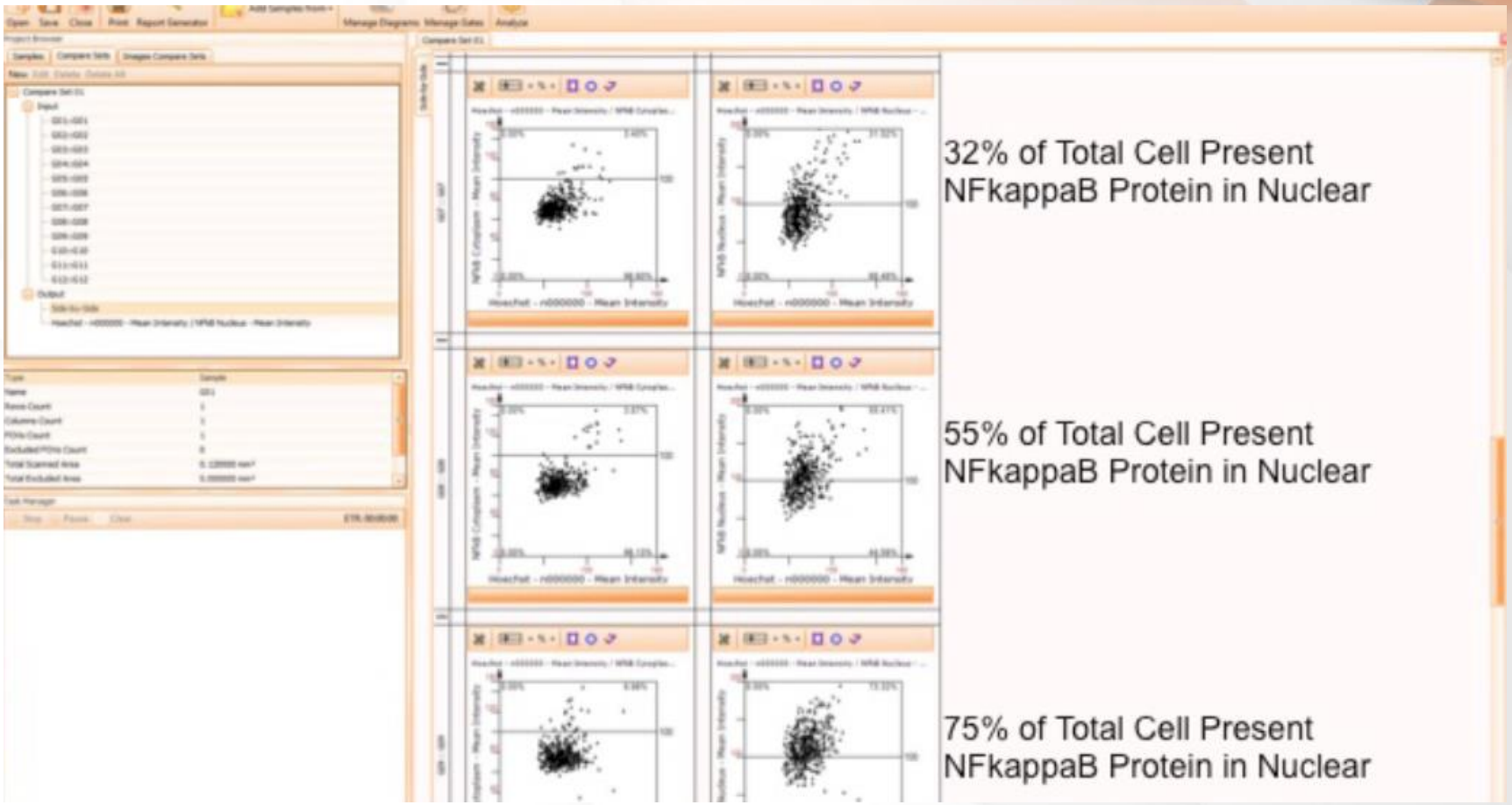
Total Area Measurement



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Analysis of marker distribution
Nuclear translocation assayof NFkB

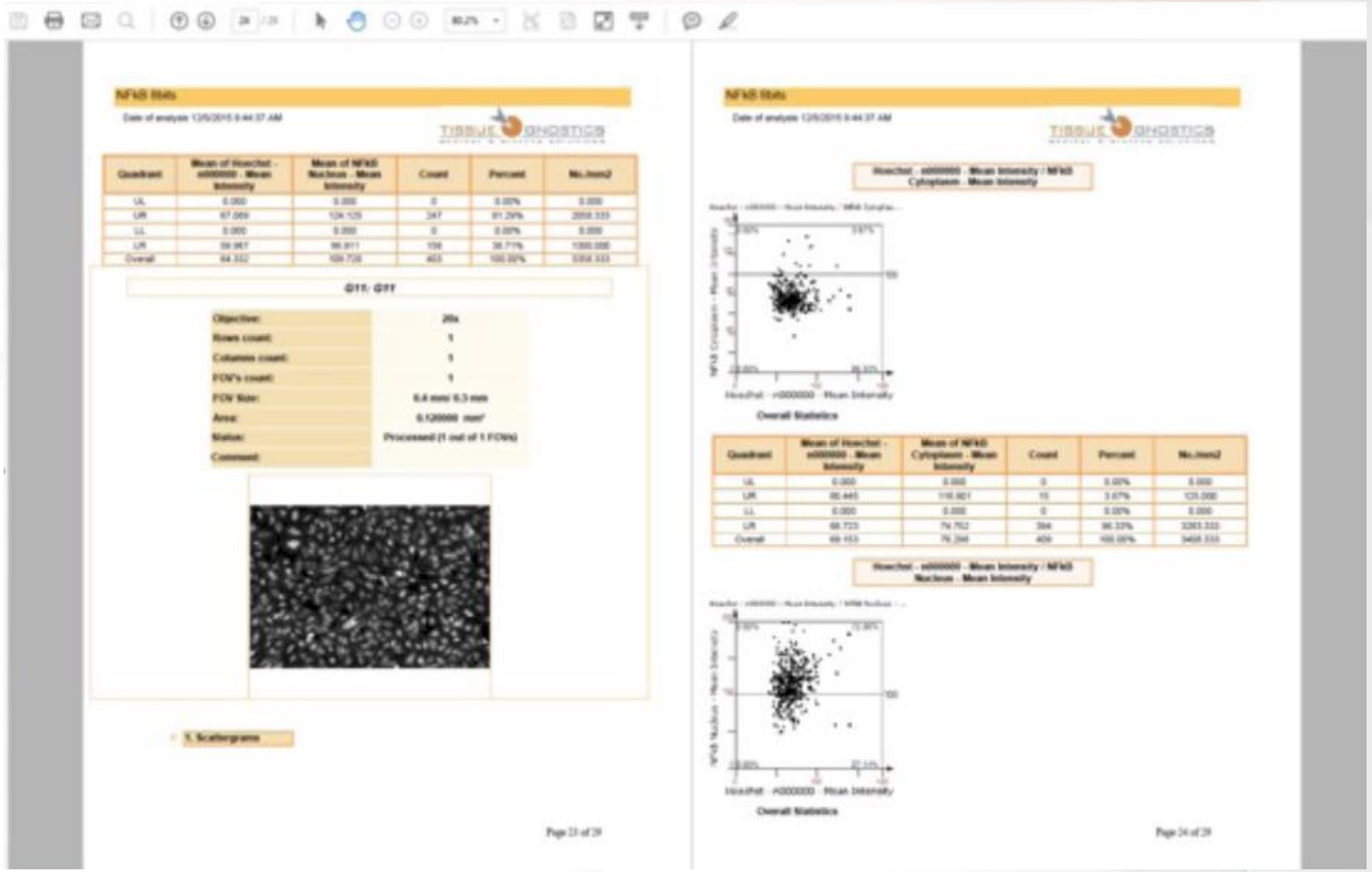
Apply Analysis Method to All Sample and Auto Generate Result



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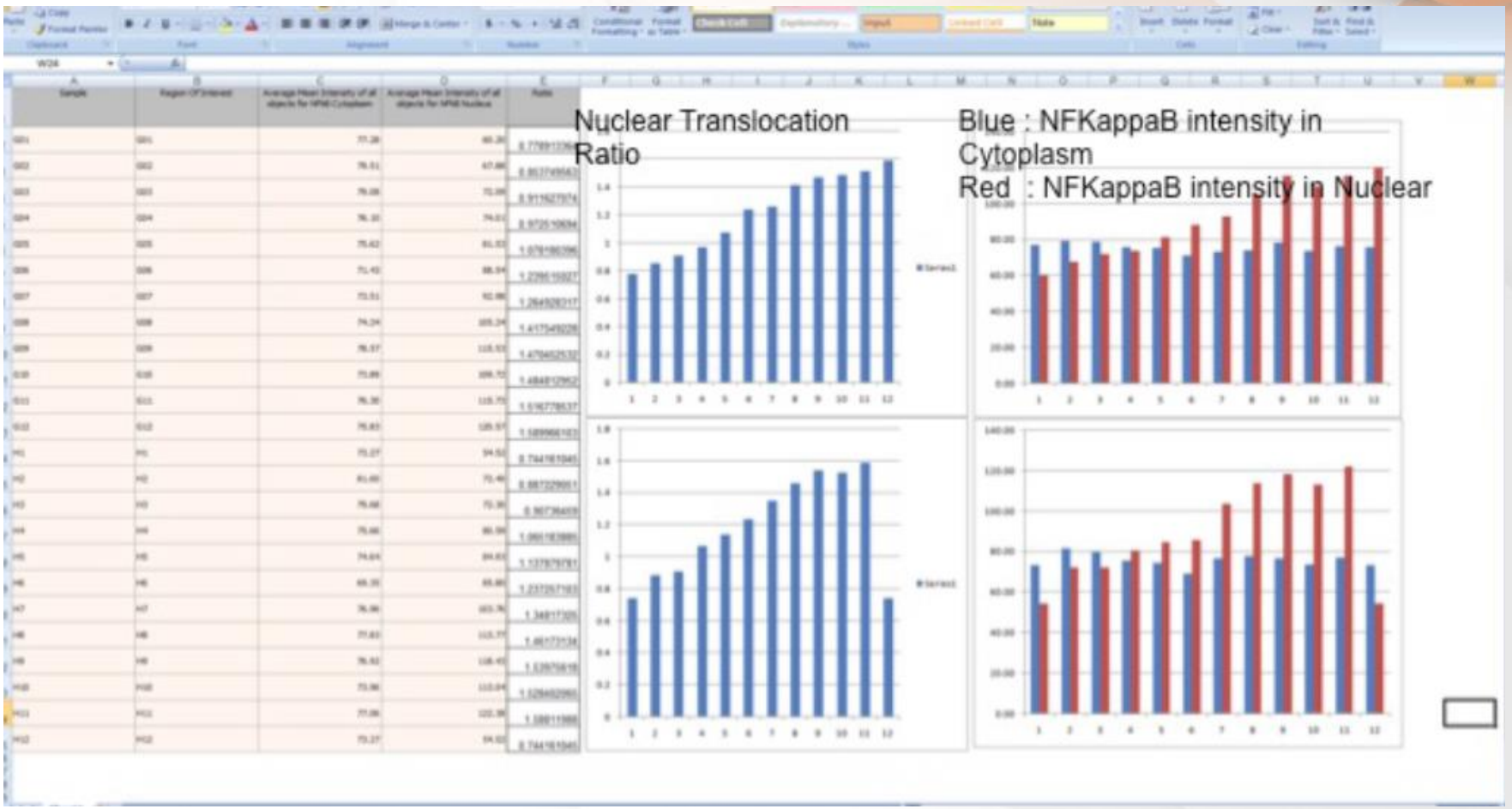
Analysis of marker distribution
Nuclear translocation assayof NFkB

Automatic Generate PDF Report



Analysis of marker distribution
Nuclear translocation assayof NFkB

Automatic Generate Statistic Report



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TissueFAXS™ Cytometry

The Microscopic Equivalent to Flow Cytometry

Application Notes

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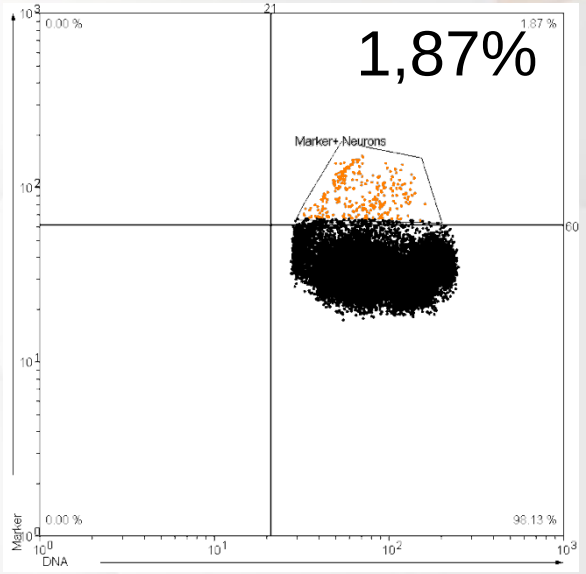
Application Note 1:
Measurement of Neuronal Markers

Aim:

Measure the moleular expression levels of certain markers in neuronal cells in a rat model.



Red: nuclei (Propidium Iodide)
Green: neuronal marker



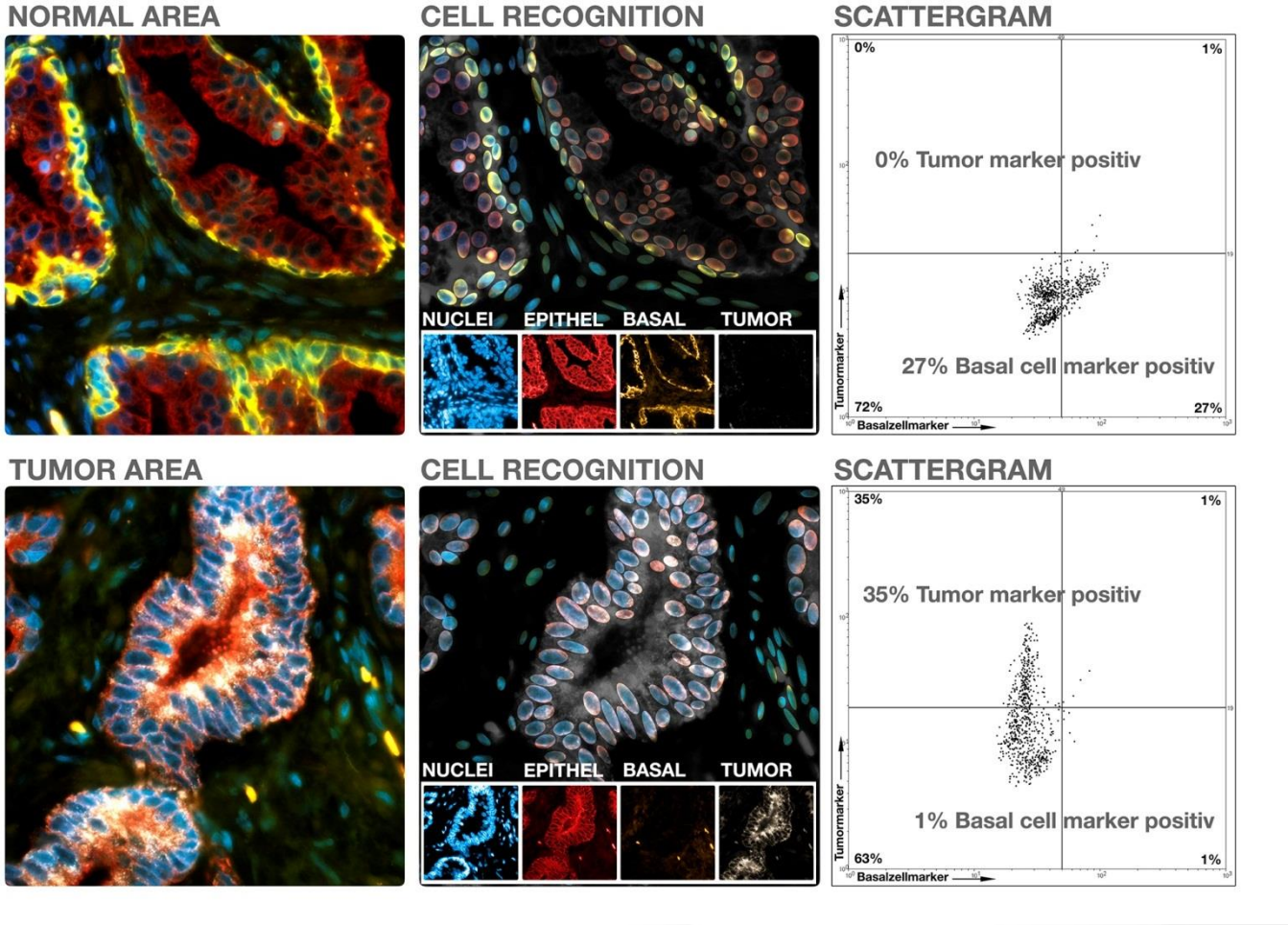
All events in scattergram					
	X-Mean	Y-Mean	Events	#/mm²	%
UL	0.00	0.00	0	0.00	0.00
UR	70.83	84.02	374	2423.47	1.87
LL	0.00	0.00	0	0.00	0.00
LR	84.12	32.62	19660	127394.17	98.13
SUM	83.88	33.58	20034	129817.64	100.00

Marker+ Neurons					
	X-Mean	Y-Mean	Events	#/mm²	%
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

TISSUE GNOSTICS
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Courtesy Prof. M. Maurer, Inst. f. Pathophysiologie, Univ. of Heidelberg, Germany

Application Note 2:
Early Identification of Malignant Transformation



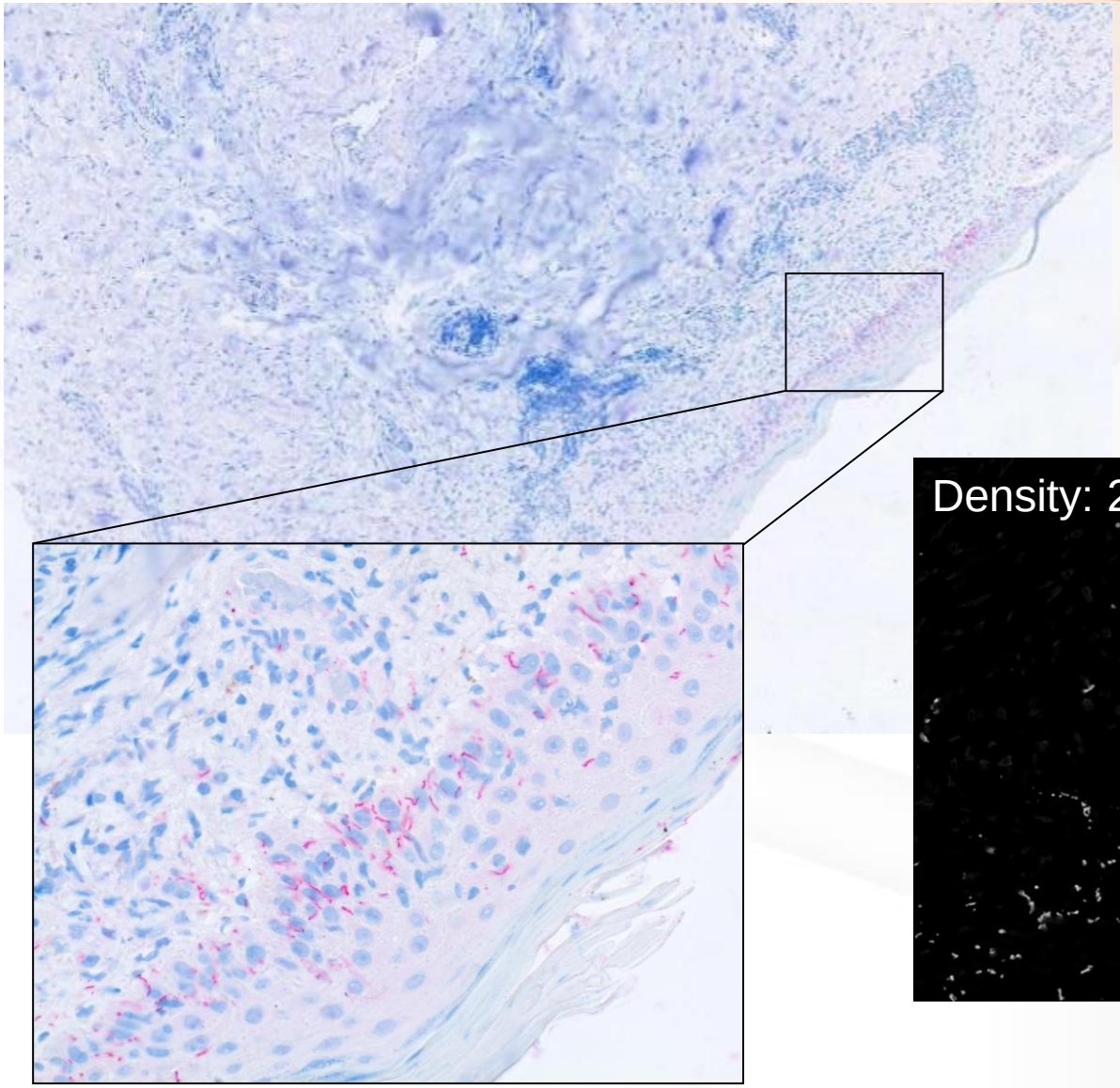
Aim:

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.

Courtesy Prof. H. Klocker, Dep. of Urology, Med. Univ. of Innsbruck, Austria

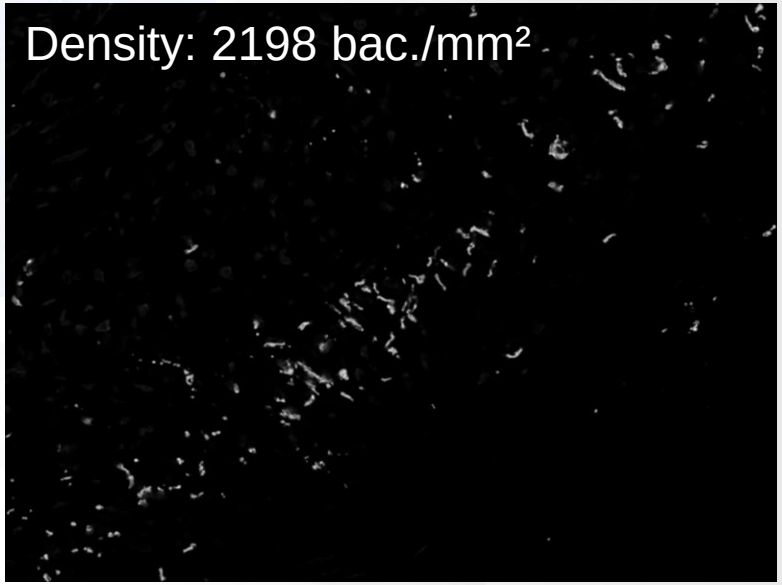
TISSUE GNOSTICS
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Application Note 3:
Counting of Bacteria (Spirochaeta)



Aim:

Automatically count bacteria per mm² in infected skin.



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Courtesy Dr. M.J. Flaig, Dep. of Dermatology, LMU Munich, Germany

Application Note 4:
Signalling Molecules in Apoptosis

PNAS PNAS

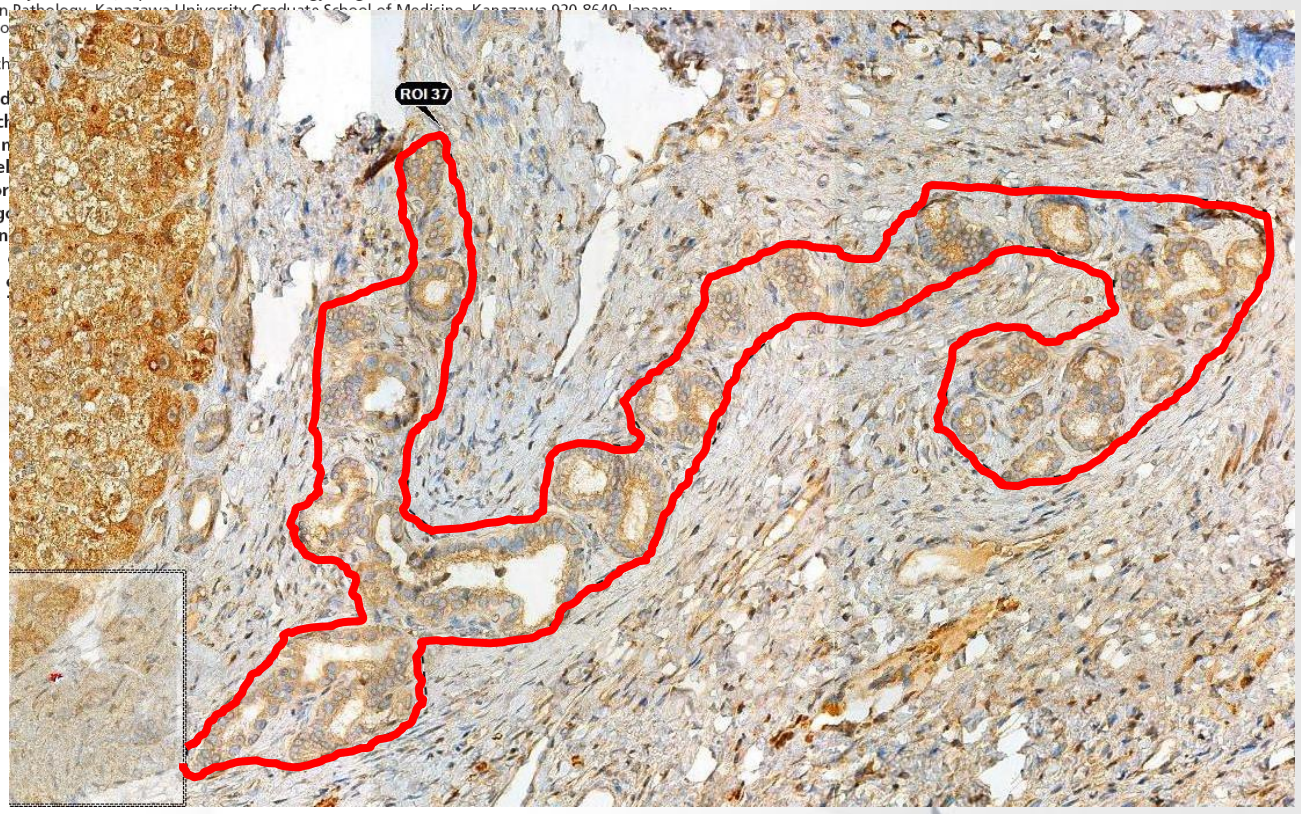
Death receptor 5 mediated-apoptosis contributes to cholestatic liver disease

Kazuyoshi Takeda*†‡§, Yuko Kojima§¶, Kenichi Ikejima‖, Kenichi Harada**, Shunhei Yamashina‖, Kyoko Okumura‖, Tomonori Aoyama‖, Steffen Frese††, Hiroko Ikeda**, Nicole M. Haynes†, Erika Cretney†, Hideo Yagita*, Noriyoshi Sueyoshi¶, Nobuhiro Sato‖, Yasuni Nakanuma**, Mark J. Smyth†§, and Ko Okumura*§

*Department of Immunology, ¶Division of Biomedical Imaging Research, Biomedical Research Center, and ‖Department of Gastroenterology, Juntendo University School of Medicine, Hongo 2-1-1, Bunkyo-ku, Tokyo 113-8421, Japan; †Cancer Immunology Program, Peter MacCallum Cancer Centre, St. Andrews Place, East Melbourne, Victoria 3002, Australia; **Human Pathology, Kanazawa University Graduate School of Medicine, Kanazawa 920-8640, Japan; and ††Department of Clinical Research, Laboratory of Tho

Edited by Leonard A. Herzenberg, Stanford University Sch

Chronic cholestasis often results in premature d
failure with fibrosis; however, the molecular mech
uting to biliary cirrhosis are not demonstrated. In
show that the death signal mediated by TNF-rel
inducing ligand (TRAIL) receptor 2/death receptor
a key regulator of cholestatic liver injury. Age
monoclonal antibody treatment triggered cholan
sis, and subsequently induced cholangitis and
injury in a mouse strain-specific manner. TRAIL-

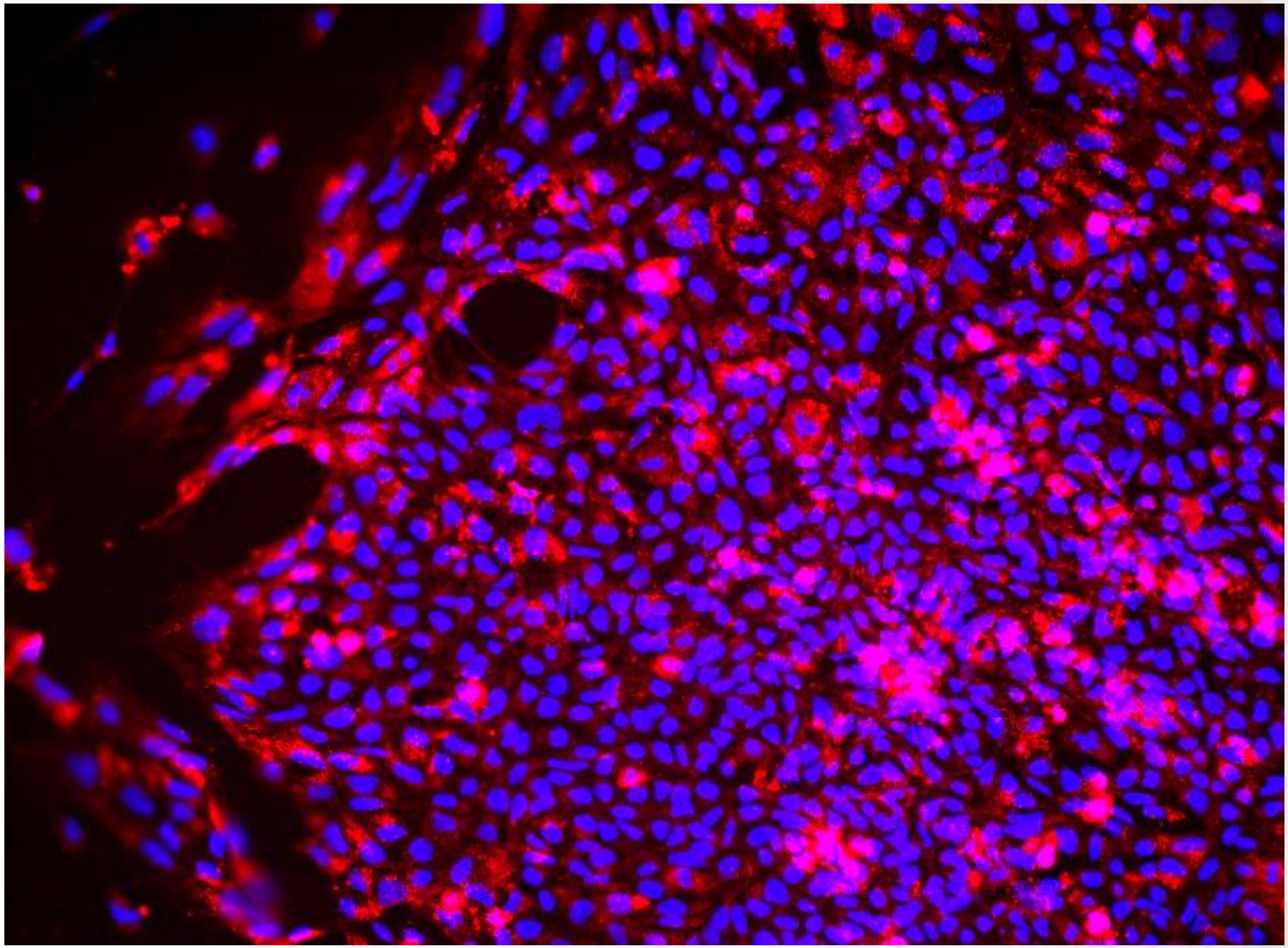


Region-specific analysis of liver tissue by HistoQuest as key feature in tissue analysis

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Takeda K, et al. 2008, Proc. Natl. Acad. Sci. USA 105(31):10895-900

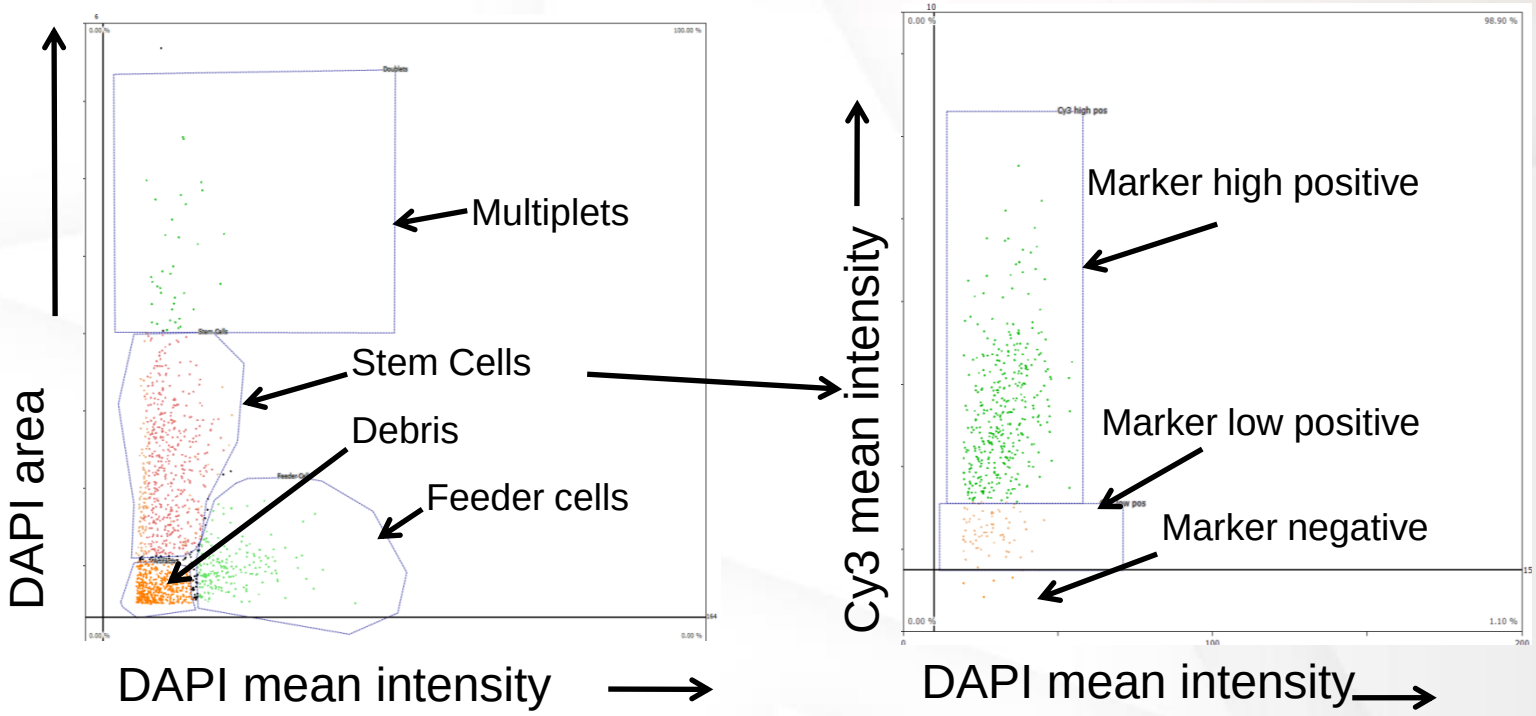
Application Note 5:
Functional Characterization of Stem Cells



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Dr. Glyn Stacey, UK Stem Cell Bank

Application Note 5:
Functional Characterization of Stem Cells



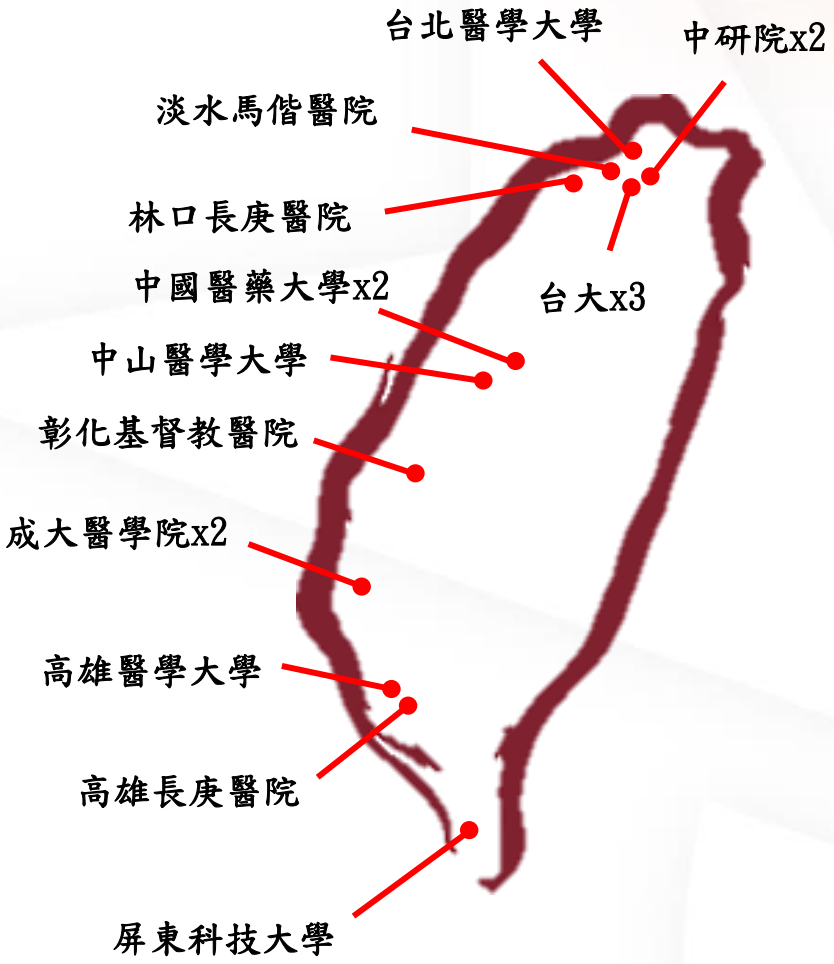
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Summary

TissueFAXS offer:

- Automated scanning of tissue sections, biopsies, cell smears
- Fluorescence and/or immunohistochemistry
- Digital overviews
- Analysis of Regions of Interest (ROI)
- Color separation
- Single cell identification even in dense tissue conditions
- Quantification of antibody staining PER CELL
- Measurement of nuclear, cytoplasmatic, membrane markers
- „FACS-like“ analysis of cells in tissue sections (dot-plots, gates)
- Forward- and backward connection to link images with data
- Observer independent, reproducible **functional** measurements

TissueFAXS全球發表超過1800篇
在台灣發表文獻超過290篇，為全球第三名

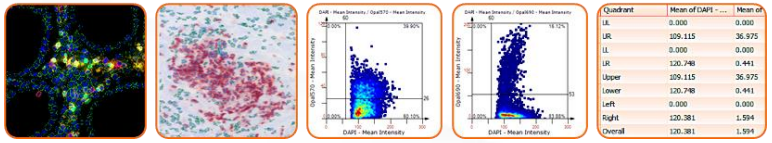


Country	Total	Percentage
USA	456	18.3%
Austria	353	14.1%
Taiwan	288	11.5%
Germany	229	9.2%
China	230	9.2%
The Netherlands	147	5.9%
UK	102	4.1%
Japan	91	3.6%
South Korea	90	3.6%
Italy	55	2.2%
Romania	51	2.0%
Czech Republic	41	1.6%
Canada	36	1.4%
Switzerland	34	1.4%
Belgium	33	1.3%
France	27	1.1%
Singapore	24	1.0%
Sweden	22	0.9%
Australia	21	0.8%

TG Ultimate Solution for Tissue Cytometry

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TissueGnostics





Cell-Bio 尚博生物科技有限公司
Cell-Bio Biotechnology Co.,Ltd.

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Thank you for your attention

Any questions?

nature
nature medicine
Clinical Cancer Research
Experimental Dermatology
PNAS
nature methods
blood
Cancer Research
Clinical Cancer Research
Gastroenterology
The American Journal of PATHOLOGY
Cellular and Molecular Biology of Disease
The Journal of Immunology
The Prostate
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JOURNAL OF INVESTIGATIVE DERMATOLOGY
HEPATOLOGY

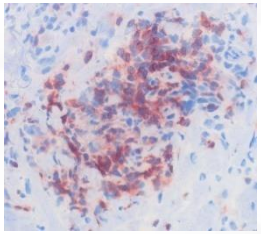
NUS National University of Singapore
National Cancer Center
Juntendo University
KOBE UNIVERSITY
National Cheng Kung University
SNUH SEOUL NATIONAL UNIVERSITY HOSPITAL
KBSI KOREA BASIC SCIENCE INSTITUTE
KING'S College LONDON
The University Of Sheffield.
Singapore Institute for Clinical Sciences
SIgN
東京大学 THE UNIVERSITY OF TOKYO
Sullivan Nicolaides PATHOLOGY
SUNG KYUN KWAN UNIVERSITY (SKKU)
ICR The Institute of Cancer Research
Memorial Sloan Kettering Cancer Center
HHMI HOWARD HUGHES MEDICAL INSTITUTE
UCL CANCER INSTITUTE
SCHOOL OF LIFE AND MEDICAL SCIENCES
FRED HUTCHINSON CANCER RESEARCH CENTER
DANA-FARBER CANCER INSTITUTE
CEDARS-SINAI
UK StemCellBank
JOHNS HOPKINS UNIVERSITY
Max Planck Institute for Metabolism Research Cologne, Germany
former name: Max Planck Institute for Neurological Research
NOVARTIS INSTITUTES FOR BIOMEDICAL RESEARCH
UCSF University of California San Francisco
BRIGHAM AND WOMEN'S HOSPITAL
University of Pittsburgh
vetmeduni vienna
Yale University School of Medicine
dkfz. DEUTSCHES KREBSFORSCHUNGSZENTRUM IN DER HELMHOLTZ-GEMEINSCHAFT
Nestlé Good Food, Good Life Nestlé Research Center
UNIVERSITÄT HEIDELBERG ZUKUNFT SEIT 1386
MEDICAL UNIVERSITY OF VIENNA
THE UNIVERSITY of York

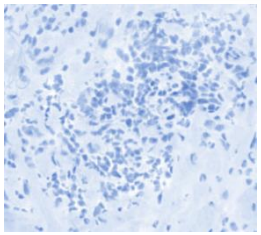
TISSUE GNOSTICS®
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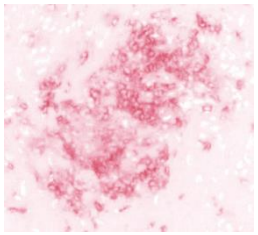
TissueFAXS Software

TissueFAXS (scanning)

TissueFAXS Viewer (Free)

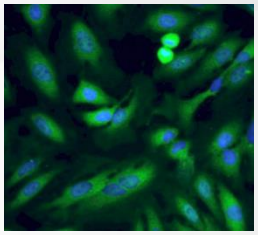


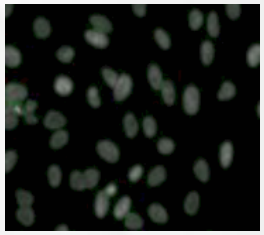


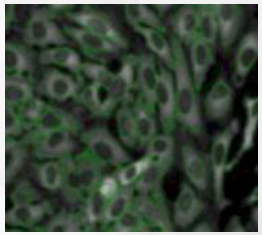


HistoQuest (Bright field)

TissueQuest (Fluorescence)







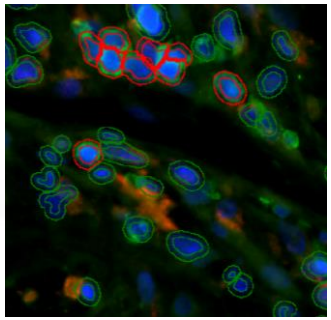
Color Separation

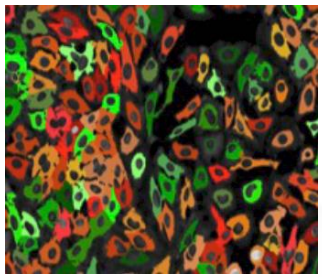
Single Cell Analysis

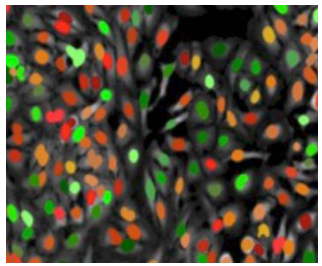
Total Area Measurement

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Analysis workflow







Pick a small region

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Color Separation (BF)

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Nucleus Detection

- Nuclei Size
- Remove small size nuclei
- Remove weakly stain nuclei

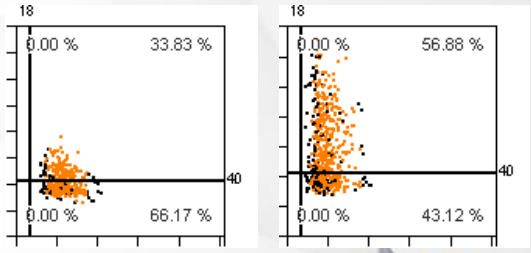
↓

Marker Detection

- Ring Mask
- Identified Cell Mask
- Nuclei Mask

↓

Setting Cut-off



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Report

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