

Immun hisztokémiai elve és kiértékelésének metodikája digitális képfeldolgozással ER, PR, KI67, HER-2



De mik azok a fehérjék?

VIGYÁZZ - EZ TEHÁT FEHÉRJE (és NEM NUKLEINSAV)!!!

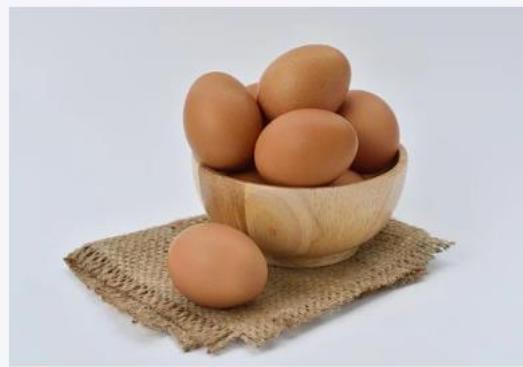
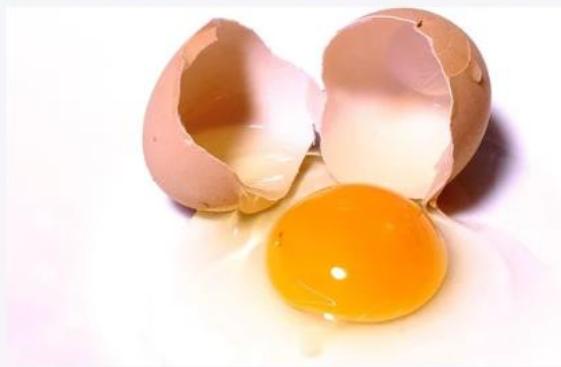
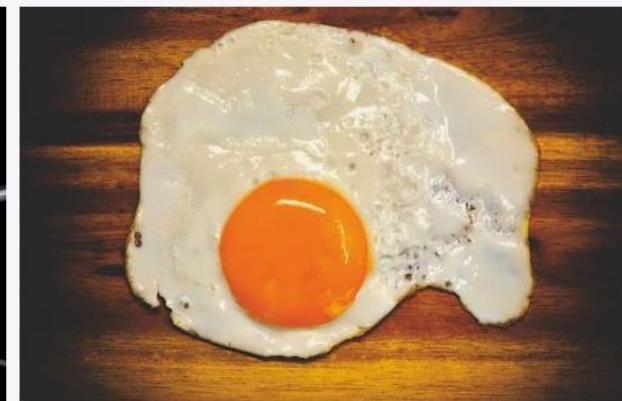
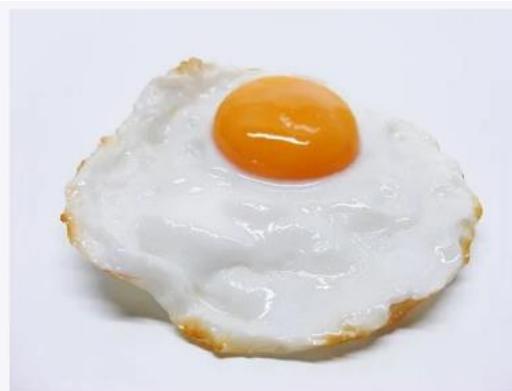
Alapkérdés: hogyan lehet megkeresni egy adott típusú fehérjét egy élőlényben (a szöveteiben, sejtjeinek falán).

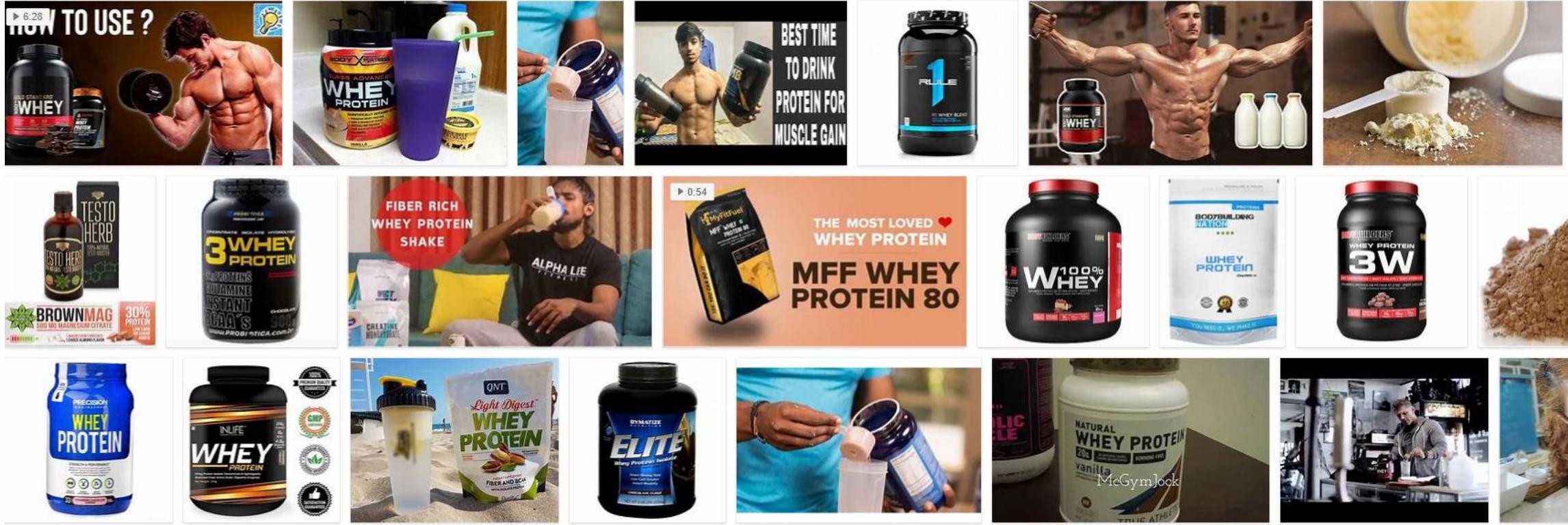
A megkeresés azt jelenti, hogy objektíven szeretnénk meghatározni:

- hol van az adott fehérje
- mihez kapcsolódik az adott fehérje (és mikor)
- mennyi van belőle
- változik-e a mennyisége az idővel
- stb.

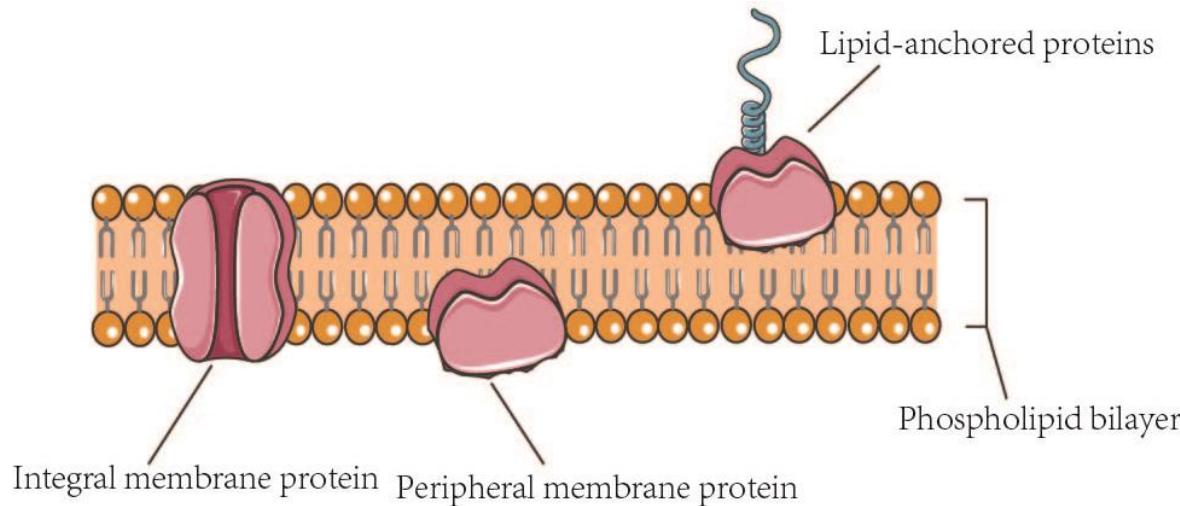
Lehetőleg valahogy számszerűsíteni és megjeleníteni is szeretnénk egyszerre...

Fehérjék

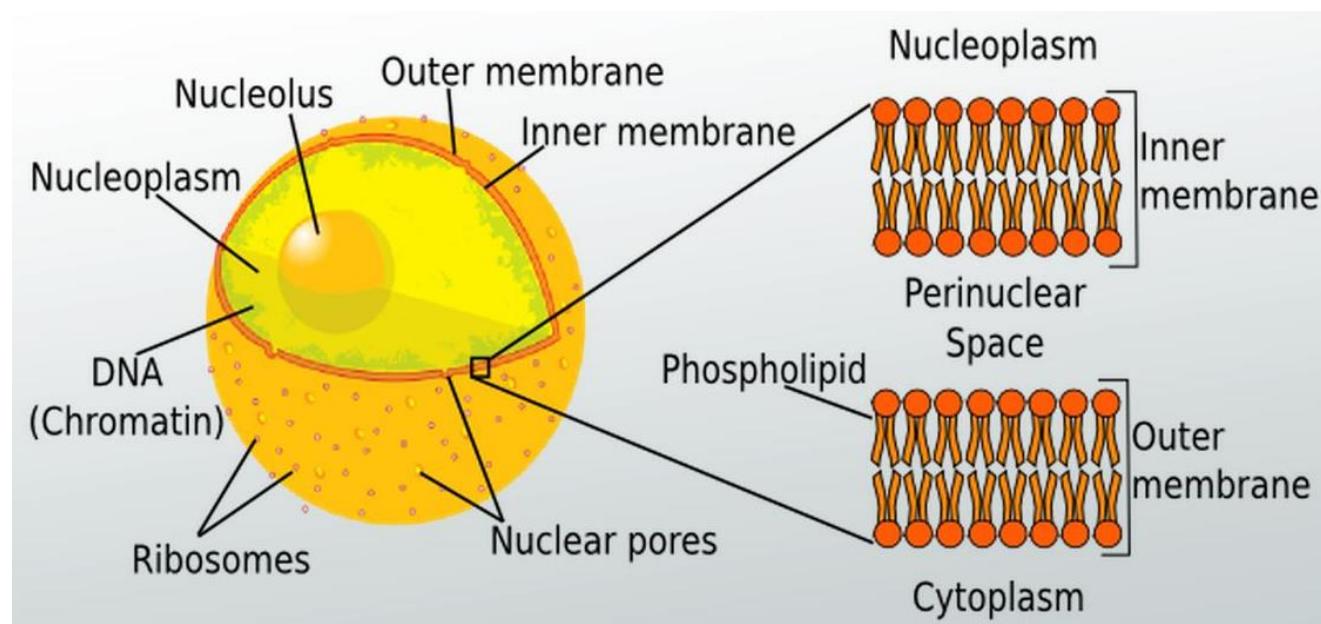




Fehérjék

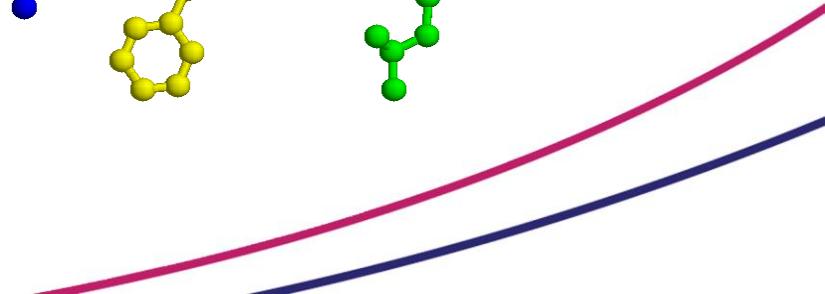
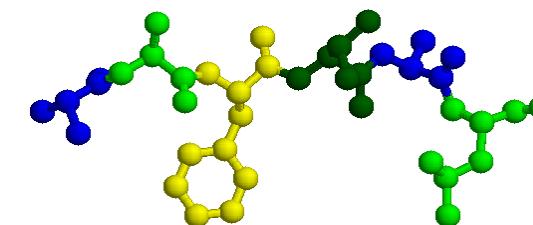


Sejt és sejtmag membrán fehérjék



A fehérjék aminosavak lineáris polimereiből felépülő szerves makromolekulák.

A fehérjék aminosav sorrendjét a gének nukleotid szekvenciája kódolja a genetikai kódszótárnak megfelelően.

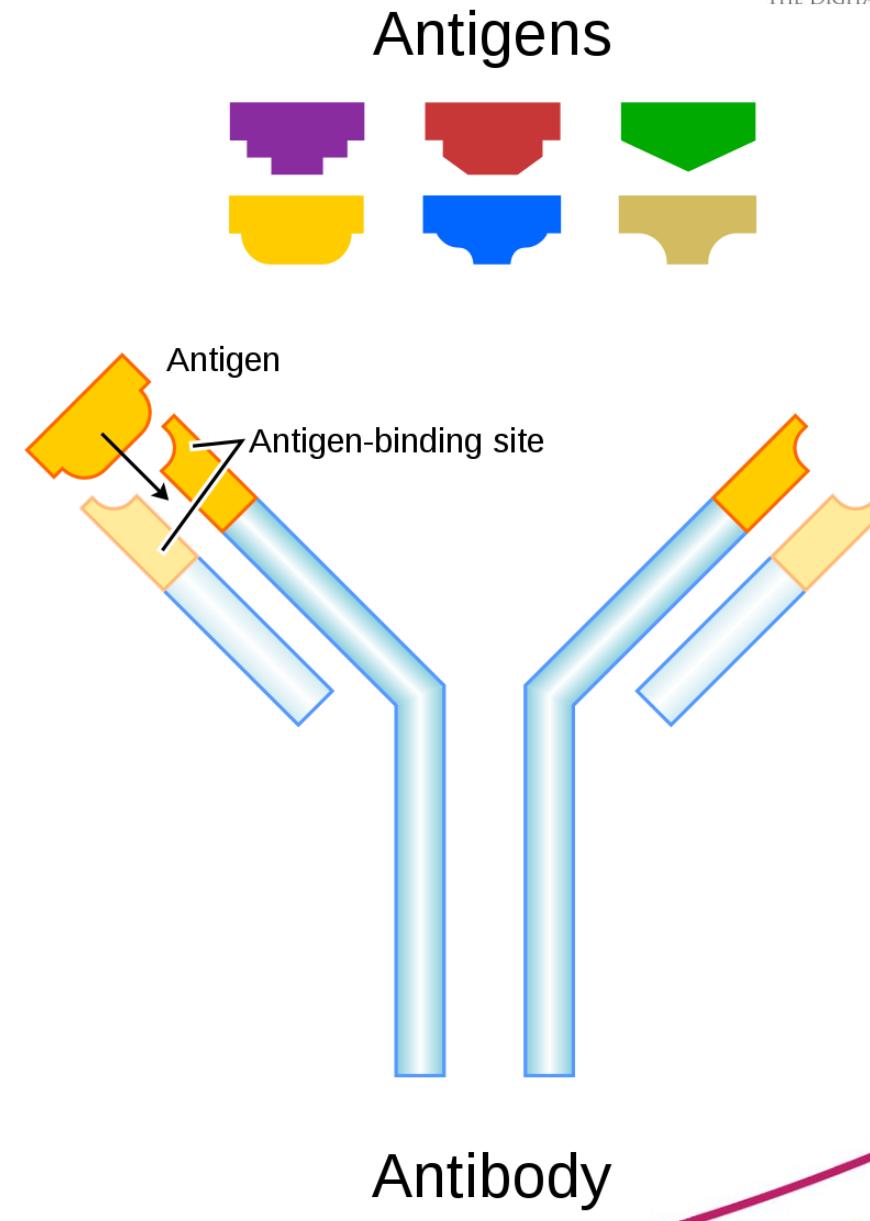


A fehérjék kimutatása

Míg adott szekvenciájú nukleinsavakat döntően hibridizációon alapuló technikákkal lehet specifikusan kimutatni, addig fehérjék specifikus kimutatására ez nem alkalmas.

A fehérjék szintézisük után feltekerednek, felveszik a rájuk jellemző térszerkezetet. Natív állapotukban adott fehérjéket elsősorban az egyéni harmadlagos szerkezetük alapján lehet megkülönböztetni egymástól.

A fehérjék specifikus kimutatására legalkalmasabbak az ellenük termeltetett **antitestek**

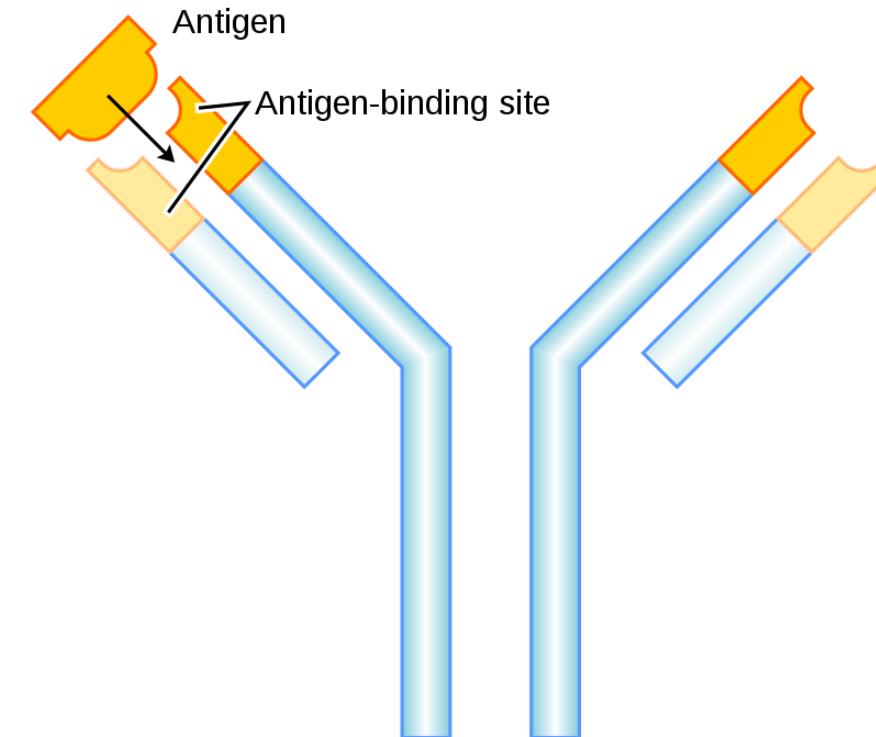
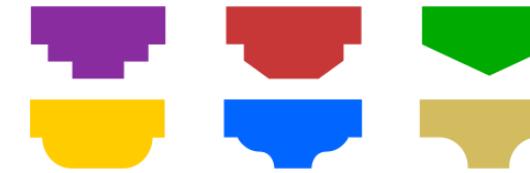


Antibody

Antitestek vagy ellenanyagok

Az ellenanyagok olyan fehérjemolekulák, amelyeket az immunrendszer termel annak érdekében, hogy felismerje és semlegesítse a szervezetbe került idegen anyagokat, mint például a baktériumokat vagy vírusokat. minden egyes ellenanyag egy idegen molekula egyedi részét (antigén) ismeri fel és kötődik hozzá.

Antigens



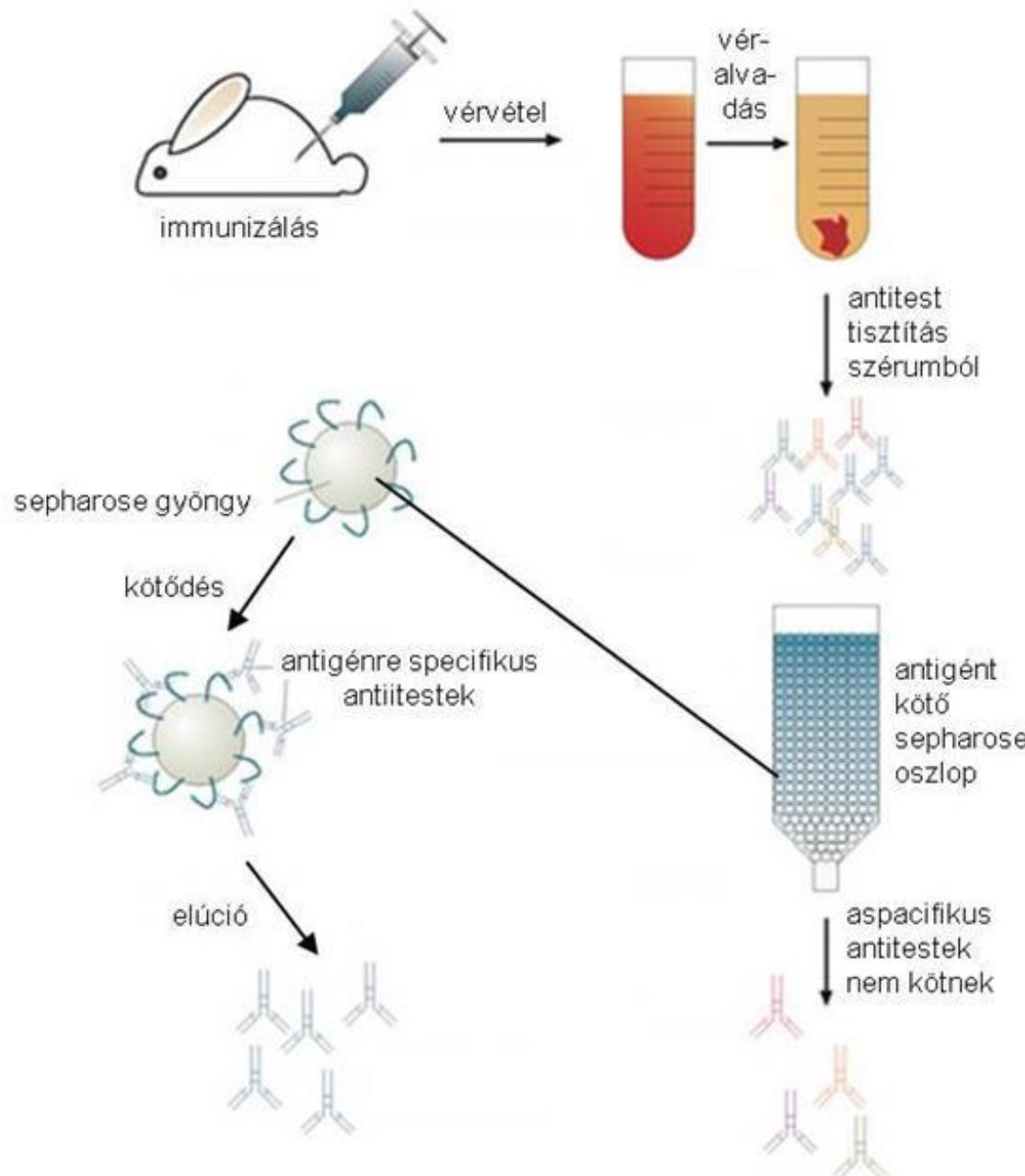
Antibody

- Antitesteket élő állatokban, vagy sejtkultúrákban termeltetnek:
 - egérben, nyúlban, kecskében, birkában, marhában, szamárban, patkányban és csirkében termeltetett antitestek a leggyakoribbak.
- Antitestek termeltetéséhez mindig antigén szükséges.
 - a kimutatni kívánt fehérje
 - az adott fehérje egy immunogén részét reprezentáló peptid

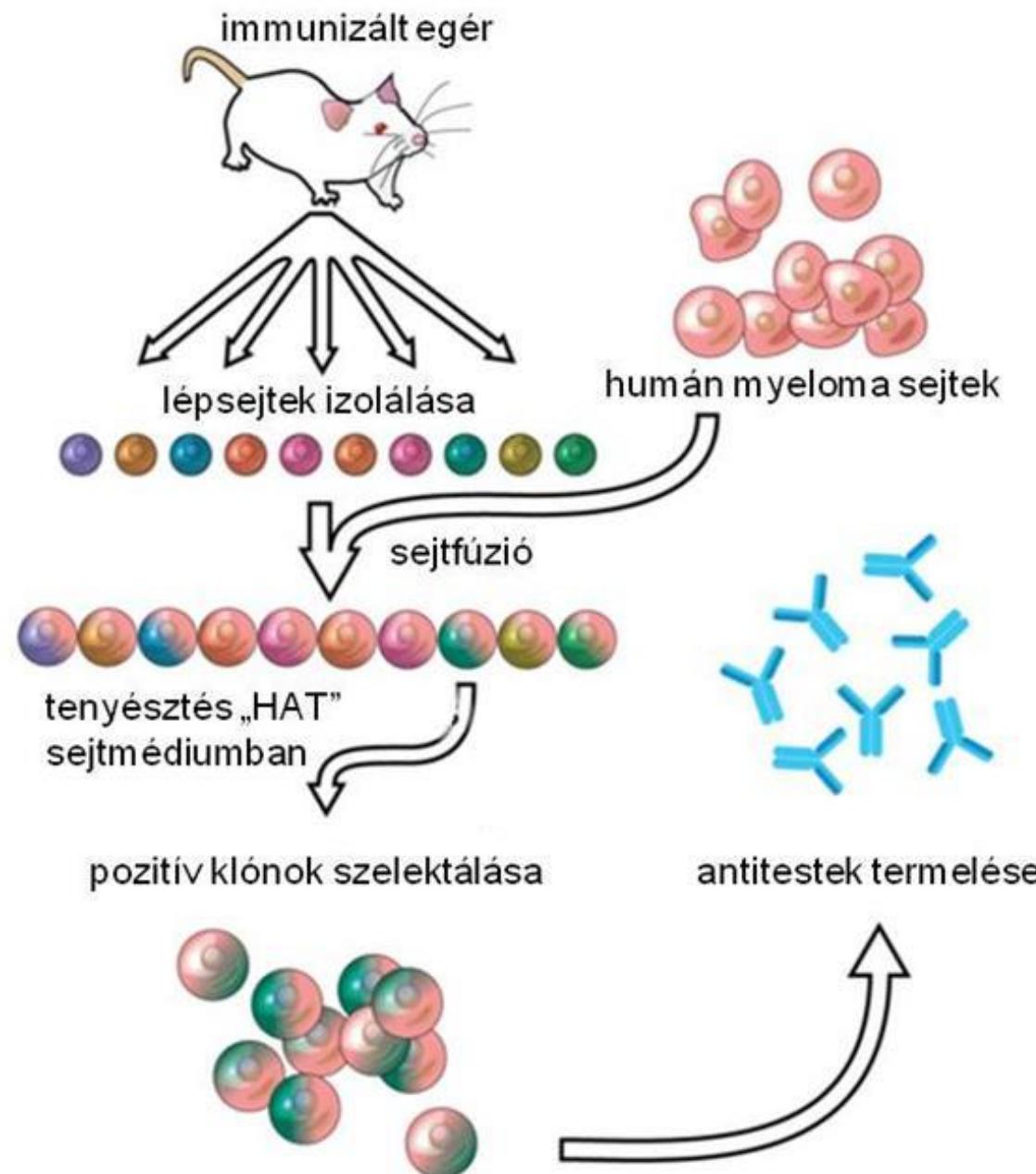
Attól függően, hogy az antitestet termelő sejtek egy, vagy több immunsejt utódai, megkülönböztetünk **monoklonális** és **poliklonális** antitesteket. A monoklonális antitestek egyformák, a poliklonális antitestek különbözőek olyannyira, hogy az sem biztos, hogy ugyanazt az **epitopot** (felismerőhelyet) ismerik fel a kérdéses fehérjén.



Poliklonális antitestek készítése

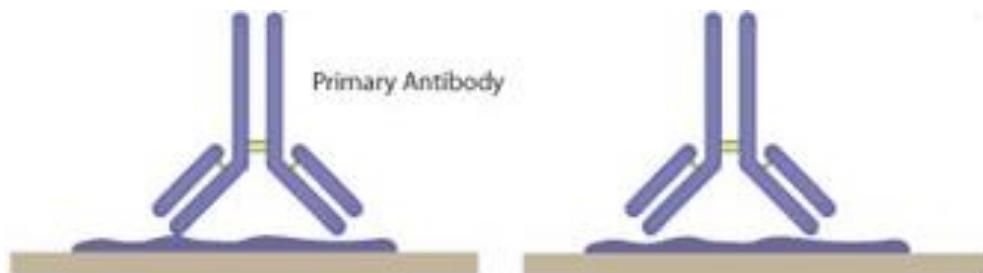


Monoklonális antitestek készítése

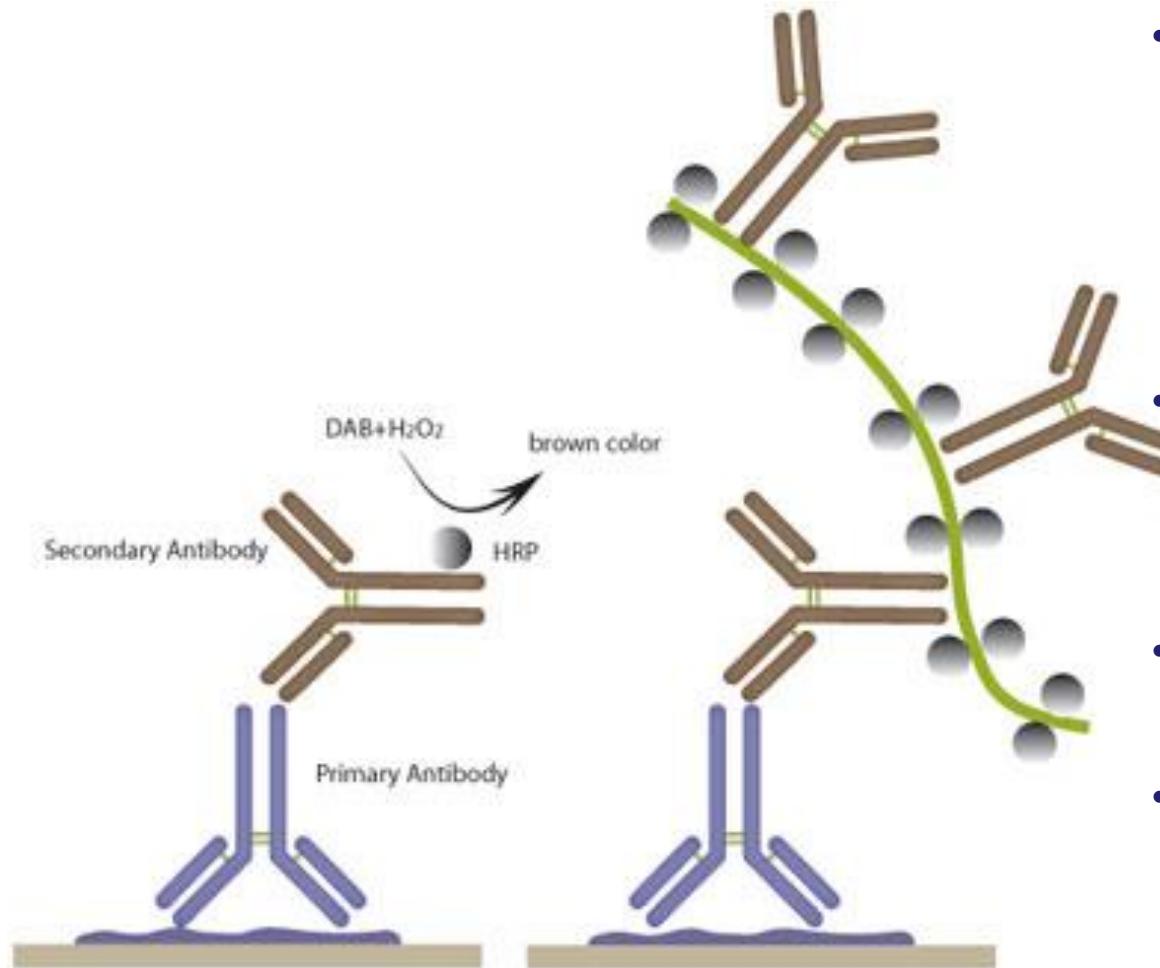


Elsődleges antitest

- Specifikus fehérjére van termeltetve
- Mivel egyedi jellegű minden drága
 - megvenni
 - vagy előállítani

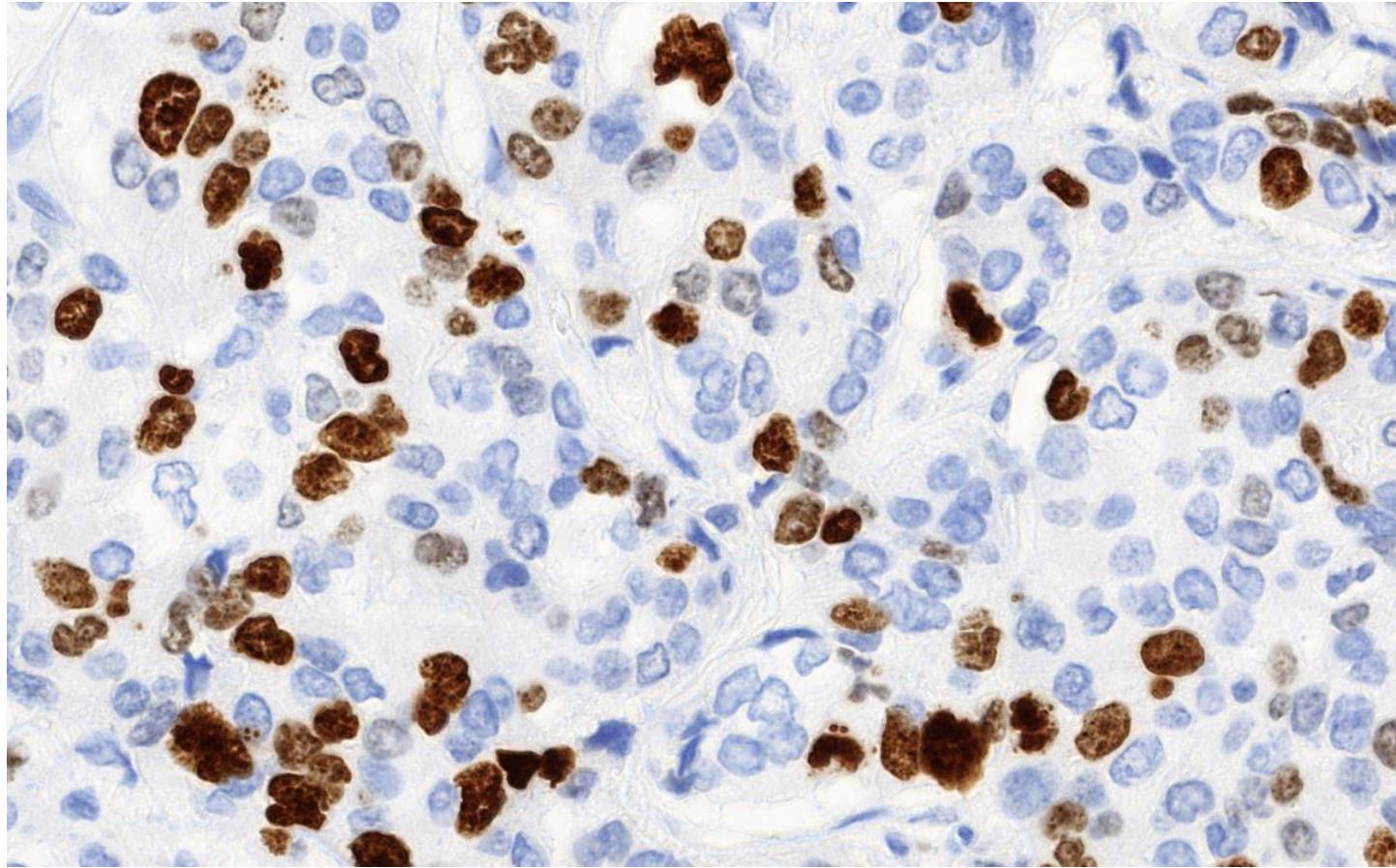


Másodlagos antitest



- Egy adott állat összes antitestjét felismeri:
 - anti-egér, anti-nyúl, anti-kecske stb.
 - az elsődleges antitestet termelő fajtól **eltérő organizmusban** termeltetetik, pl.nyúlban termeltetett anti-egér antitestet
- **fluoreszcens festékkel**, vagy valamilyen enzimmel (**torma-peroxidáz, alkalikus foszfatáz**) szokták konjugálni
- dextran polymer használható jelerősítéshez
- Mivel nagyon sok elsődleges antitesst kompatibilis:
 - olcsó
 - tömegtermék

Háttérfestés vs. specifikus festés



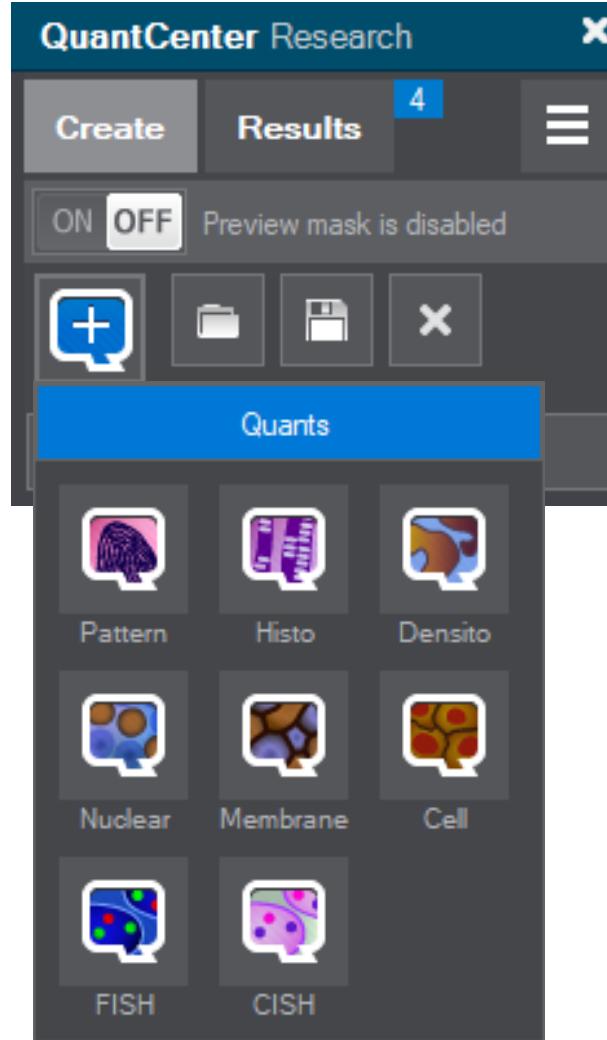
Barna: specifikus festés DAB

Kék: háttérfestés Hematoxylin

Quant Center



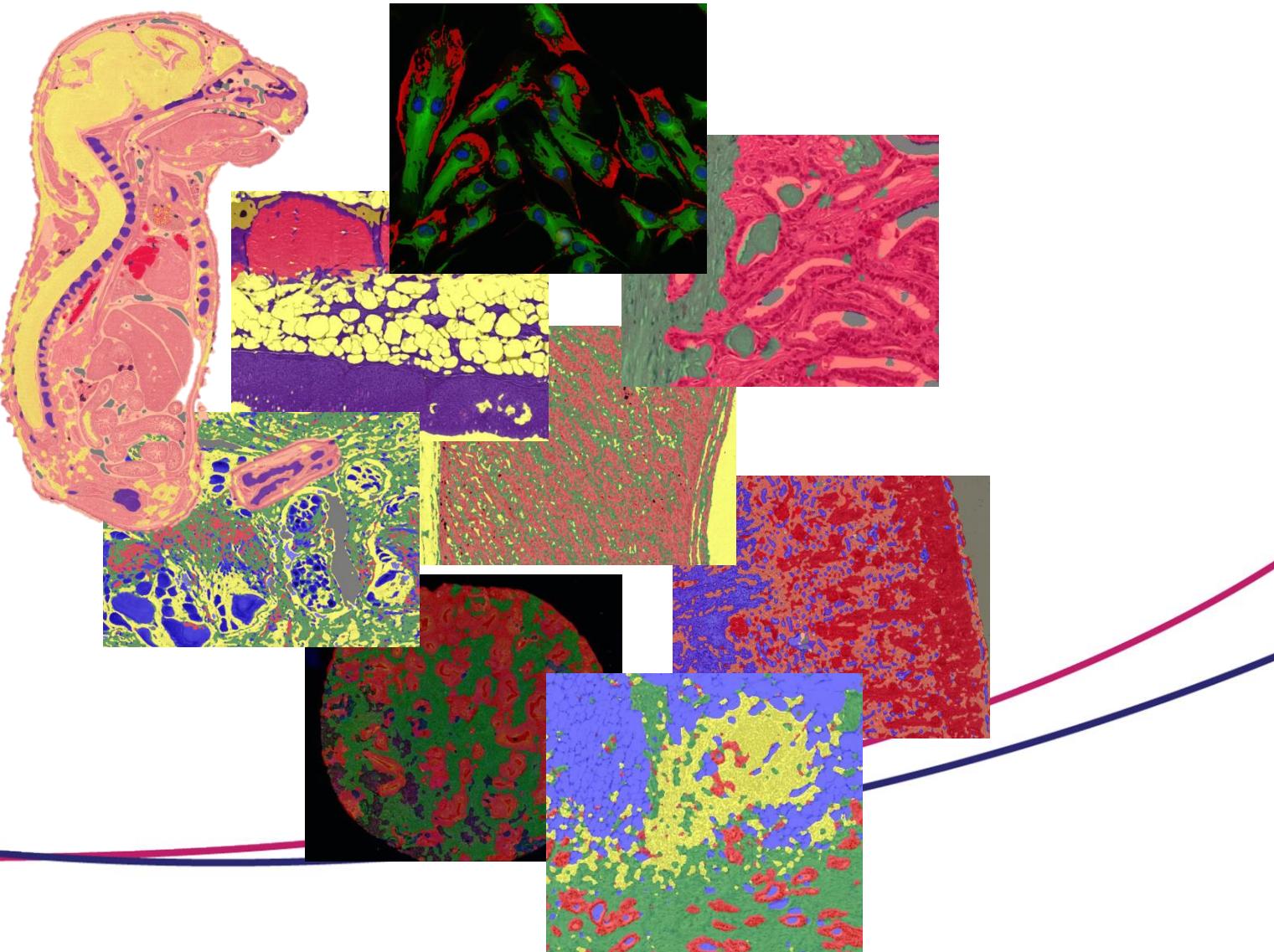
Számos képfeldogozó modul elérhető



Pattern Quant
Histo Quant
Densito Quant
Nuclear Quant
Membrane Quant
Cell Quant
FISH Quant
CISH Quant

PatternQuant – Tissue Classification

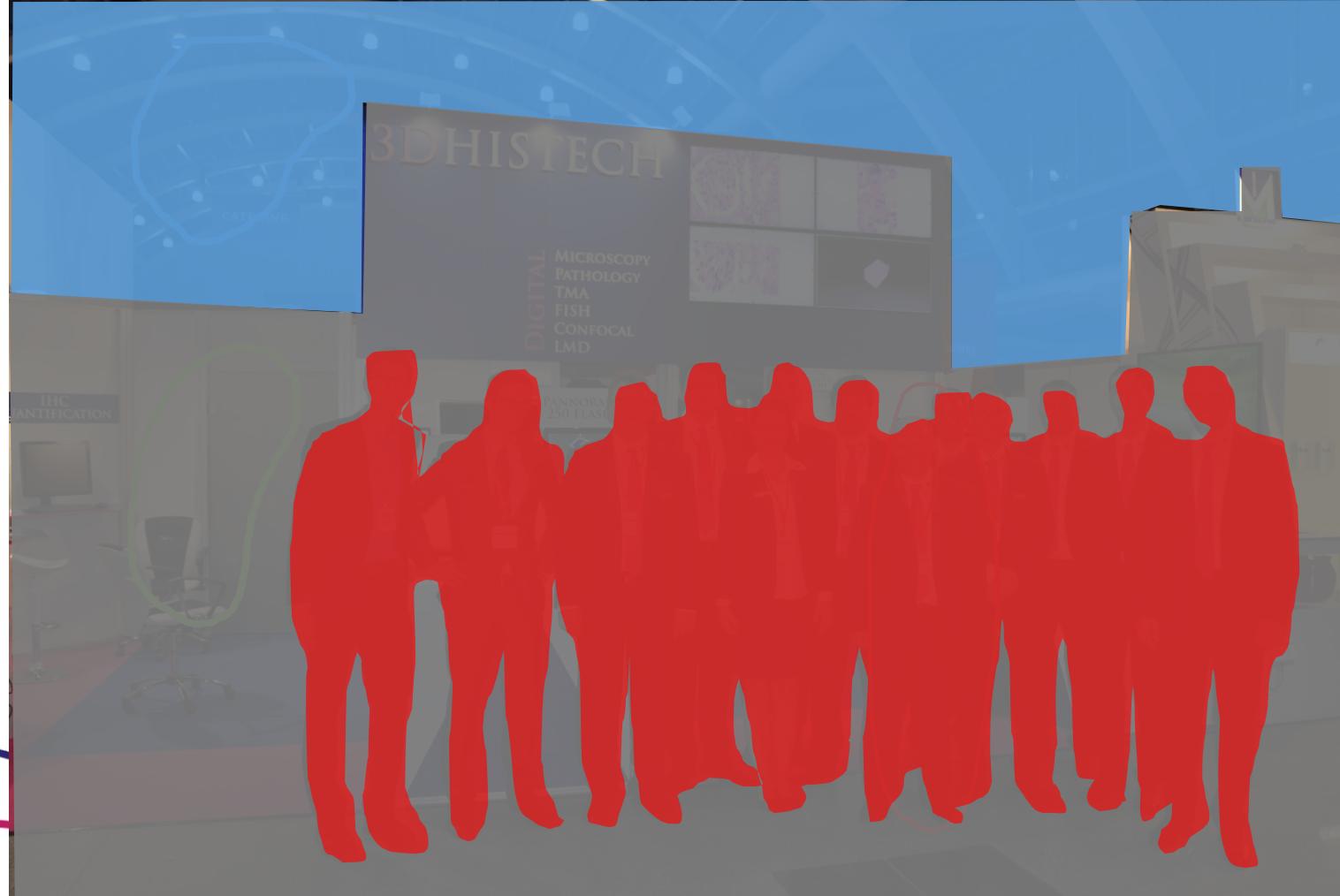
- Pattern recognition
- Tissue segmentation
- Tissue classification
- Presegmentation



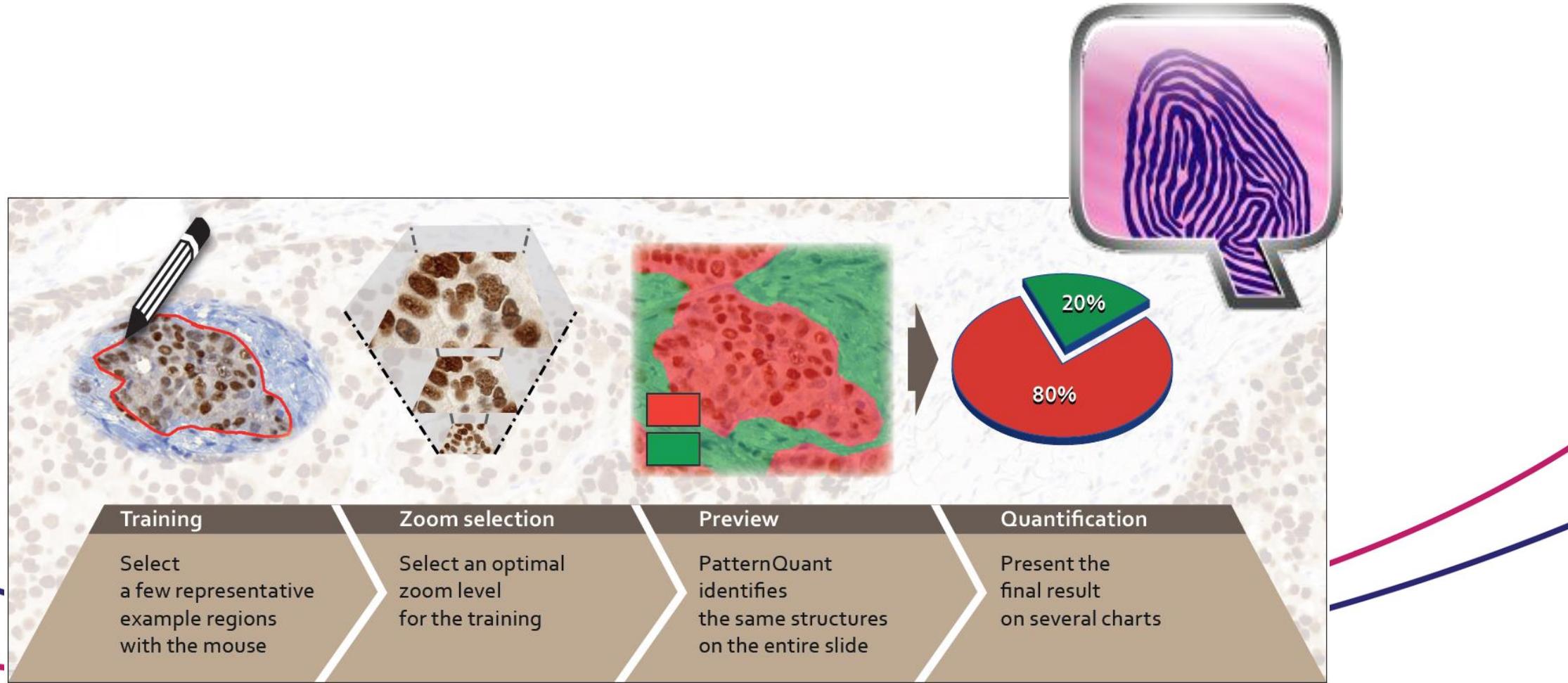


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THE DIGITAL PATHOLOGY COMPANY

Pattern recognition in Image Analysis

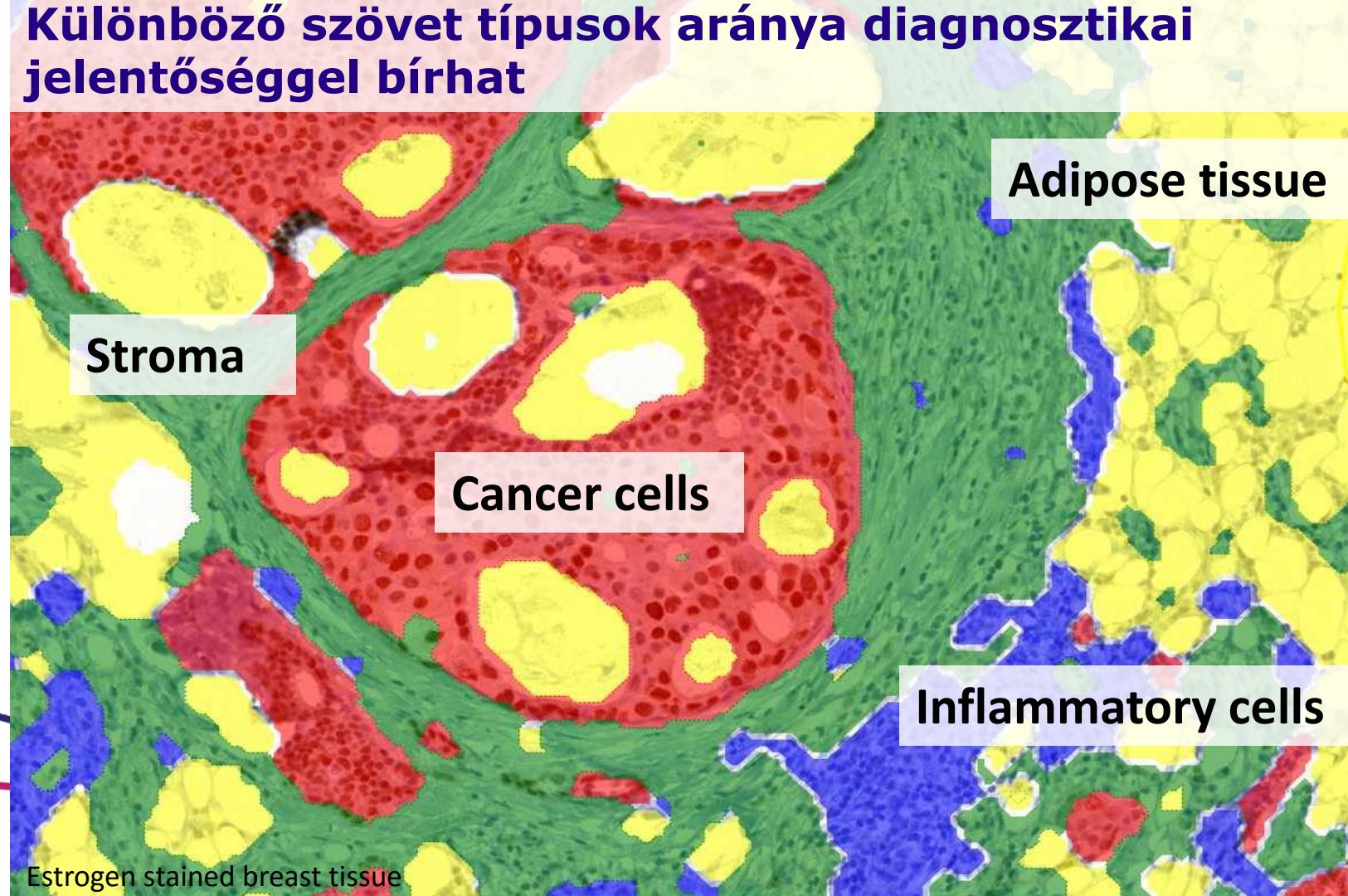


Pattern Quant

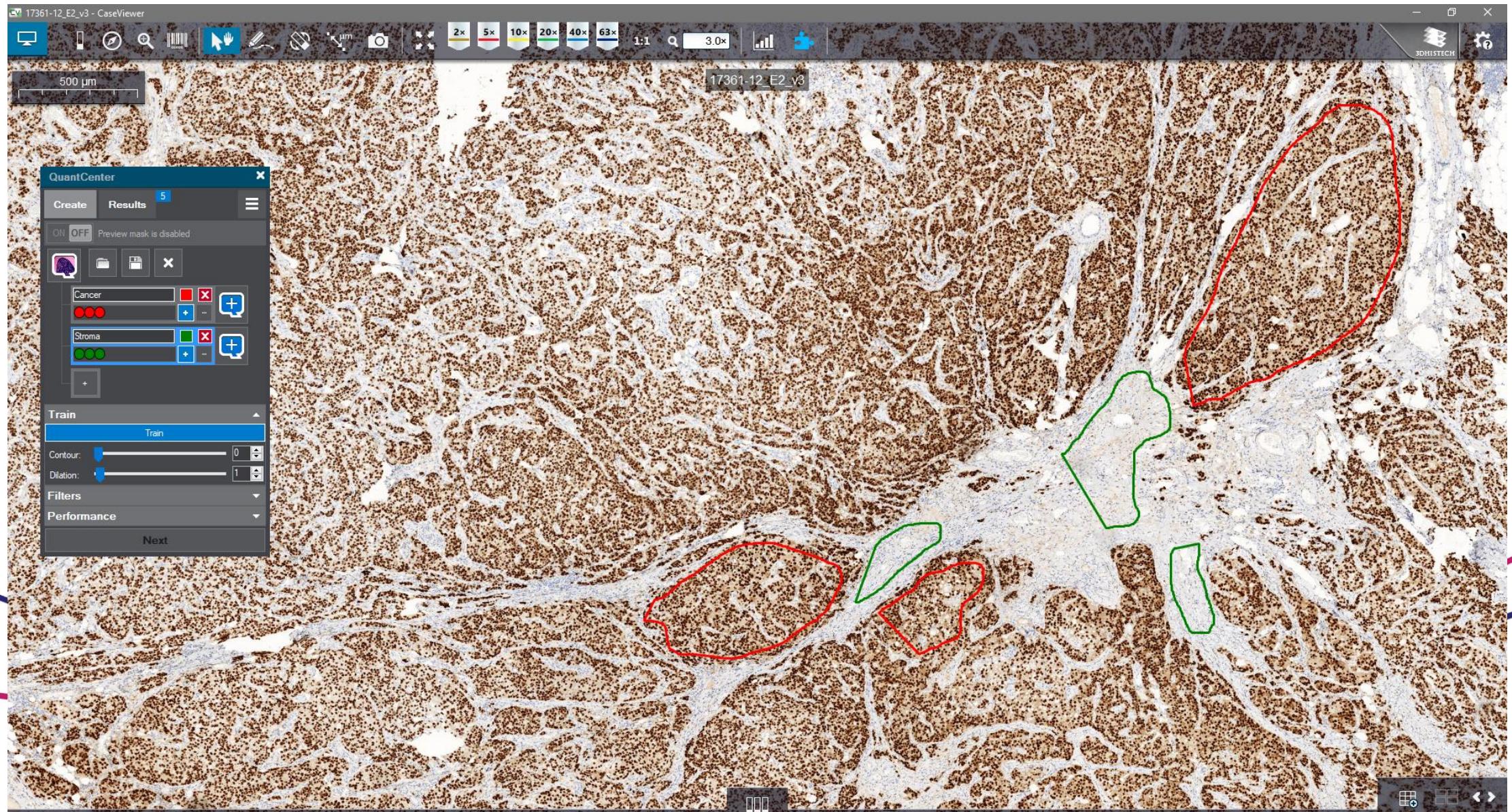


Szövet szegmentáció

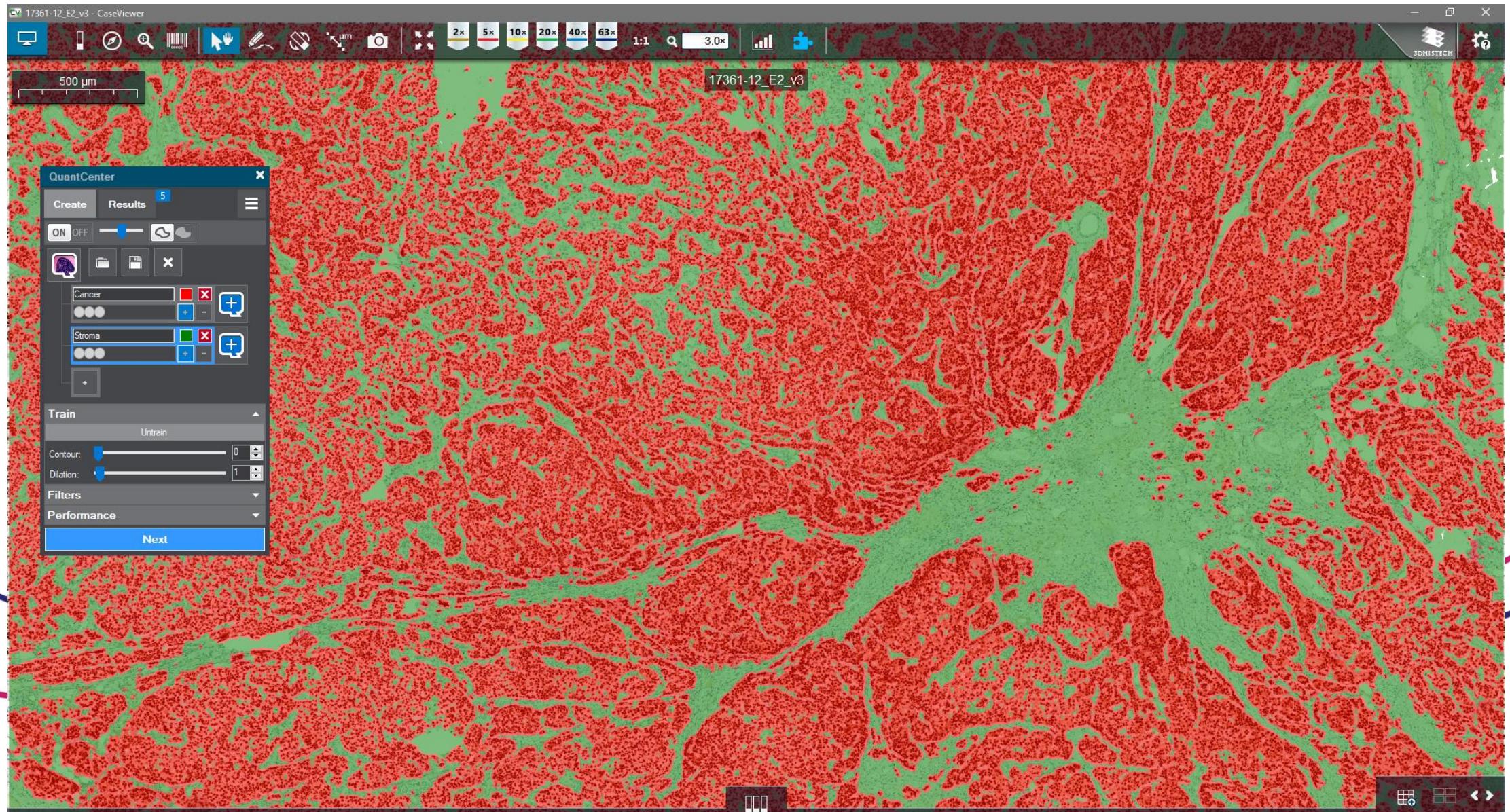
Különböző szövet típusok aránya diagnosztikai jelentőséggel bírhat



Pattern Quant



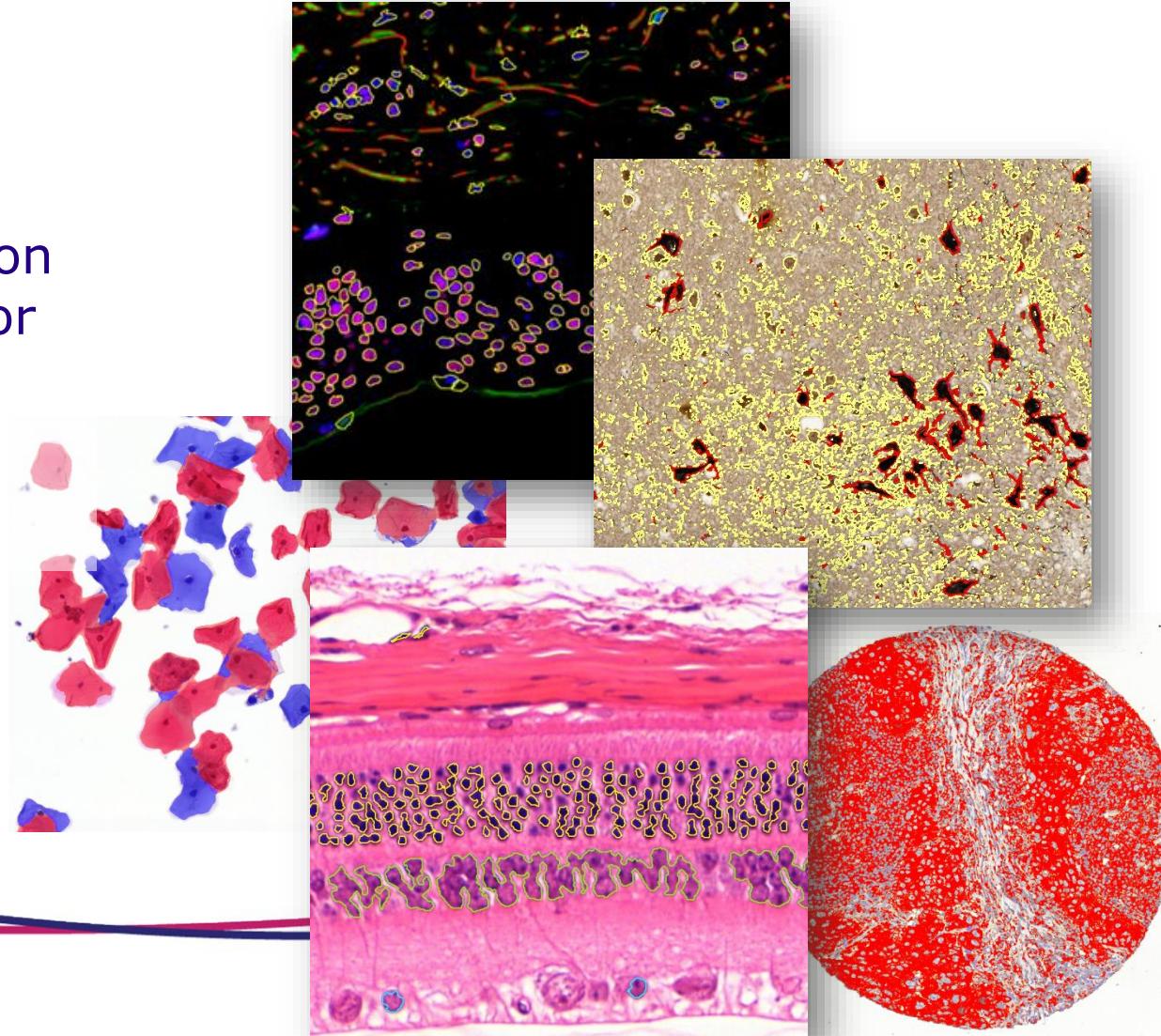
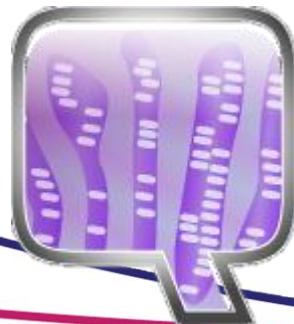
Pattern Quant



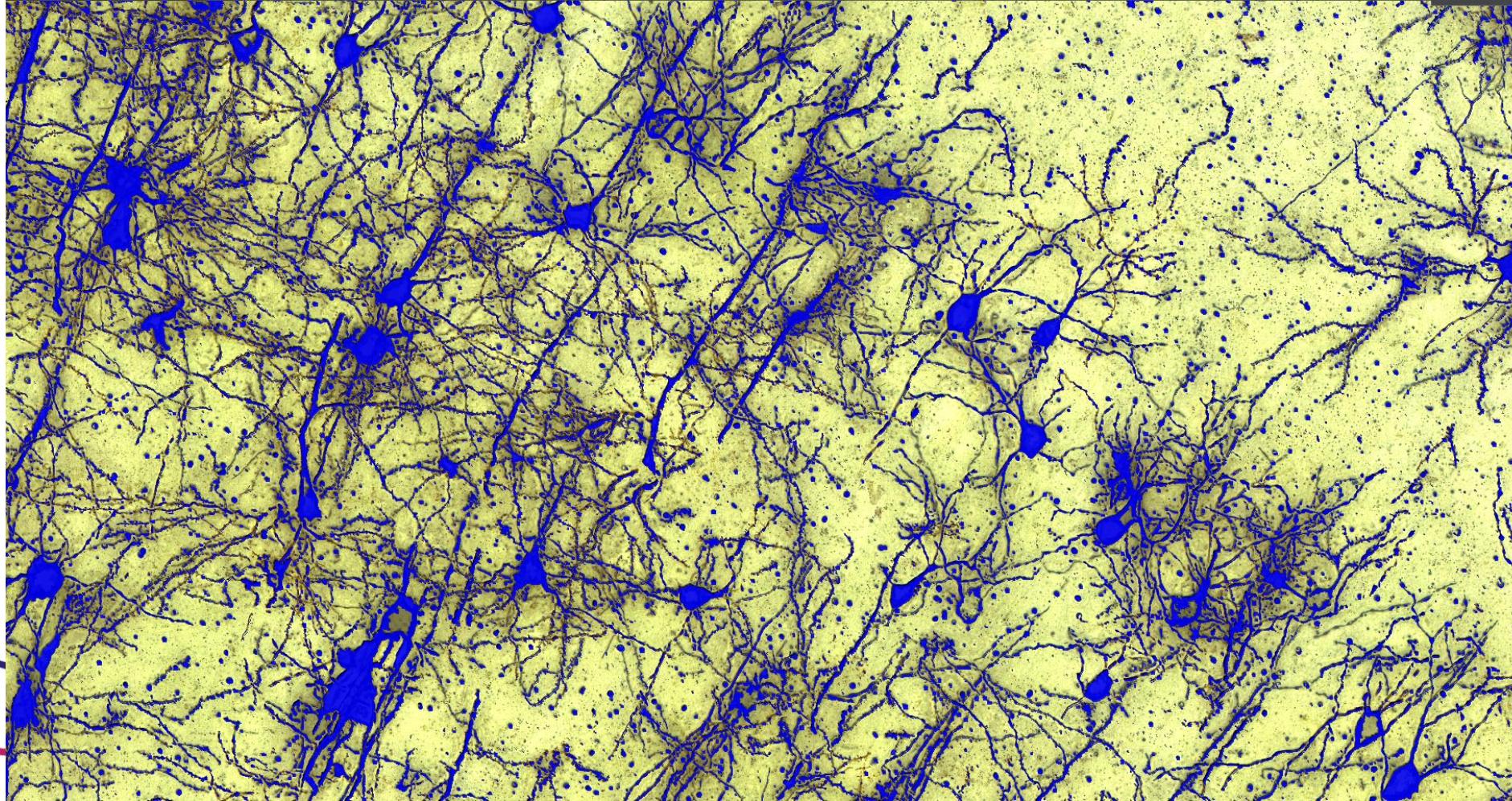
HistoQuant – Tissue segmentation

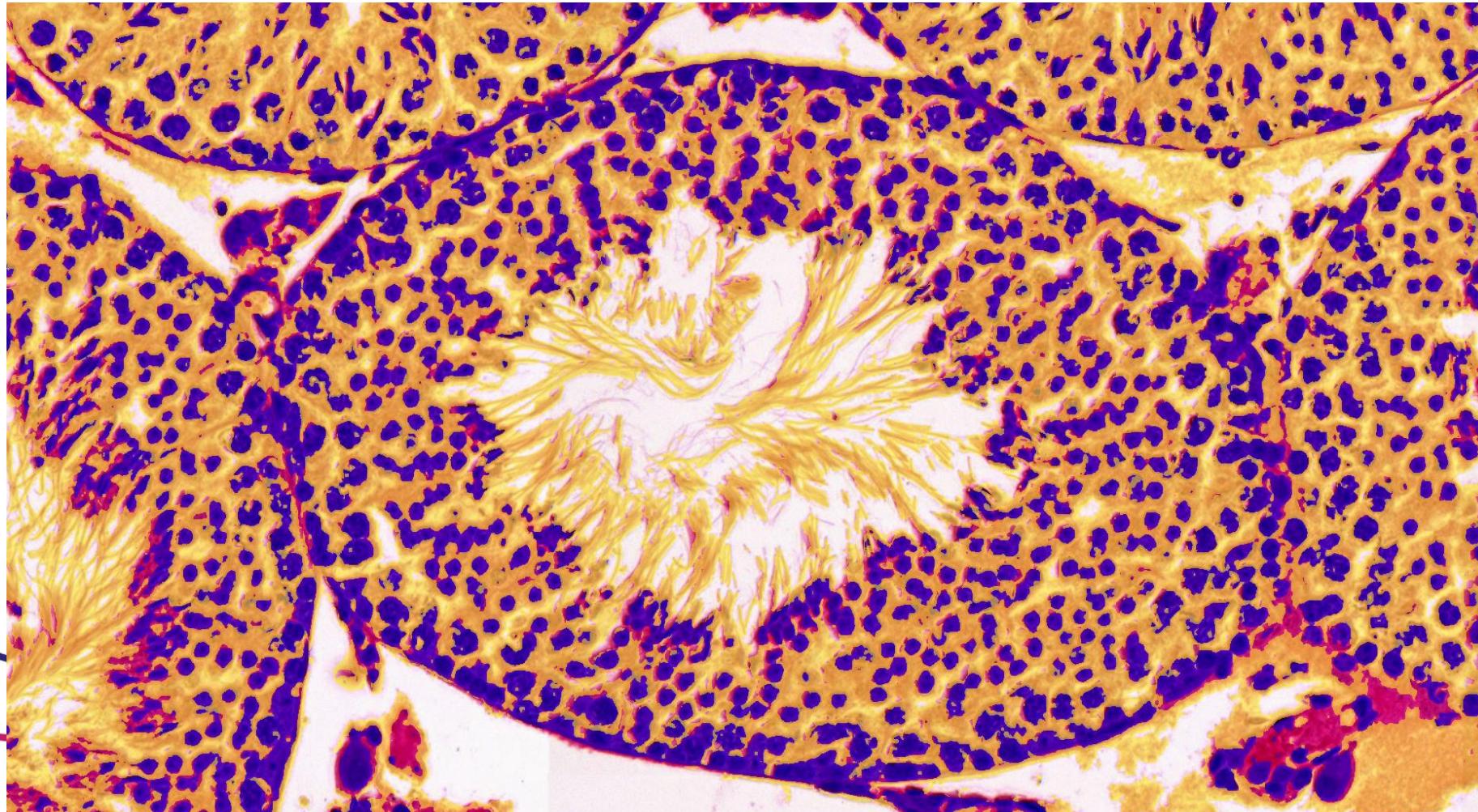
Keywords:

- General solution
- Object segmentation based on stain color
- Fluorescent stain examinations
- Presegmentation

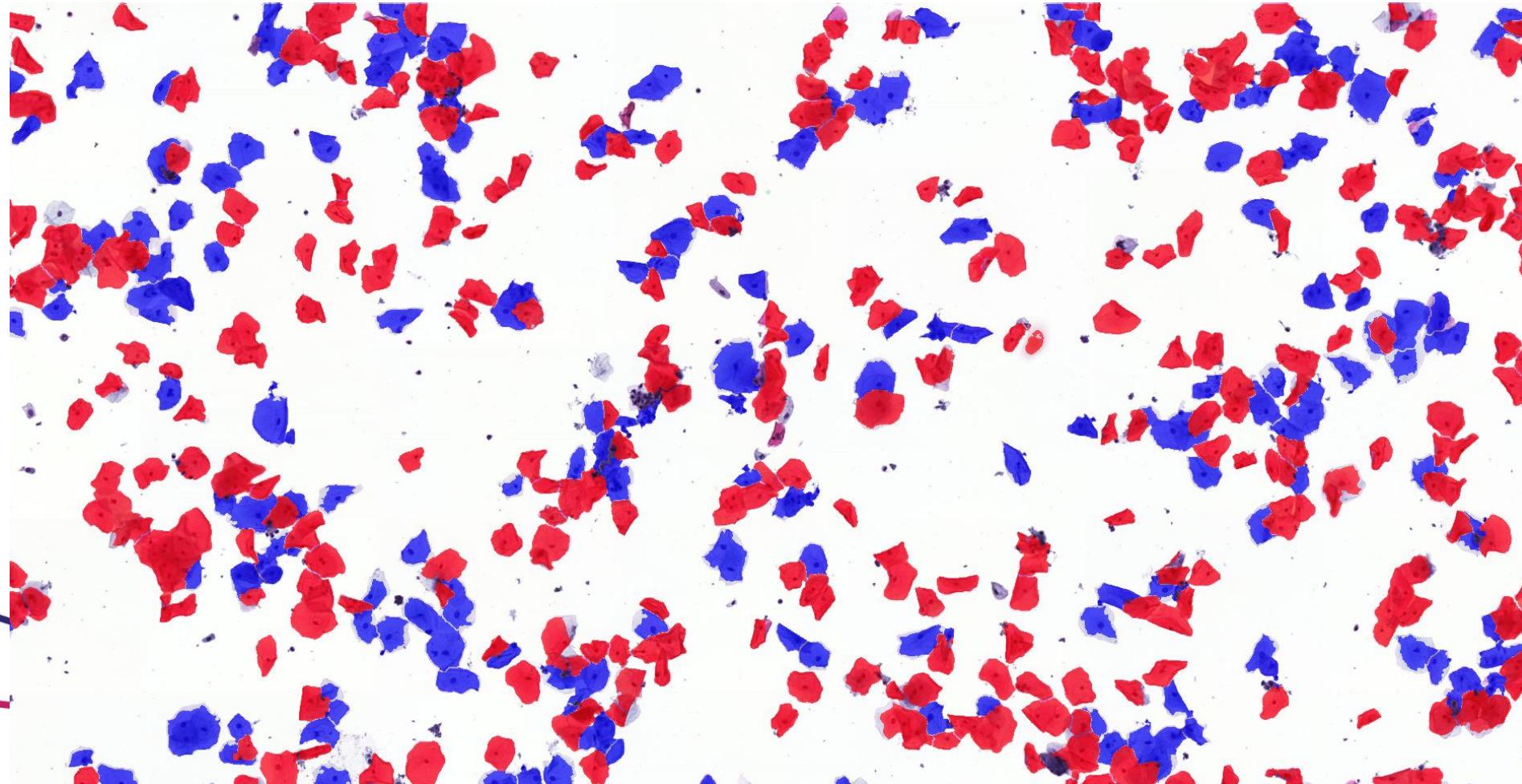


HistoQuant in practice

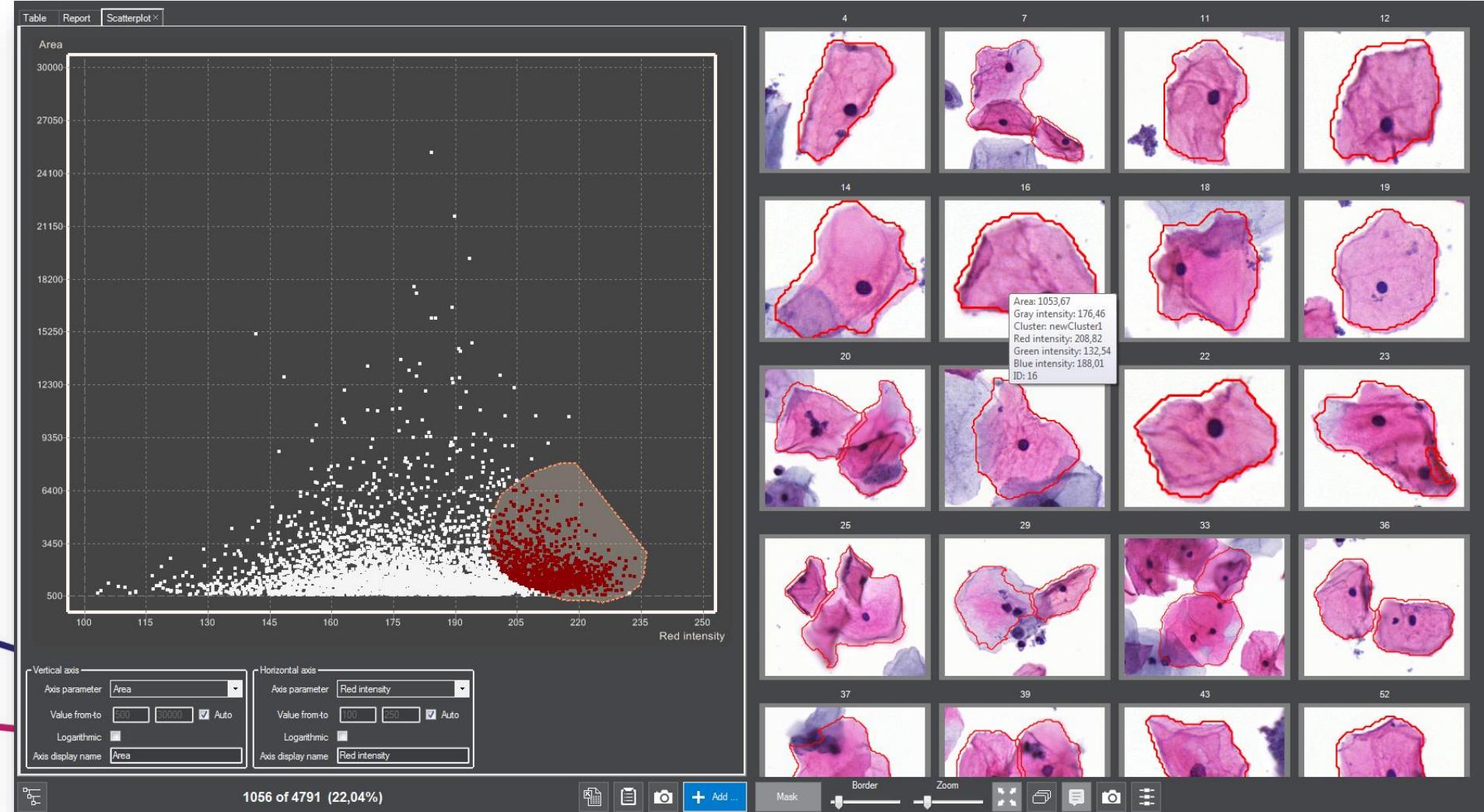




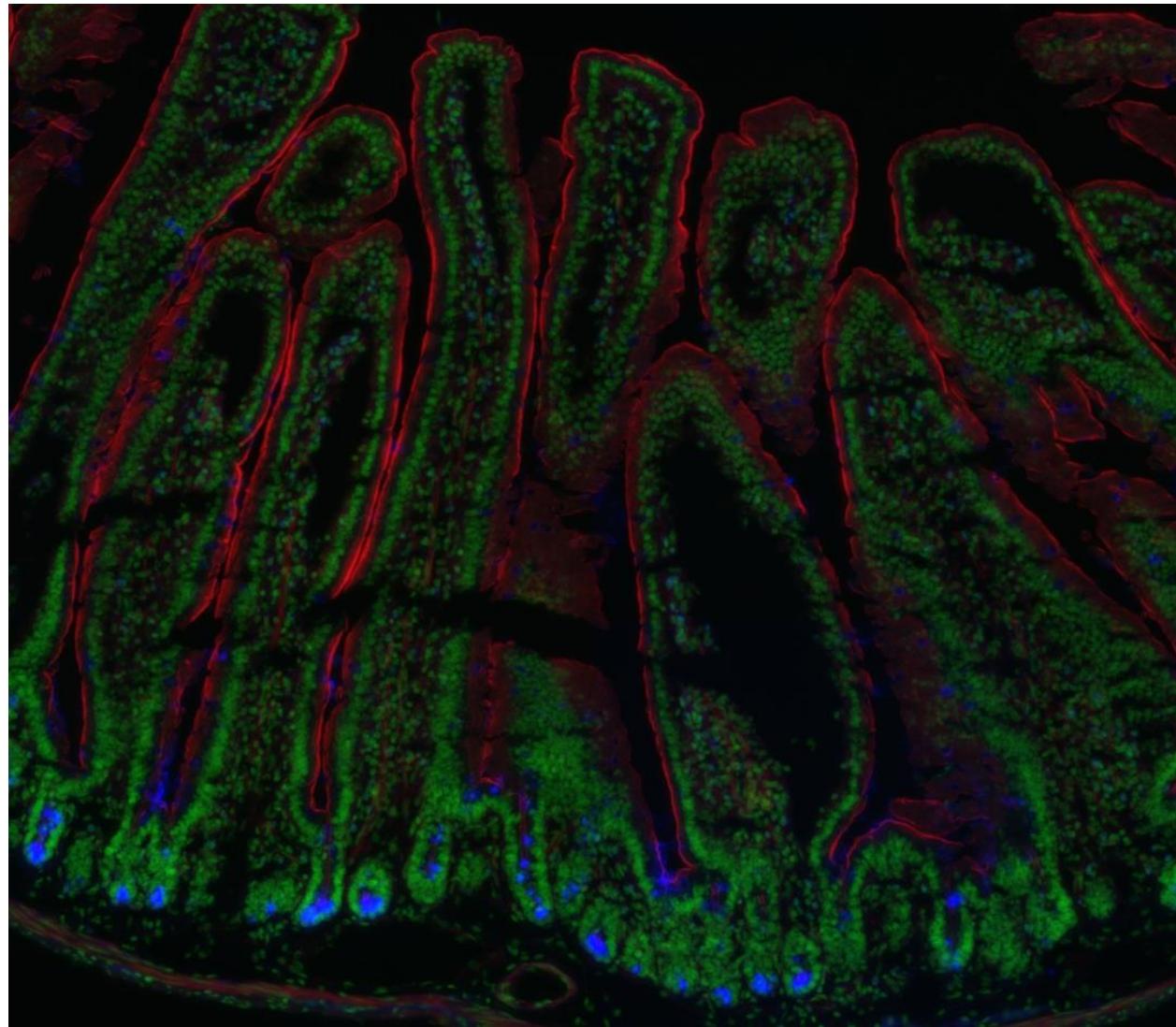
HistoQuant in practice



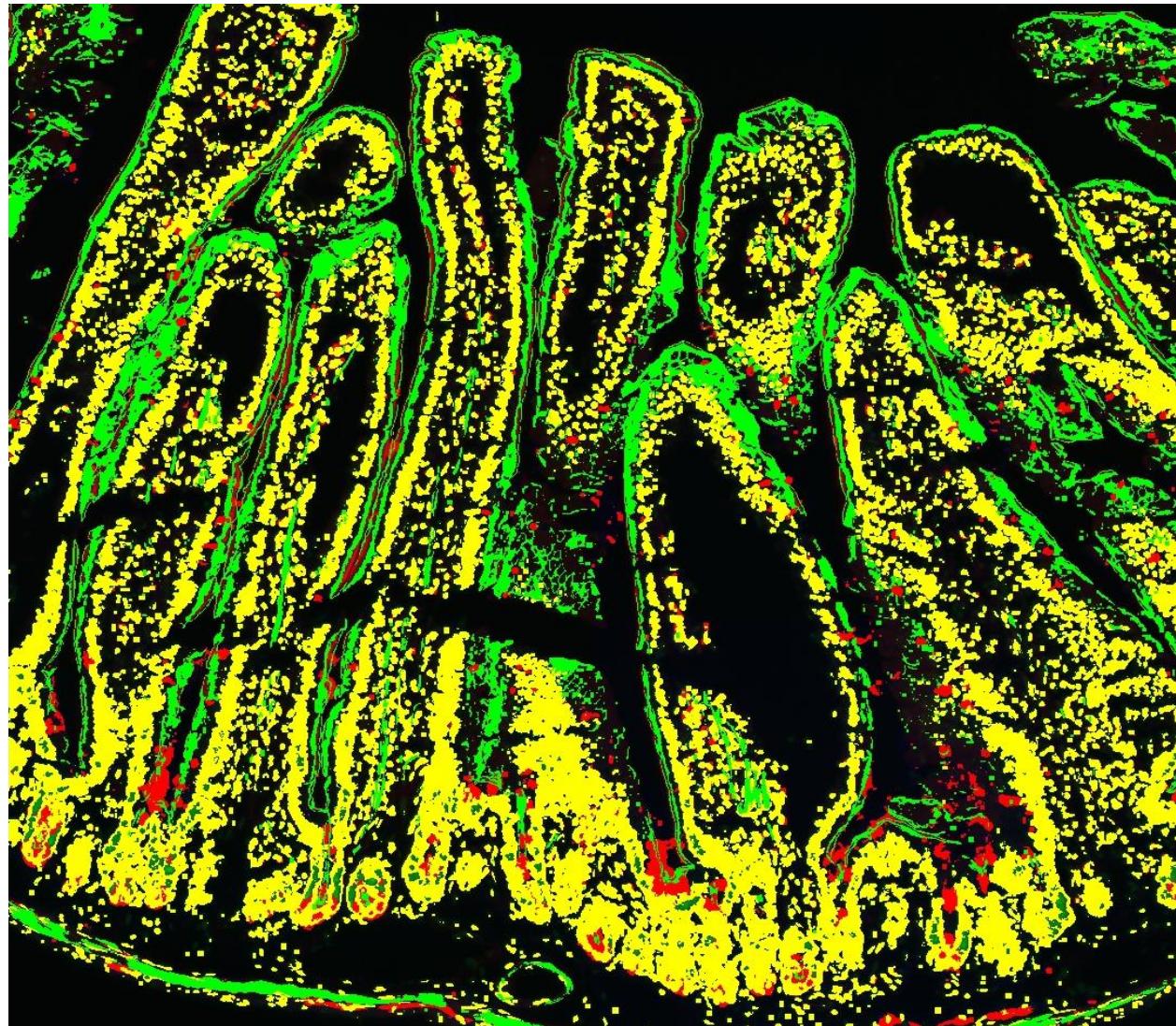
HistoQuant in practice



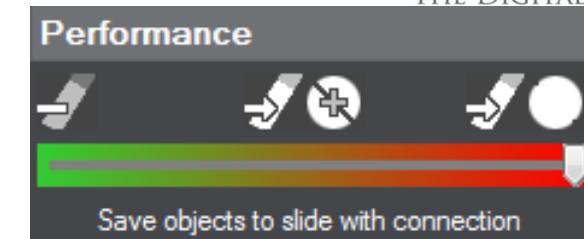
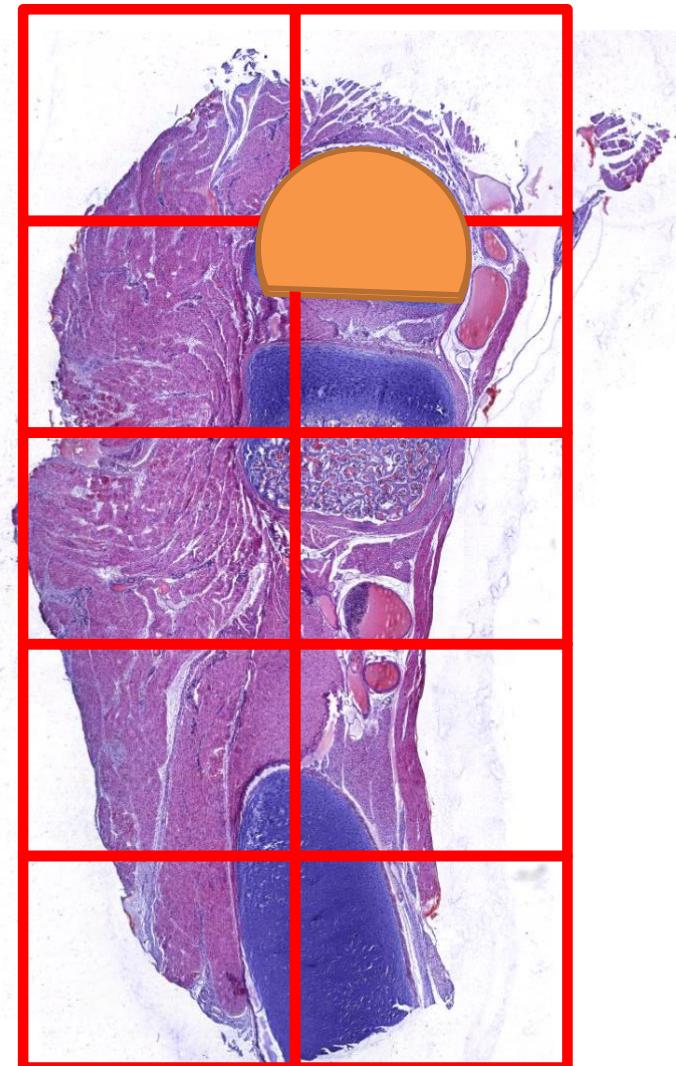
HistoQuant – Fluorescent application



HistoQuant – Fluorescent application



Whole slide optimization Object connection



Tile-based quantification

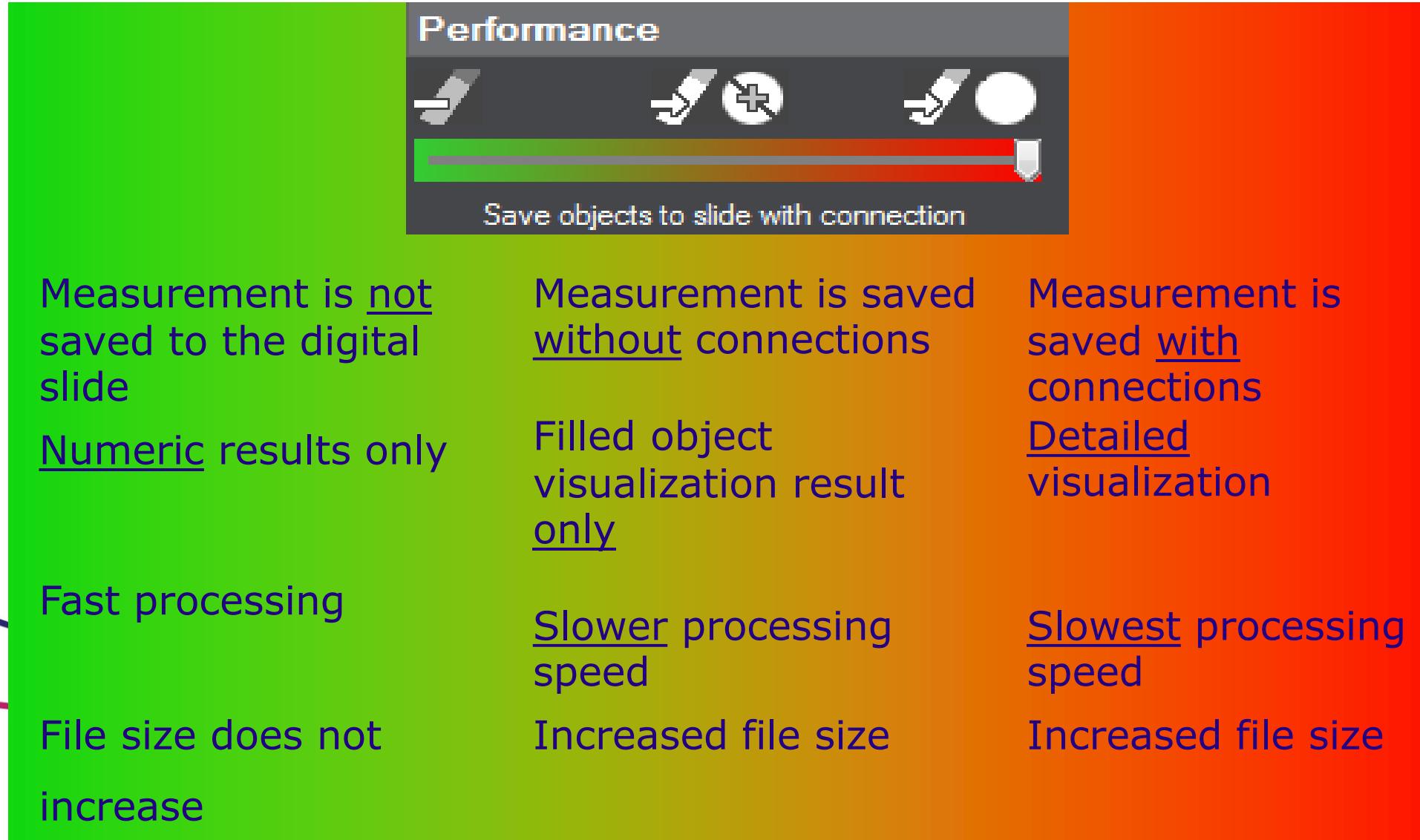
Object connecting process is
needed

Resource dependency

Measurement optimization

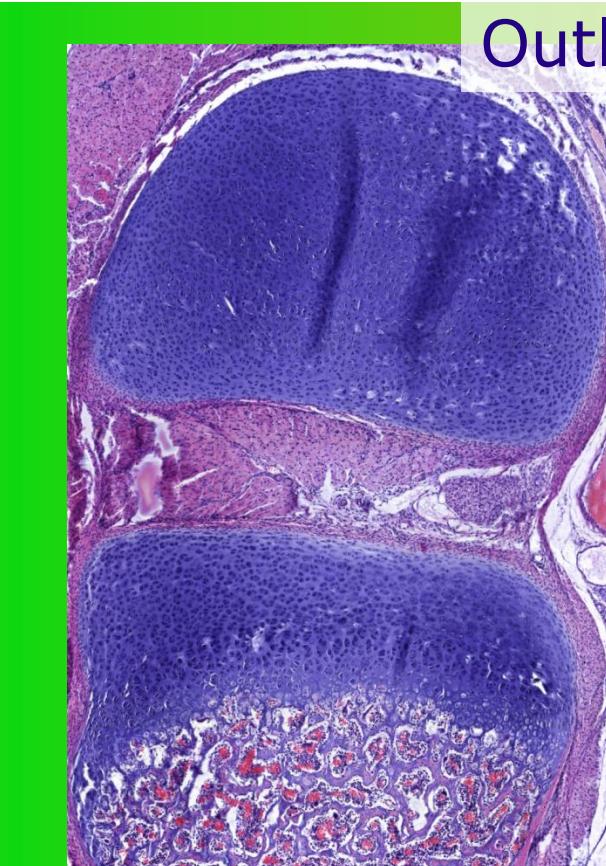
Whole slide optimization

Object connection



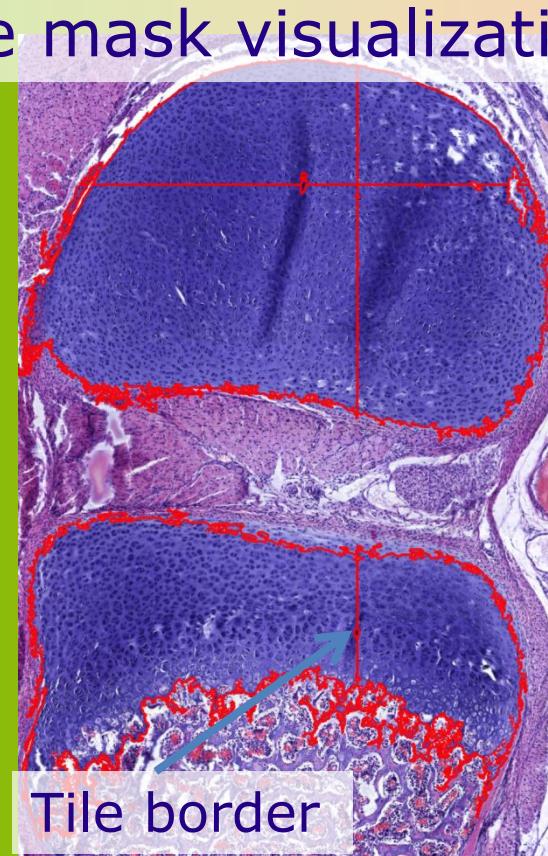
Whole slide optimization

Object connection

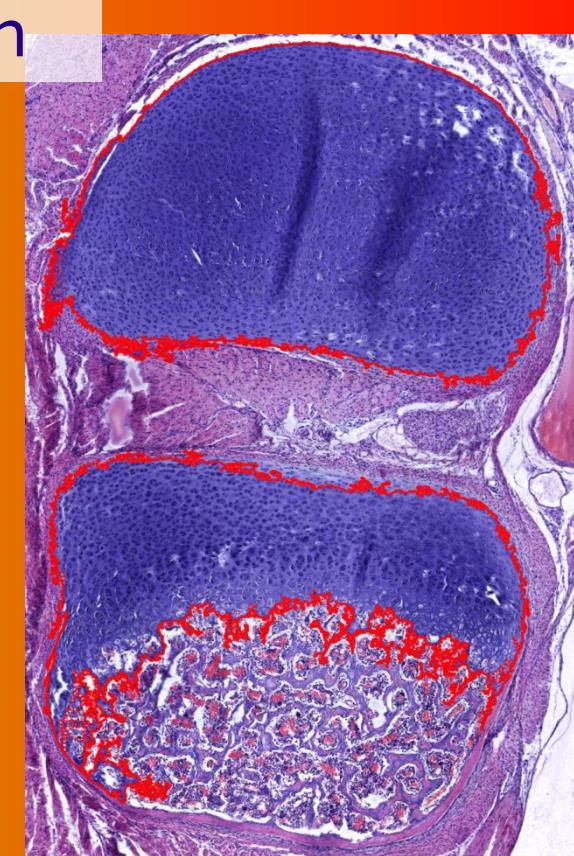


„Save objects
to slide”
is disabled

Outline mask visualization



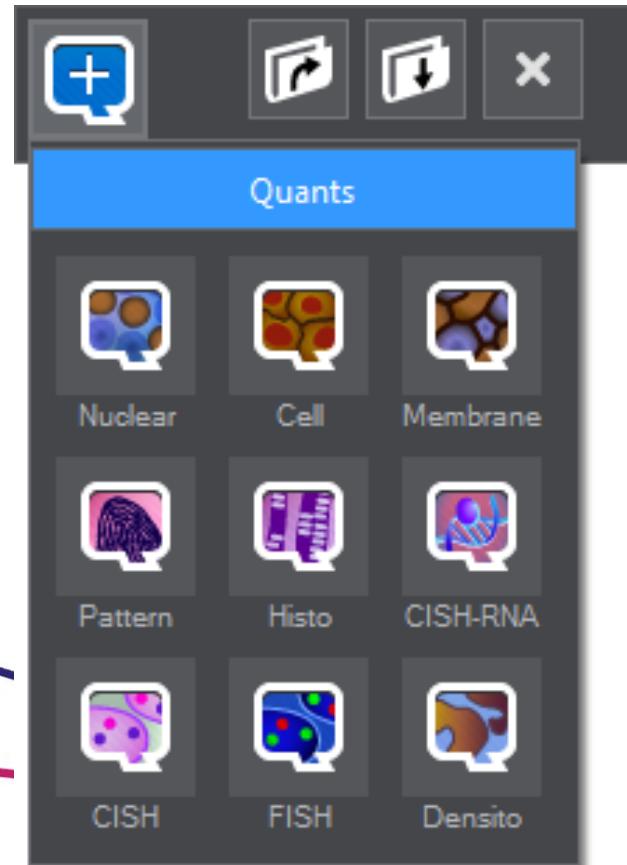
Save objects to
slide without
connection



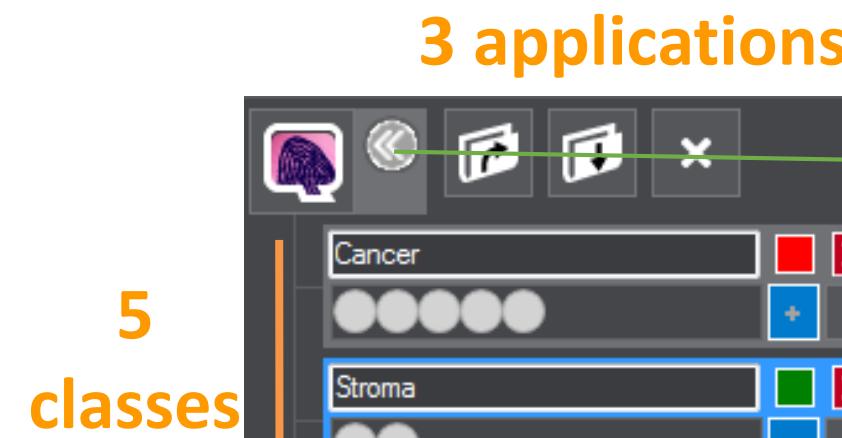
Save objects to
slide with
connection

Presegmentation in practice

Create your own image analysis **scenario** by linking the algorithms into a chain

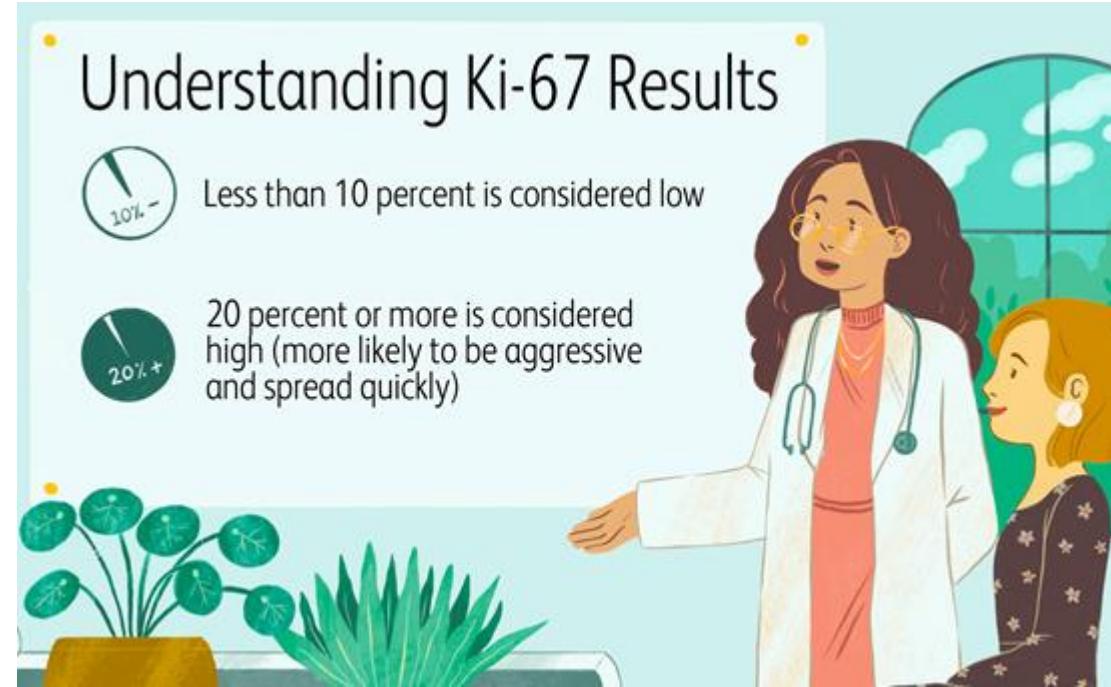


Define multi-level measurement by using Scenario Builder!



Types of breast cancer

- **Hormone Receptor-Positive Breast Cancer:**
 - **Estrogen, progesterone therapy**
- **HER2-Positive Breast Cancer:**
 - **Herceptin therapy**
- **Triple-Negative Breast Cancer:**
 - **generic therapy only (surgery, chemotherapy)**
- **Ki-67 test:**
 - **Ki-67 is a cancer antigen (protein) that's found in growing, dividing cells but is absent in the resting phase of cell growth**
 - **role in predicting chemotherapy response and prognosis**



Quantification of IHC samples in routine

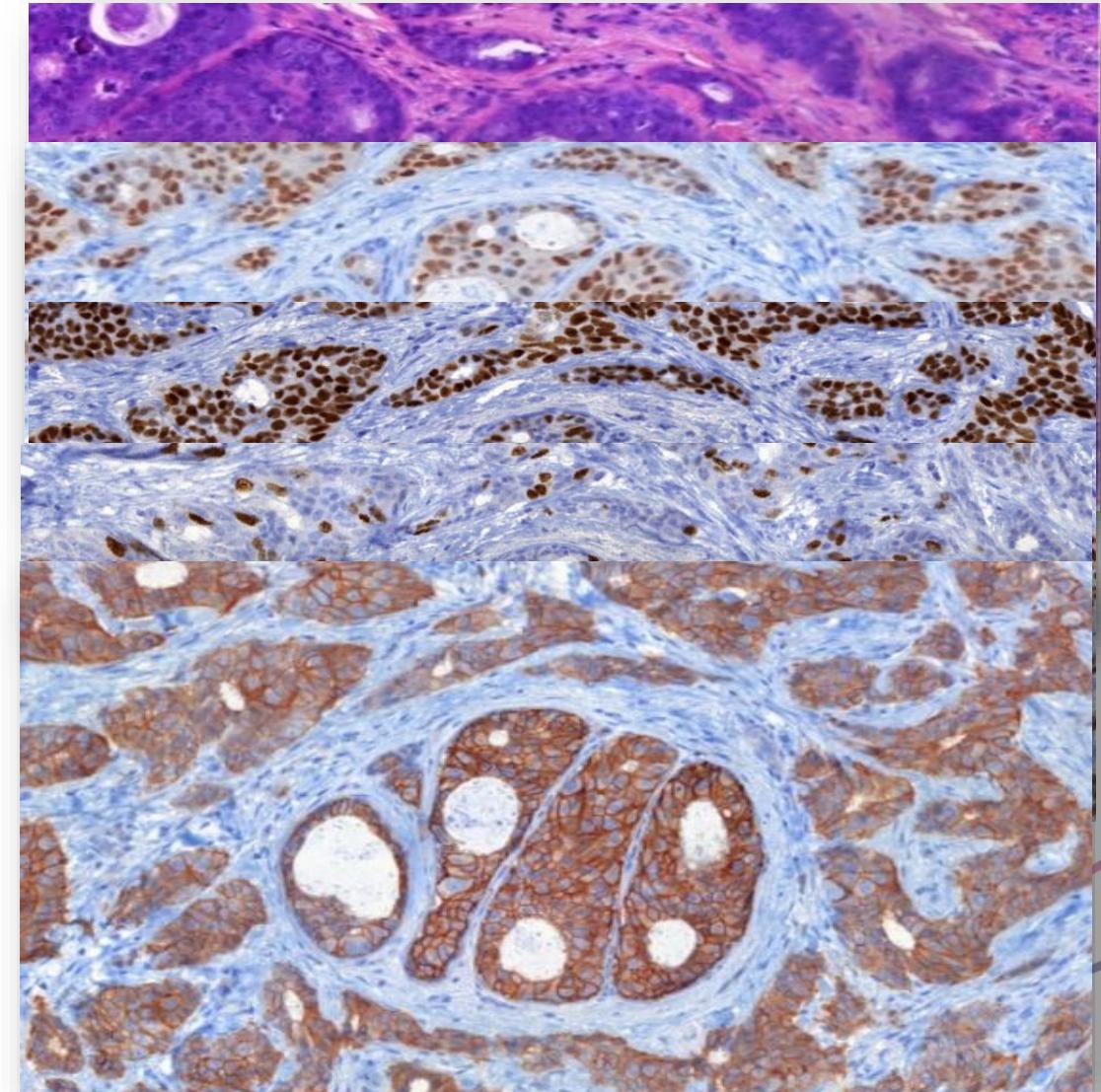
Breast Pathology

Breast IHC panel:

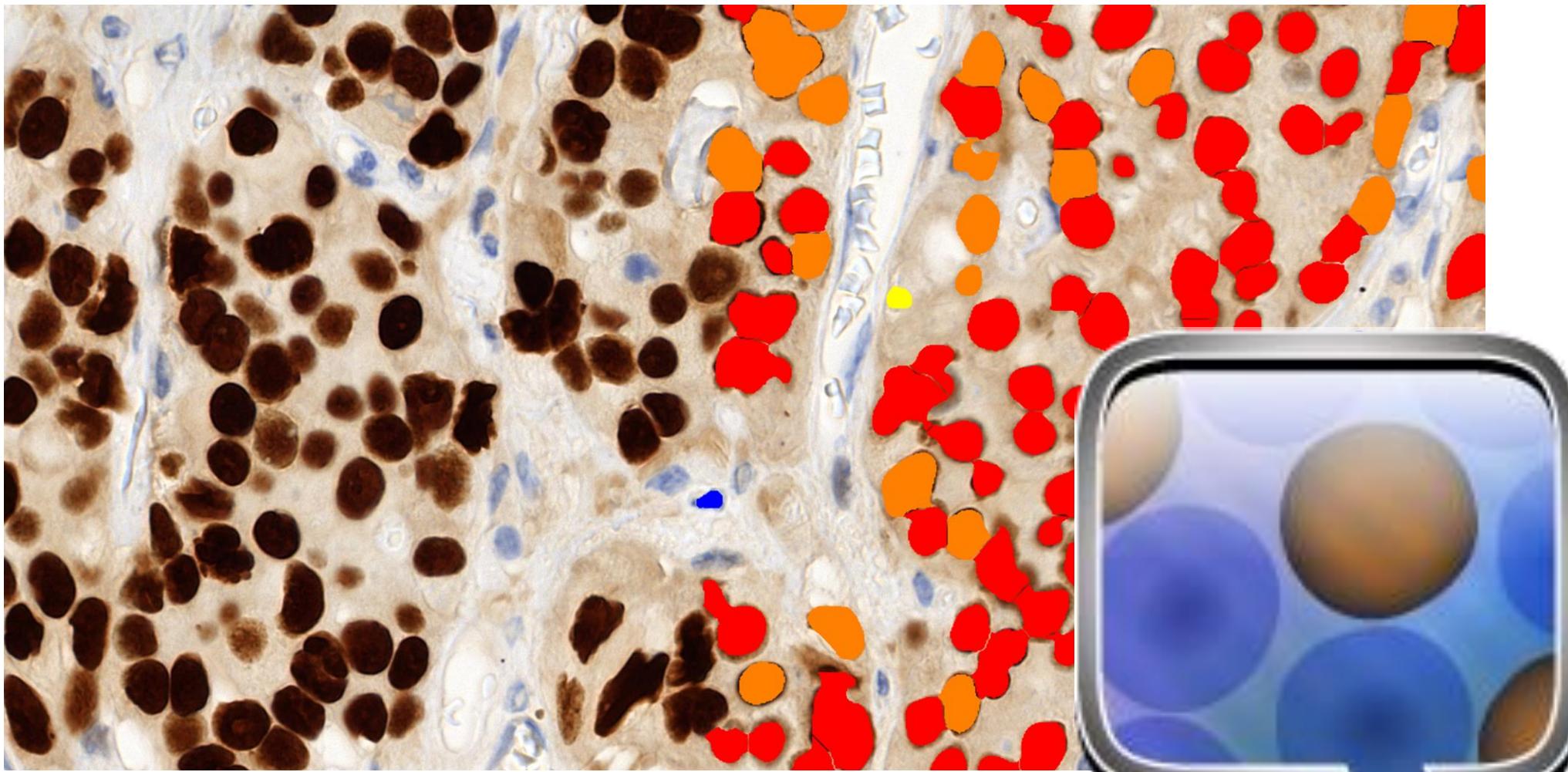
Nuclear markers

Membrane marker

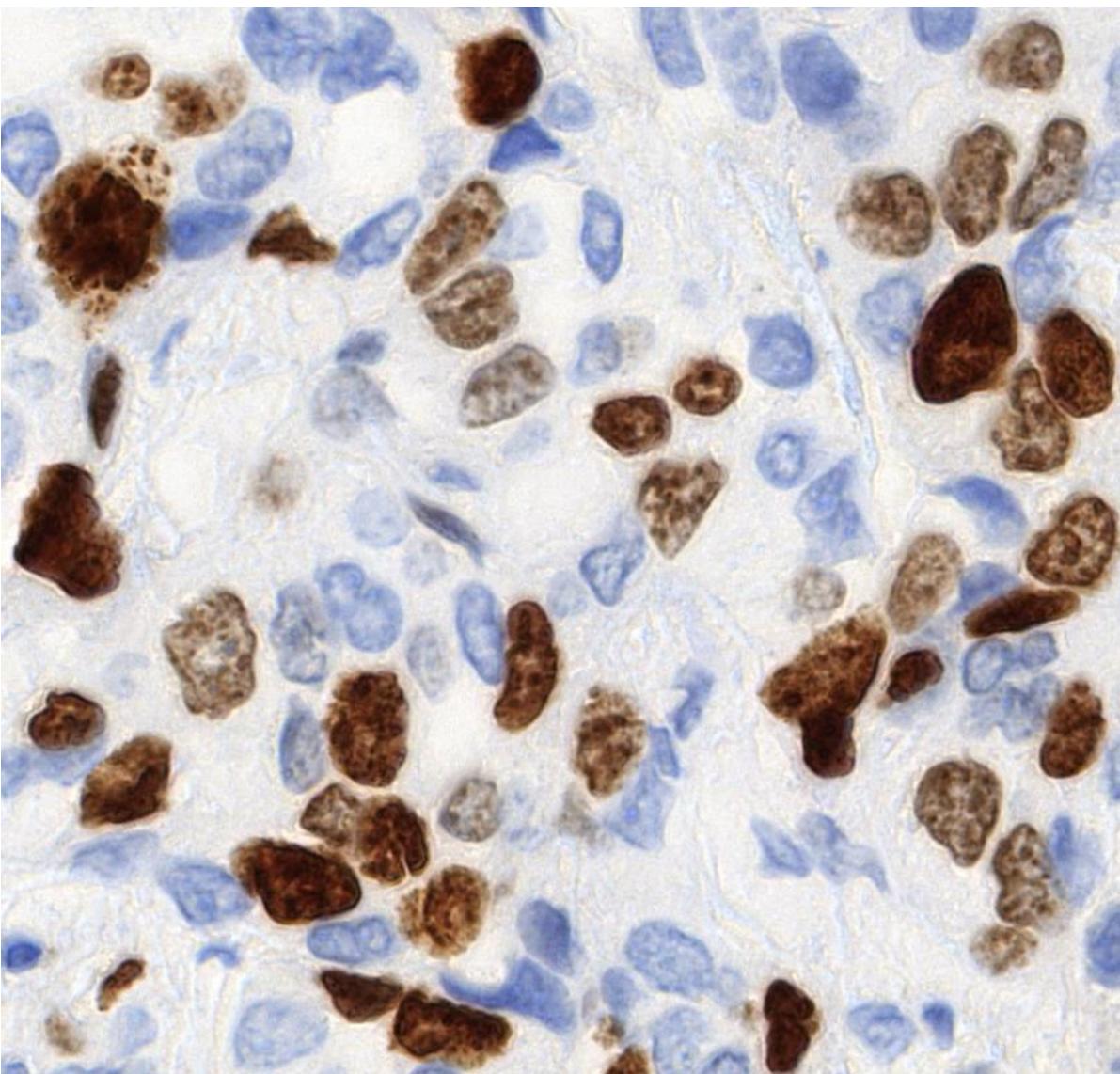
HE
Estrogen
Progesterone
Ki67
HER2



Nuclear Quant



About the IHC algorithm



IHC stains:

- different stain procedures → different intensity
- different intensity → different score value

Solution:

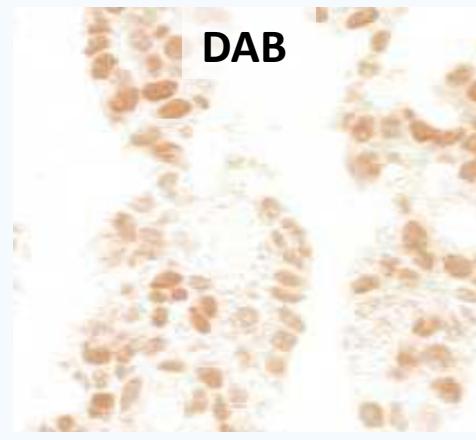
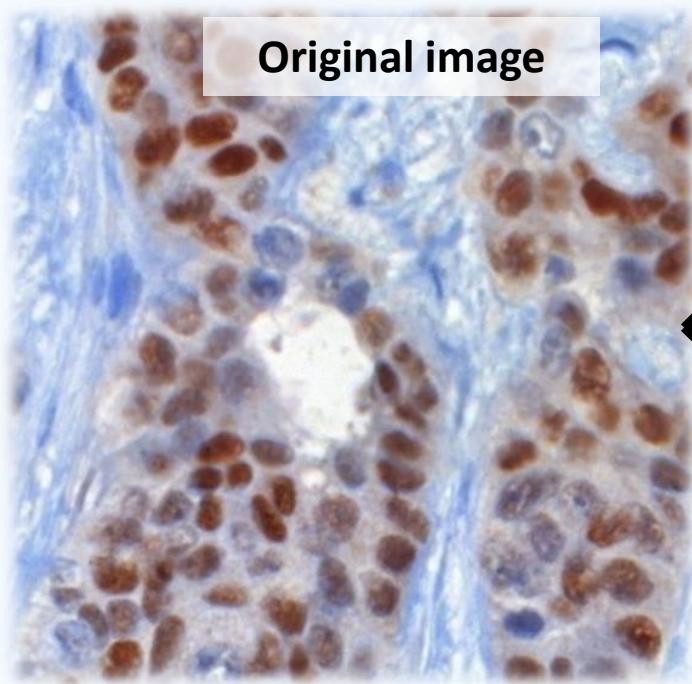
Color separation – Color deconvolution process

Advantages:

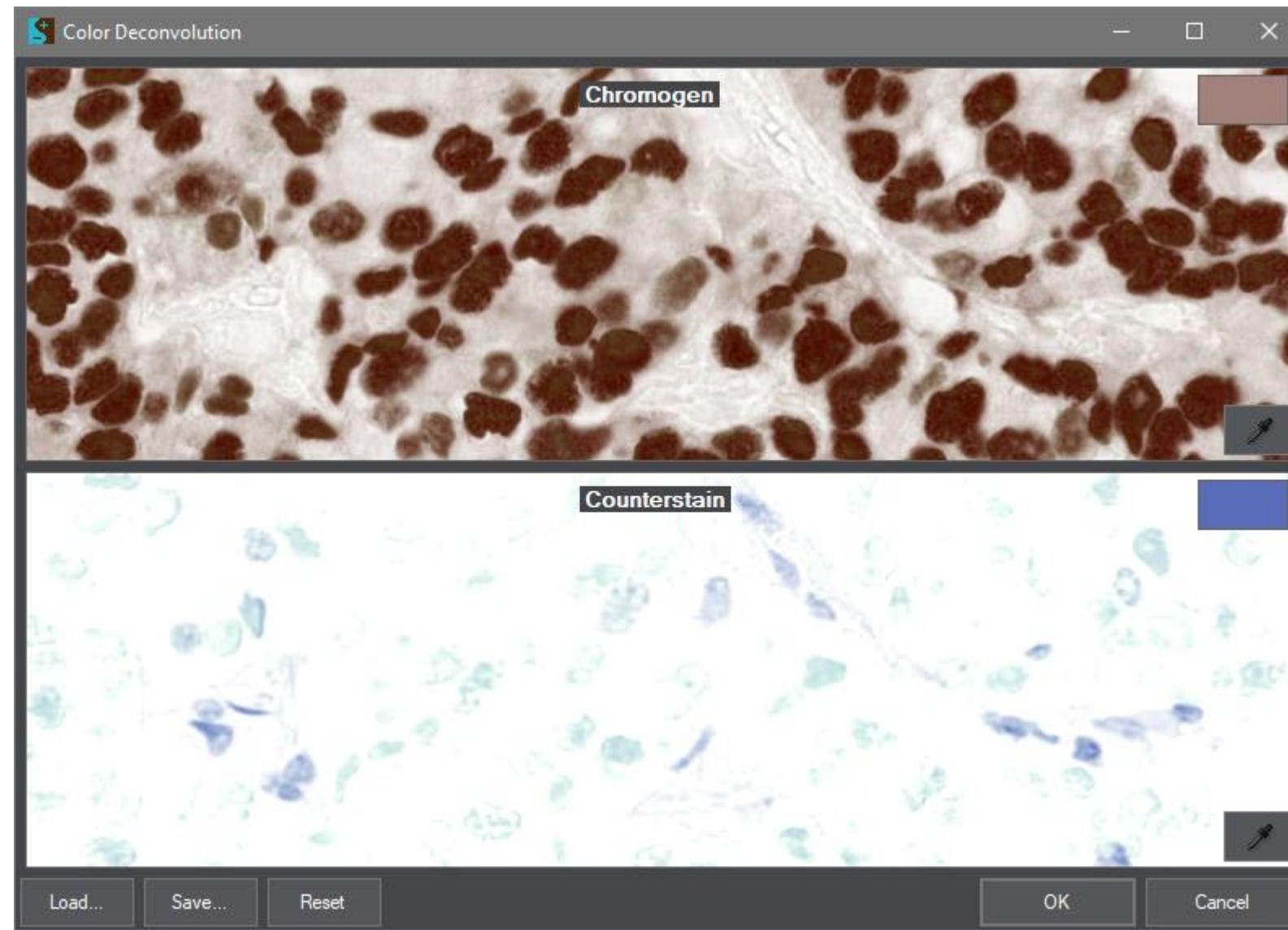
- The algorithm can be calibrated
 - to the local laboratory protocol in the customer side
 - to different stainers

About the algorithm

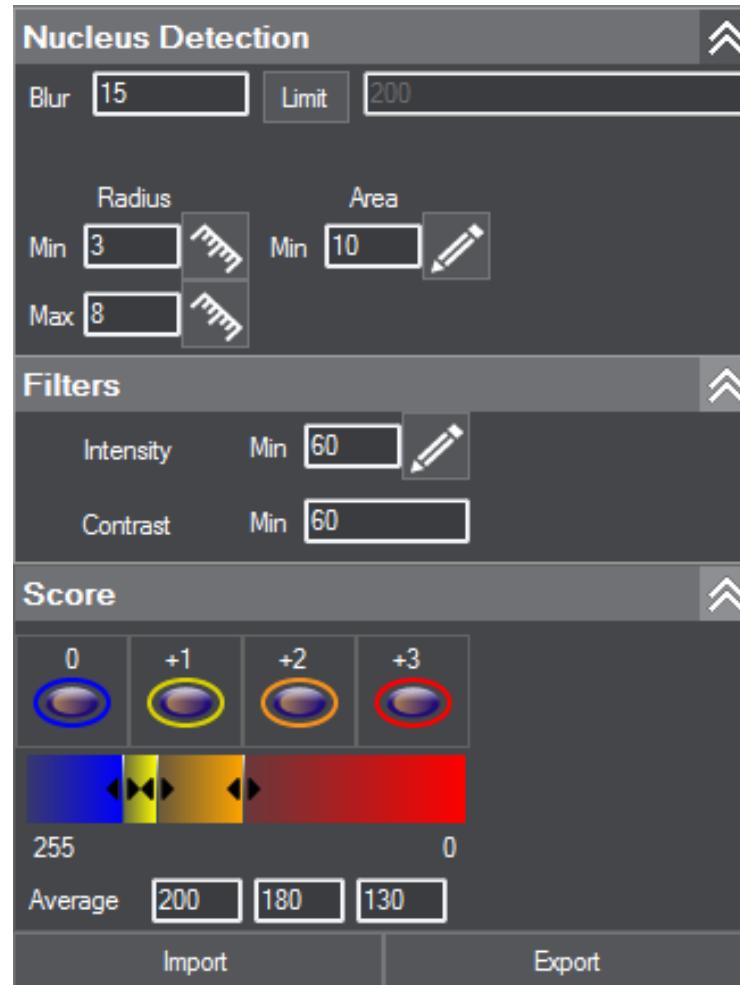
Color Separation



Color Deconvolution



Other settings



The morphological parameters are optimized to breast tissue

The intensity has to be calibrated specially to the local sample quality

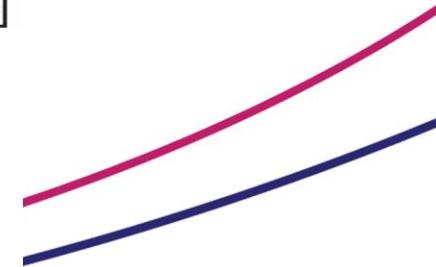
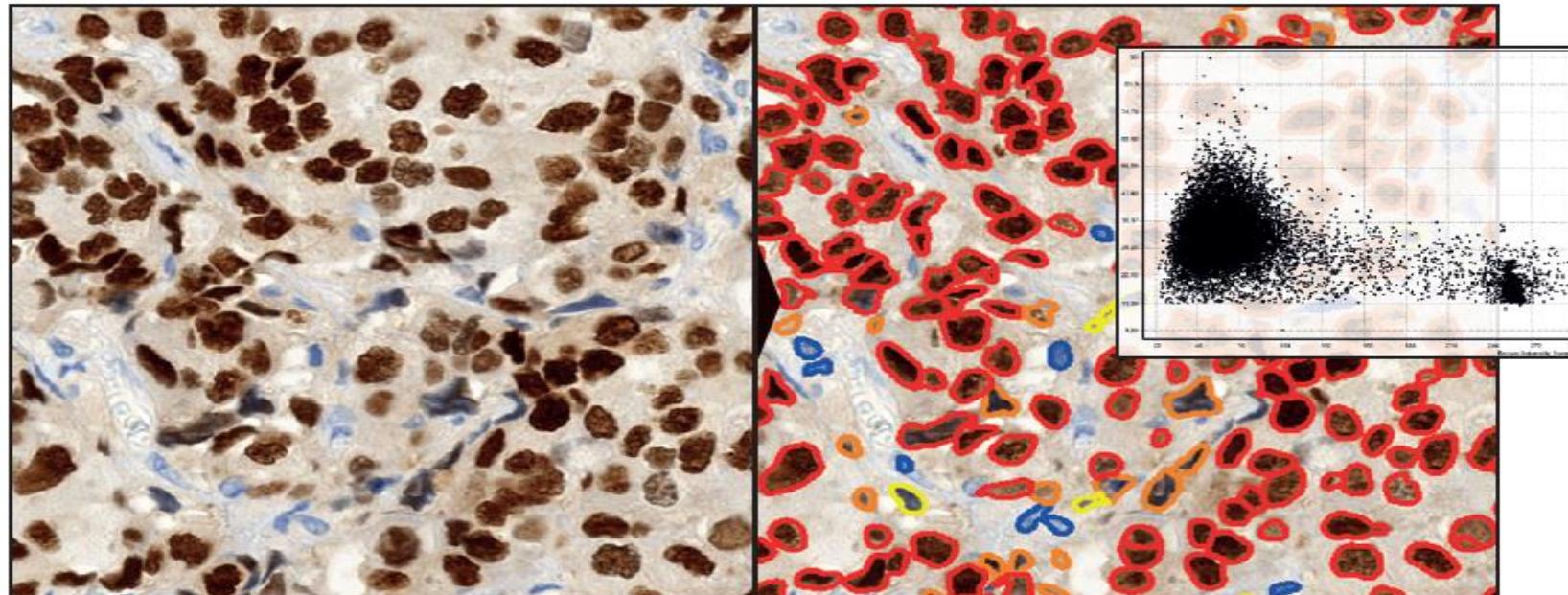
Score definition:

- Calibration to the pathologist
- Threshold based classification

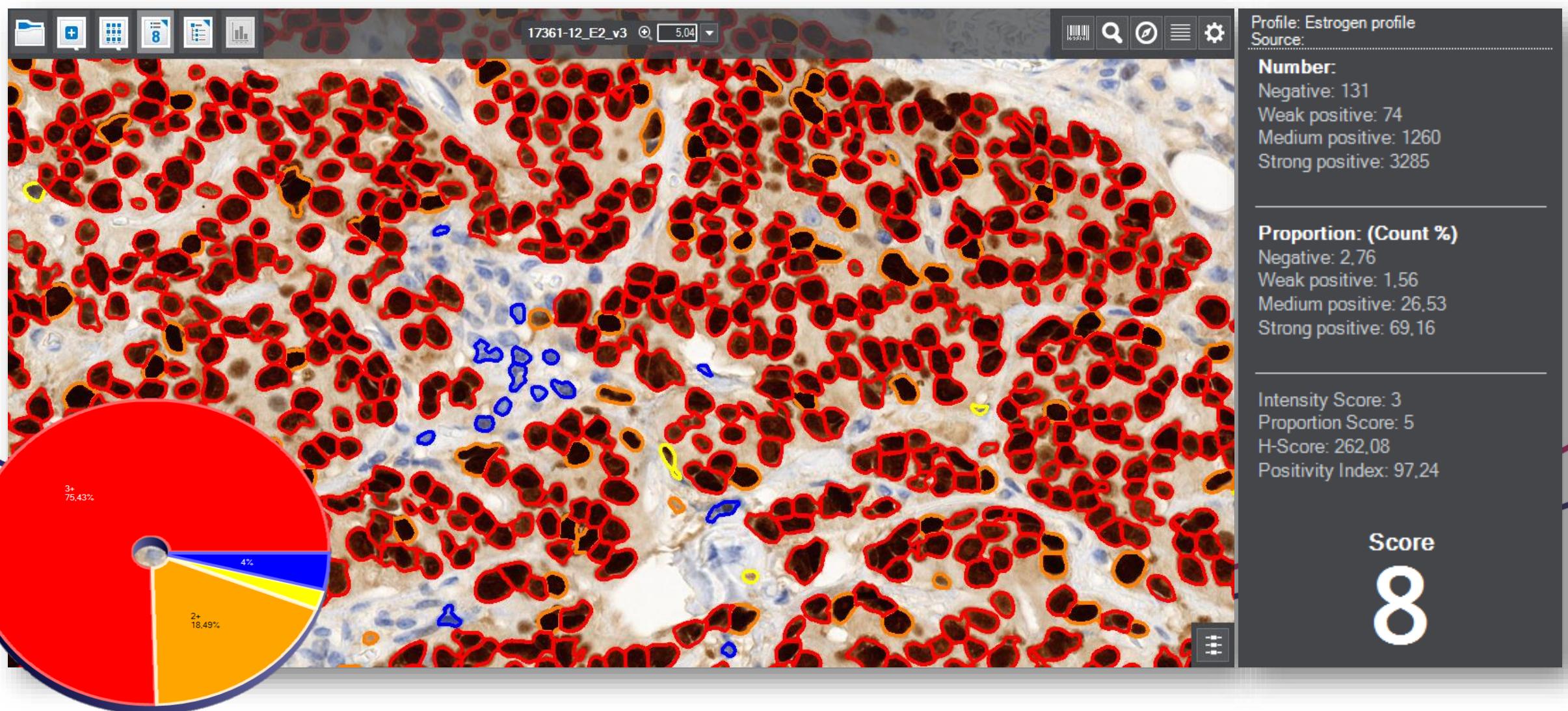
NuclearQuant application



- Estrogen and Progesterone stain
- **IVD** approved
- tested on a clinical trial
- color profile based cell nuclei detection



The result



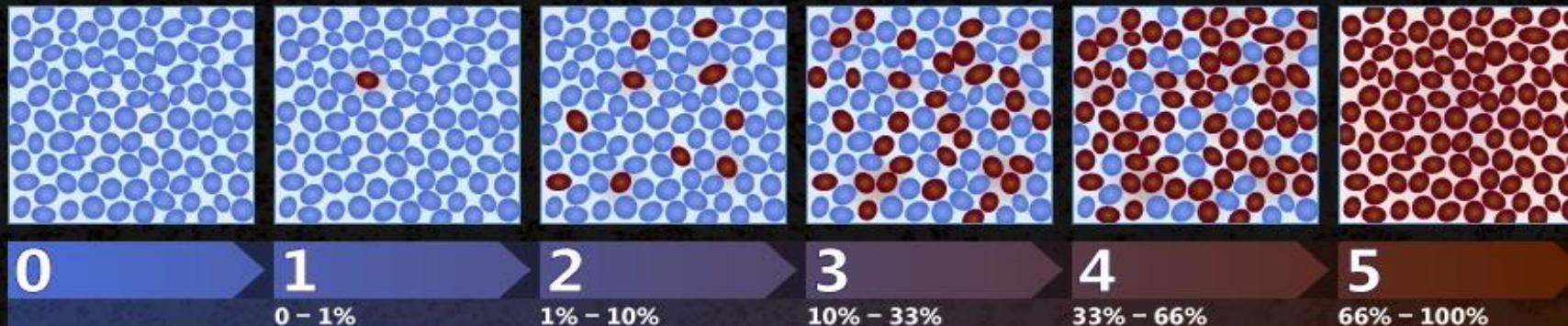


Scoring

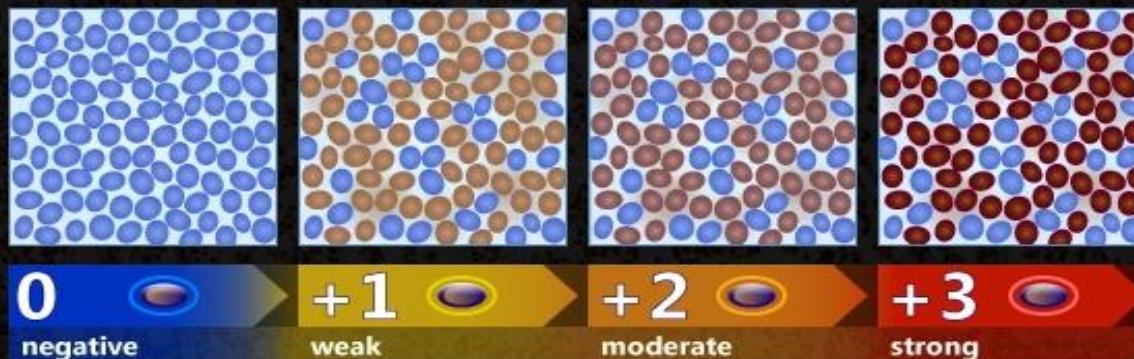
Breast Cancer

Allred Scoring System for the semiquantitative assessment of steroid receptor Estrogen and Progesterone positivity

Proportion Score



Intensity Score



Total score =
Proportion score + Intensity score
(between 0–8)

Under 3 score the tumors are negative

Profile: Estrogen profile
Source:

Number:

Negative: 131
Weak positive: 74
Medium positive: 1260
Strong positive: 3285

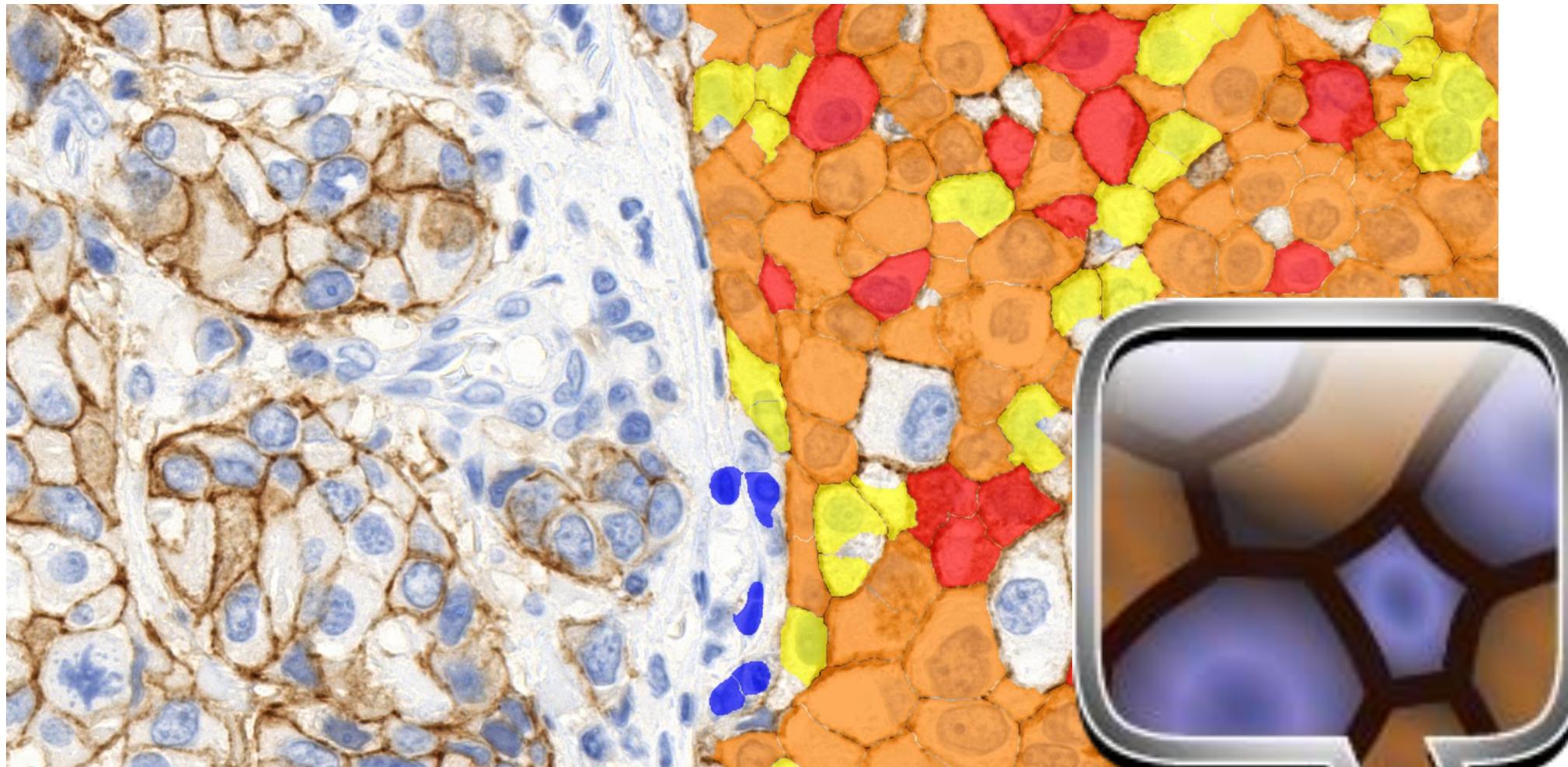
Proportion: (Count %)

Negative: 2,76
Weak positive: 1,56
Medium positive: 26,53
Strong positive: 69,16

Intensity Score: 3
Proportion Score: 5
H-Score: 262,08
Positivity Index: 97,24

Score
8

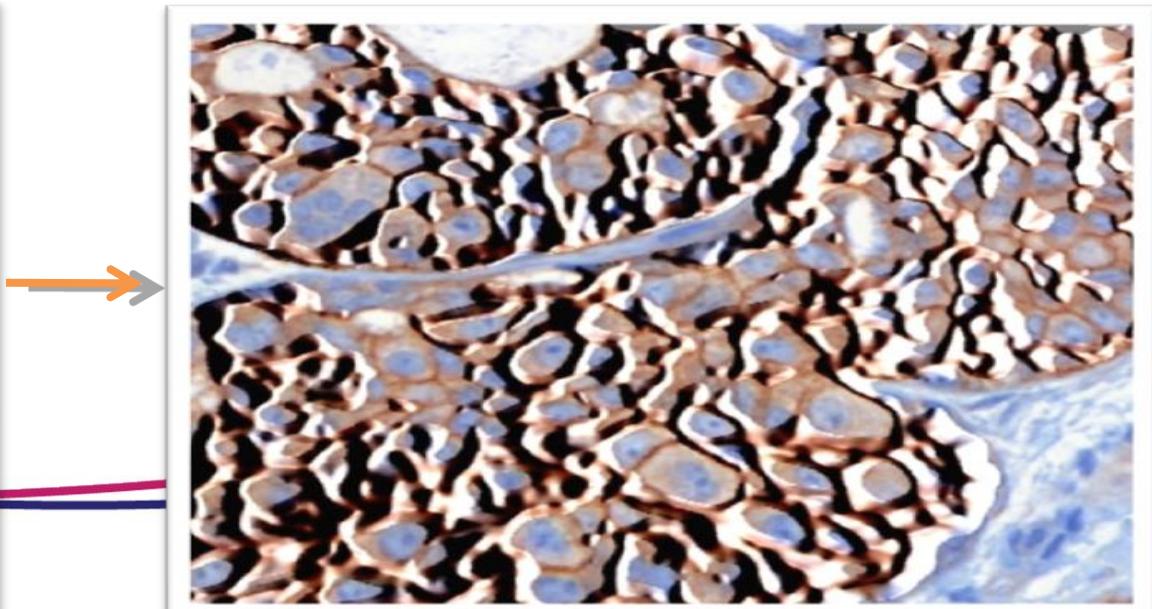
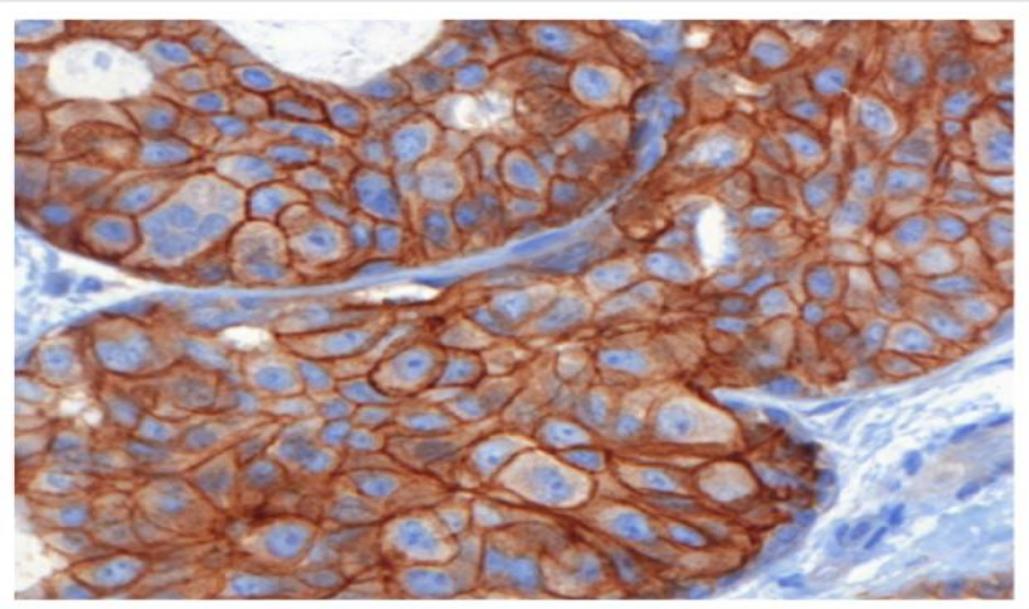
Membrane Quant



MembraneQuant

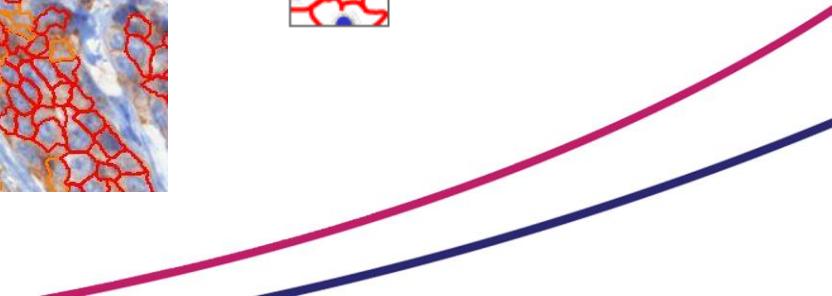
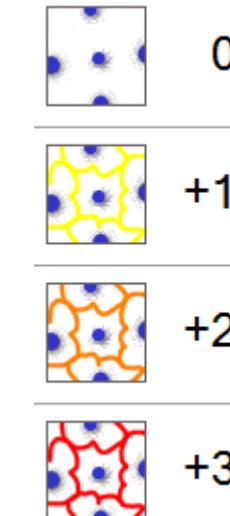
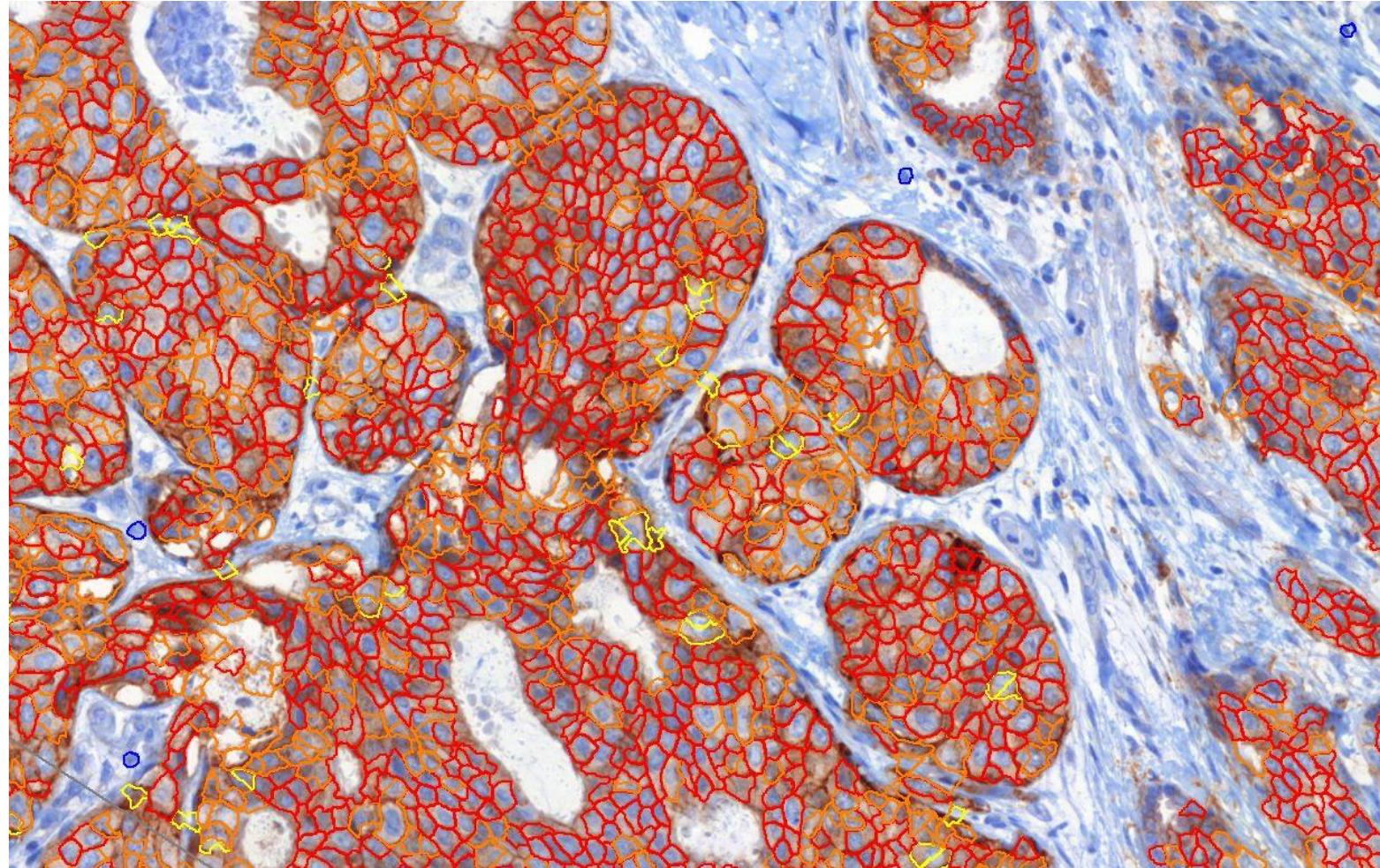


- Her2 stain analysis
- IVD approved
- tested on several clinical trial
- color profile based detection

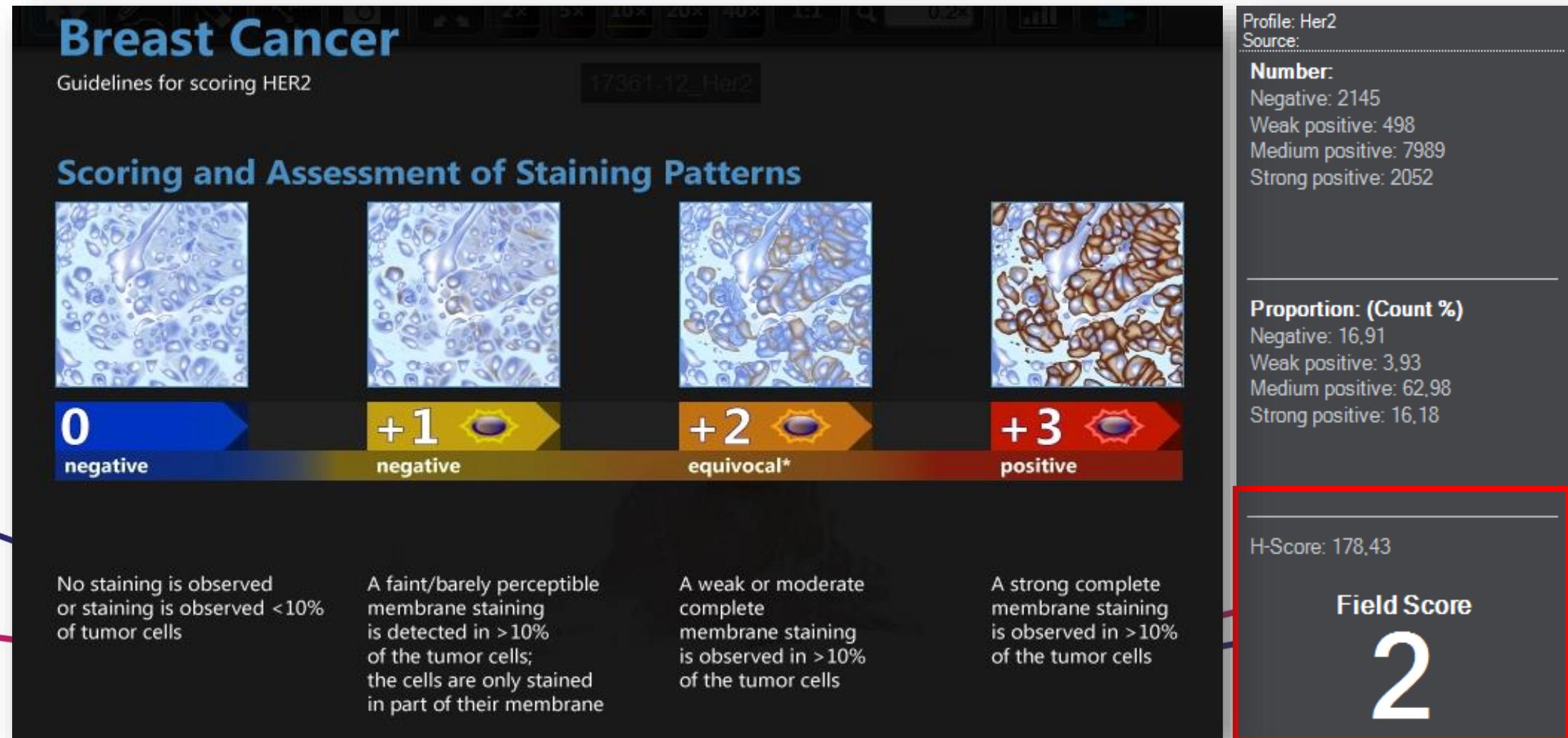


Membrane Quant

IHC quantification of membrane markers

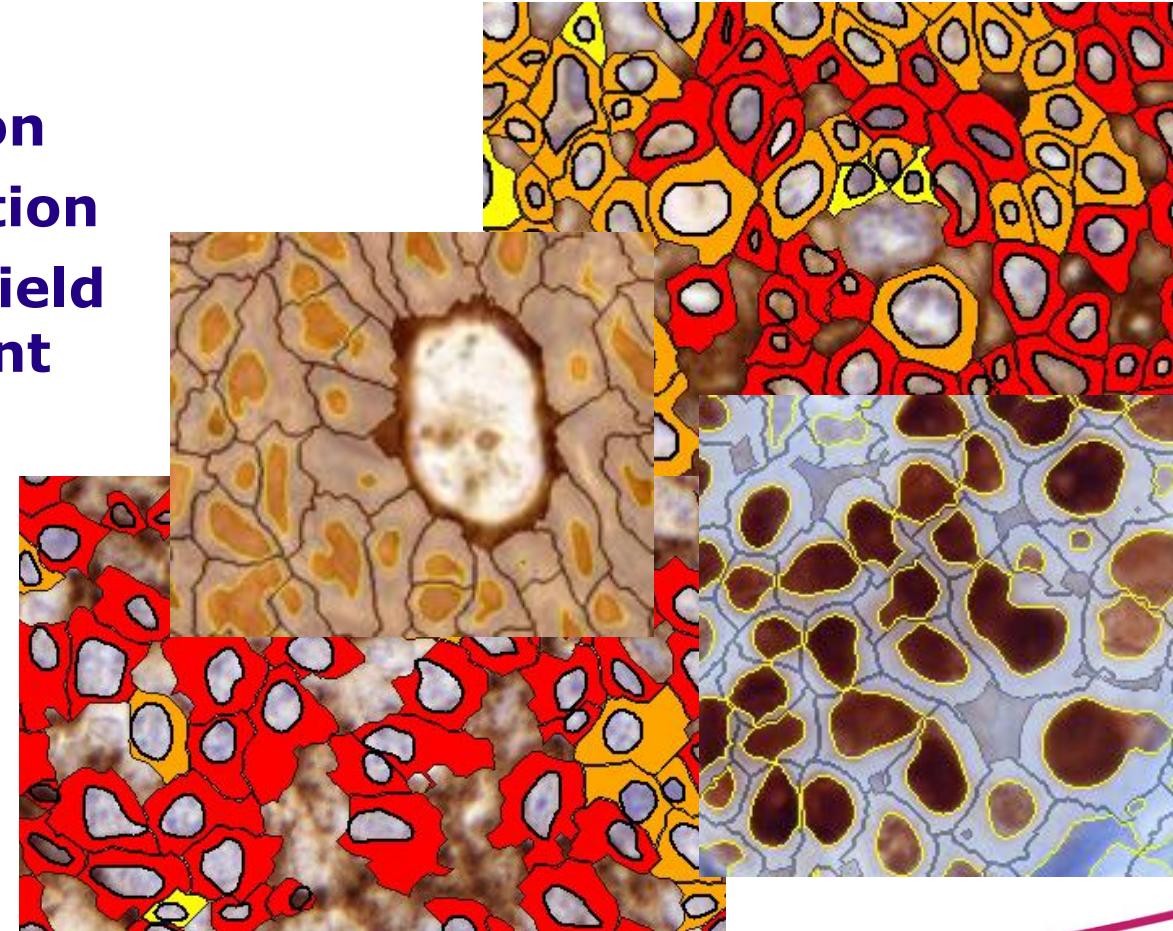


Her2 scoring

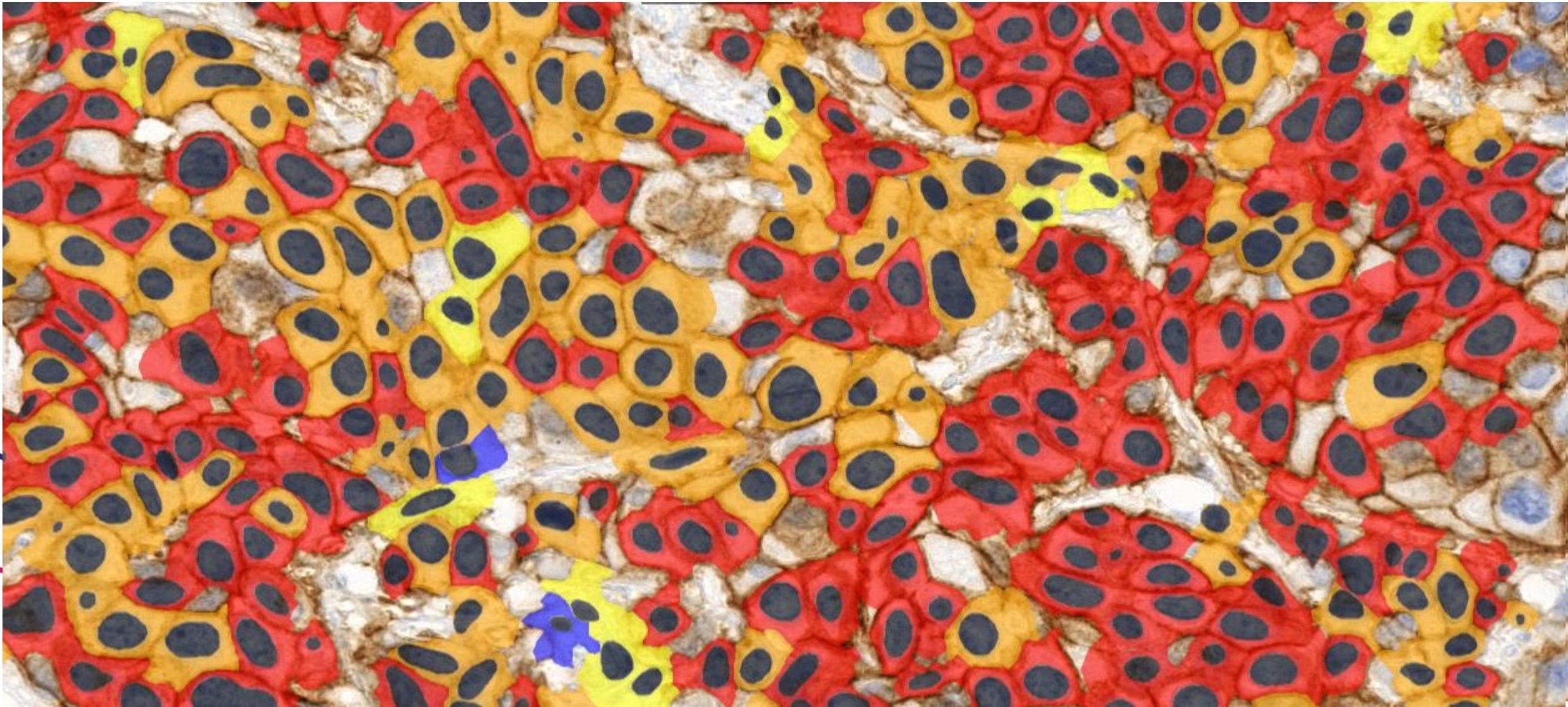


CellQuant

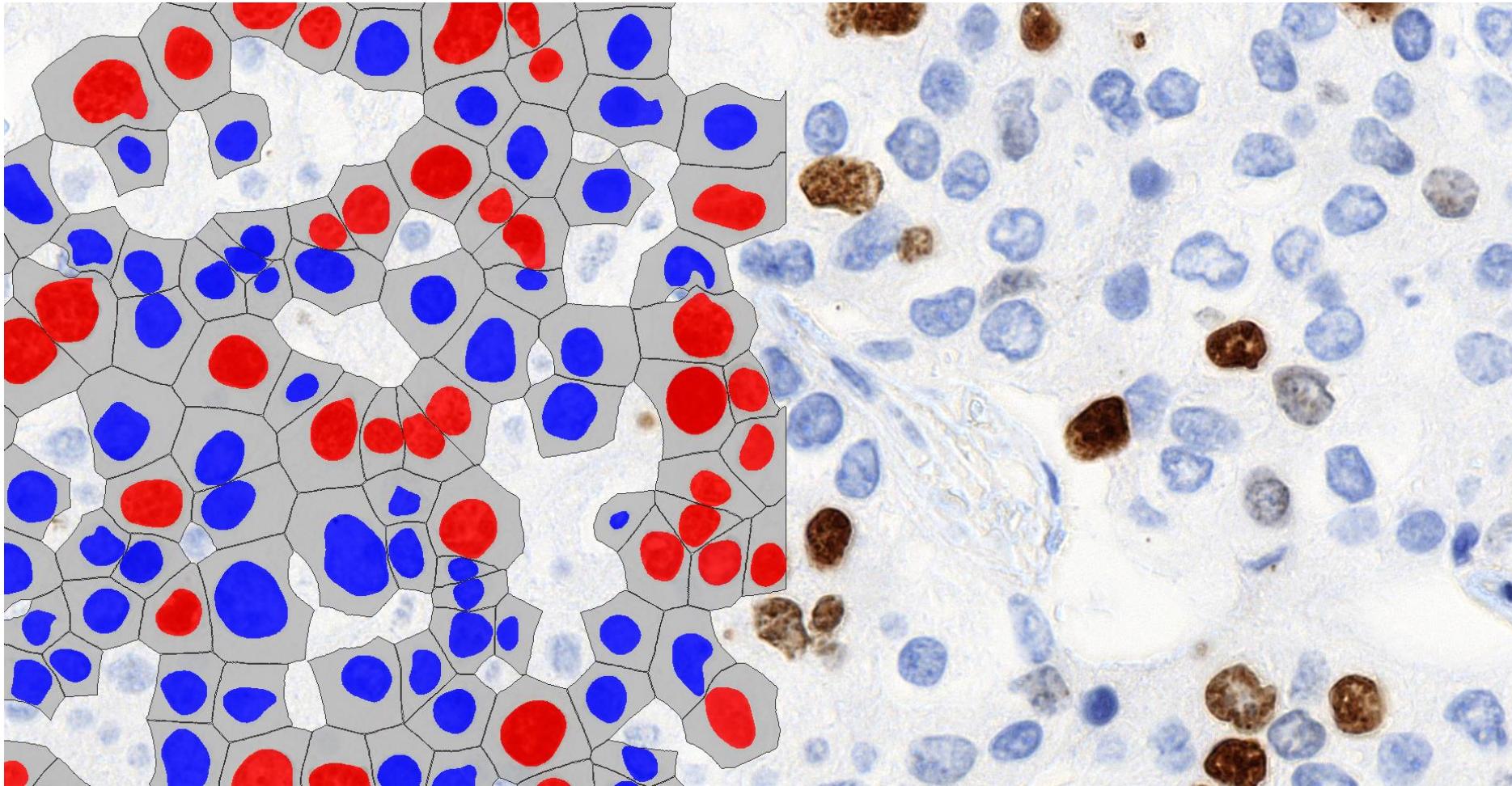
- **IHC Quantification**
- **Whole cell detection**
- **Works on brightfield and on fluorescent slides**



CellQuant – Cytoplasm detection and scoring



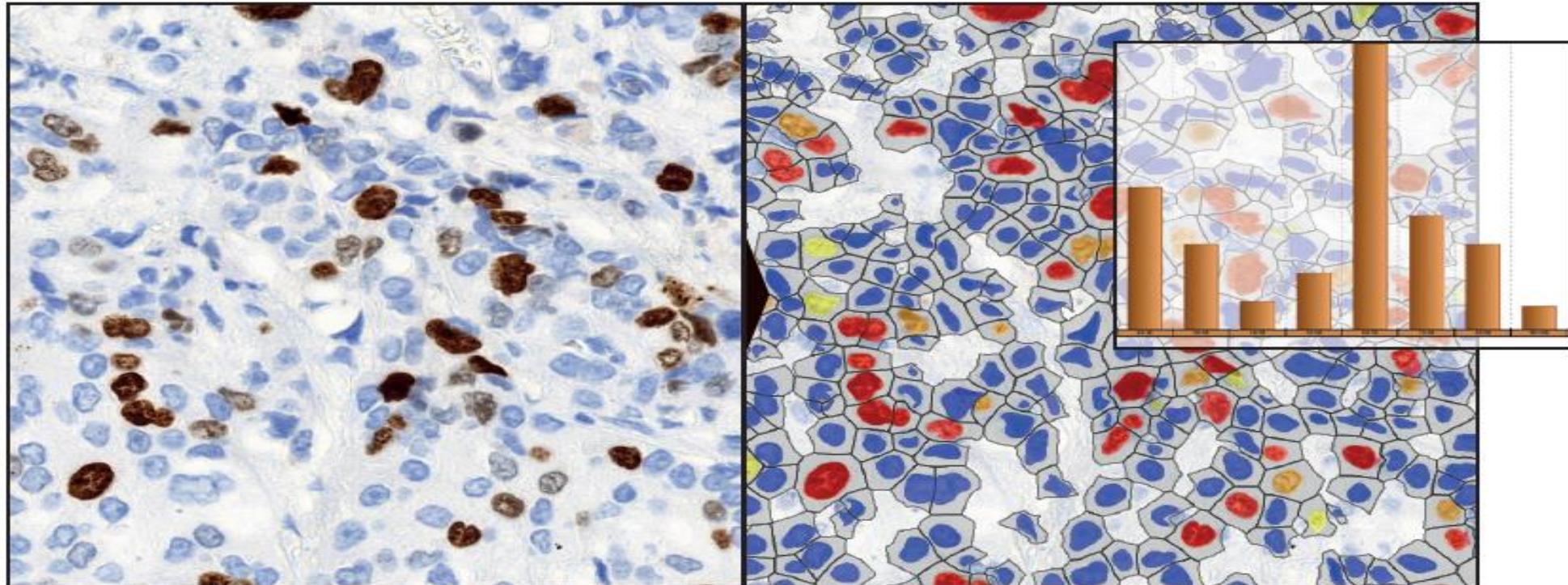
CellQuant – Nuclei detection



CellQuant - for research purposes



- Nuclei/Cytoplasm/Membran stain
- For research use only
- Adequate for Ki67 stain analysis



Adequate solution for Ki67 quantification

Ki67- proliferation marker

Cells with high mitotic rate



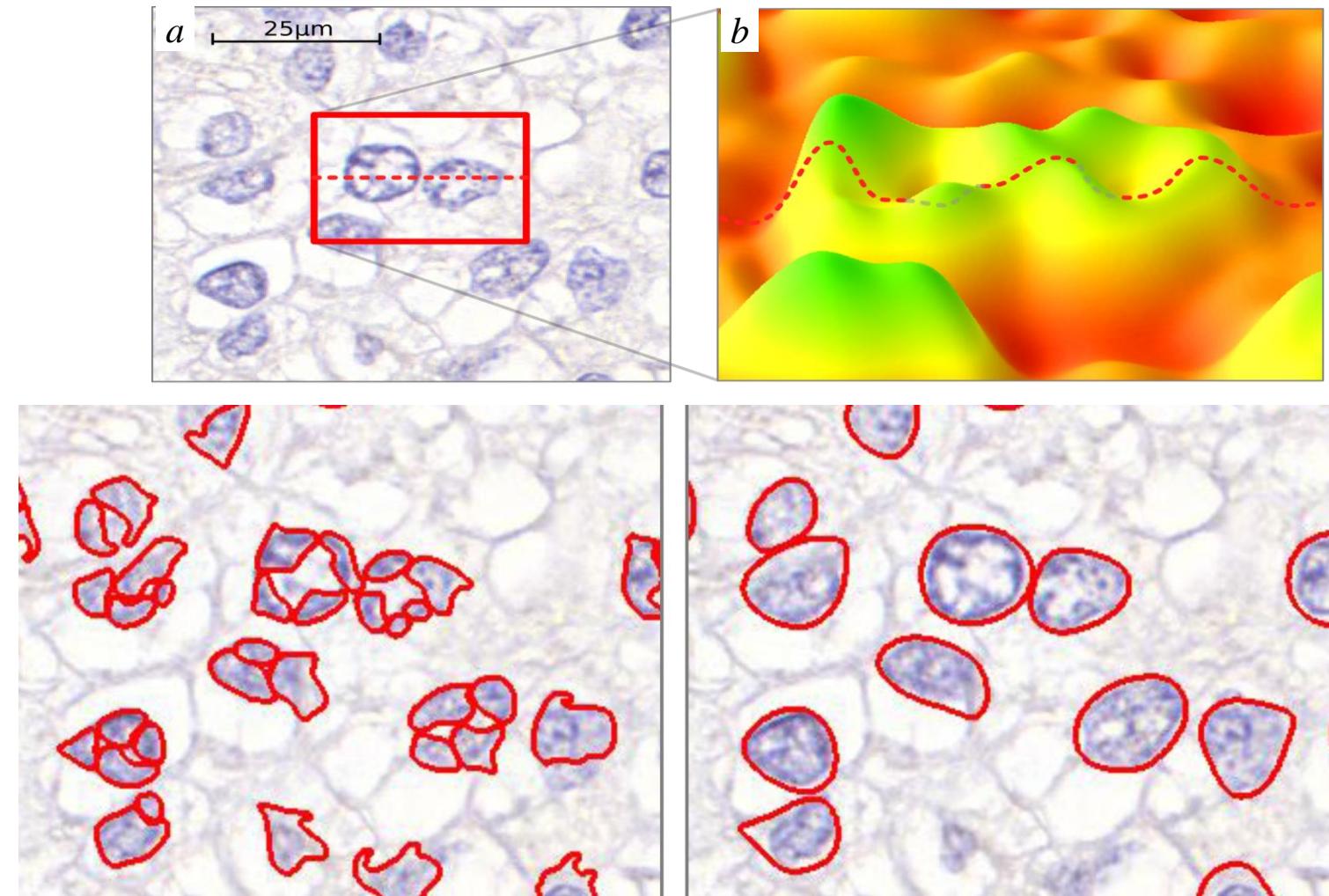
DNA condensation



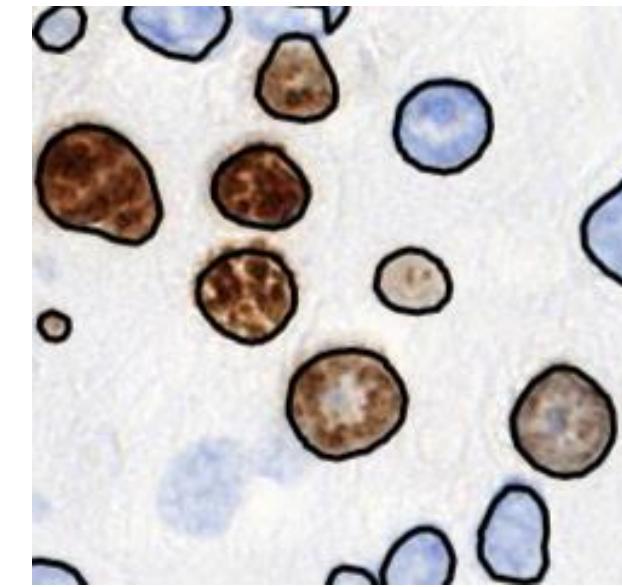
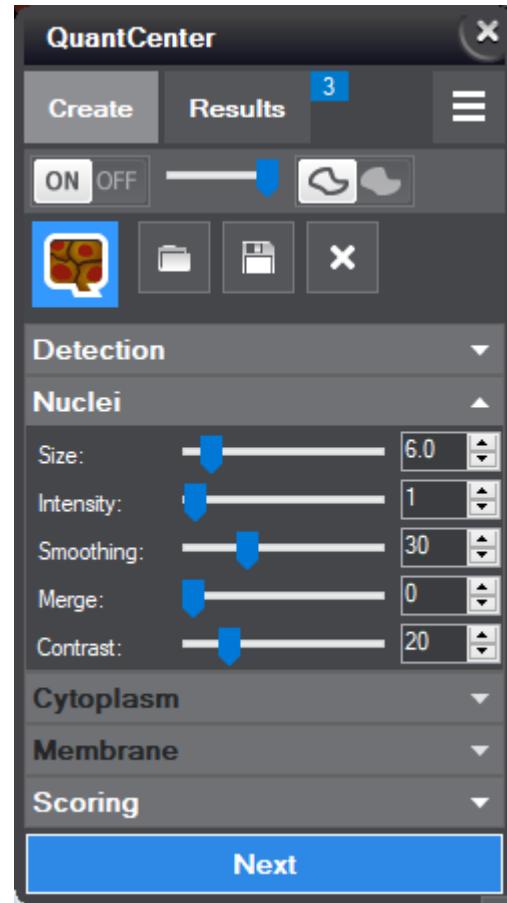
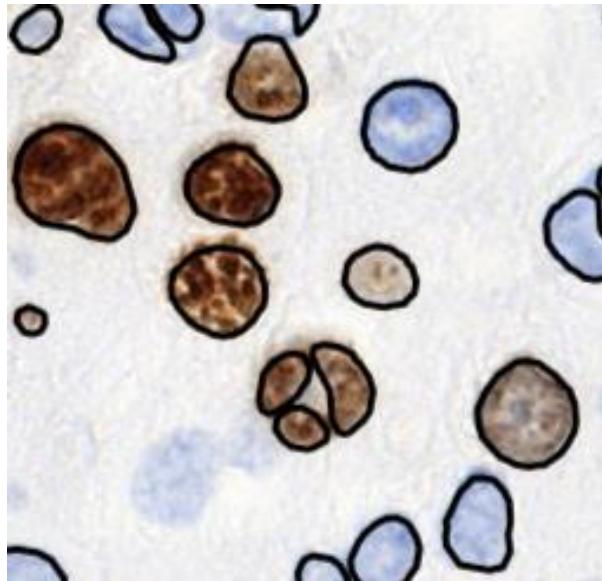
Heterogen cromatin



Holes into the nuclei



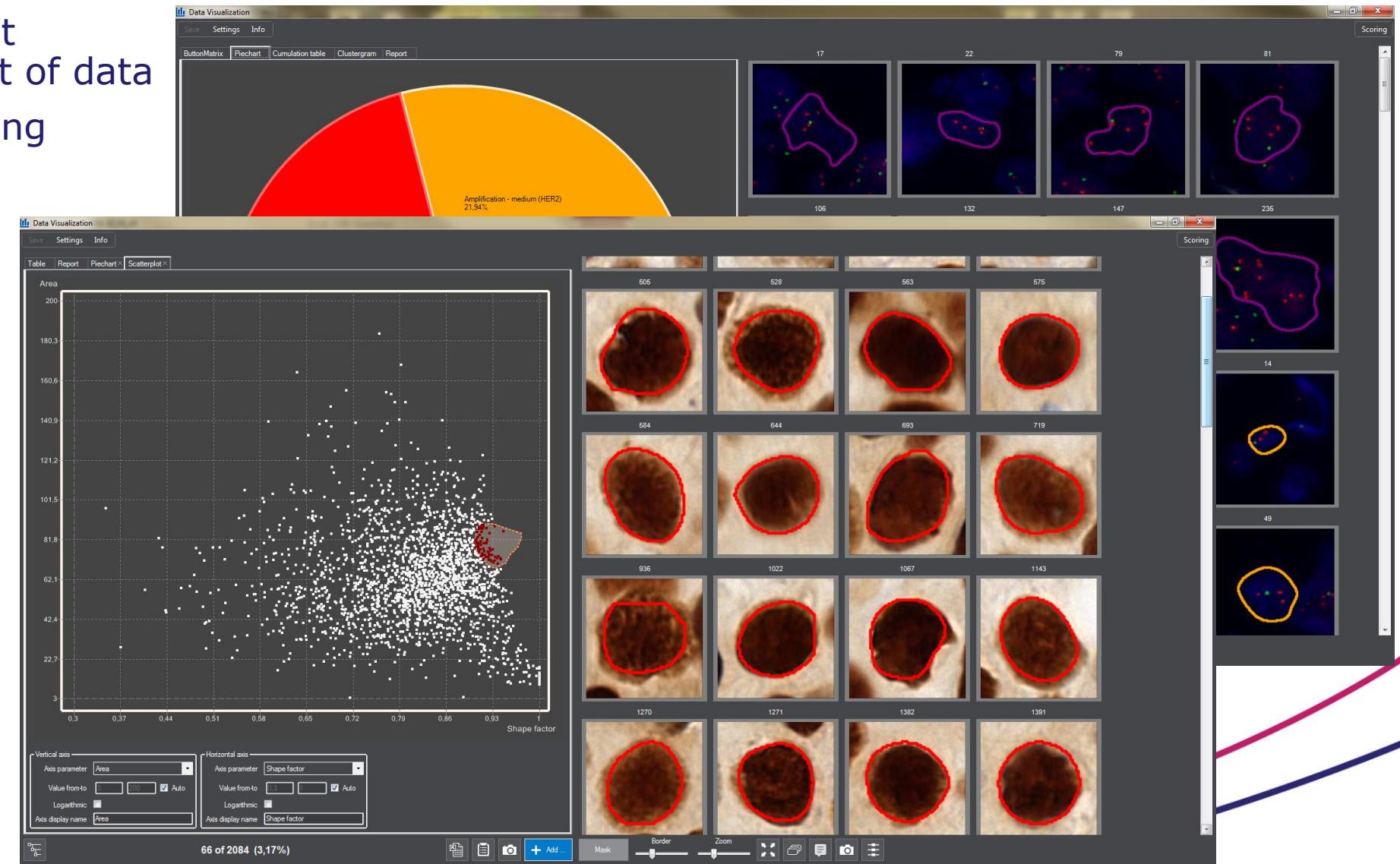
Adequate solution for Ki67 quantification



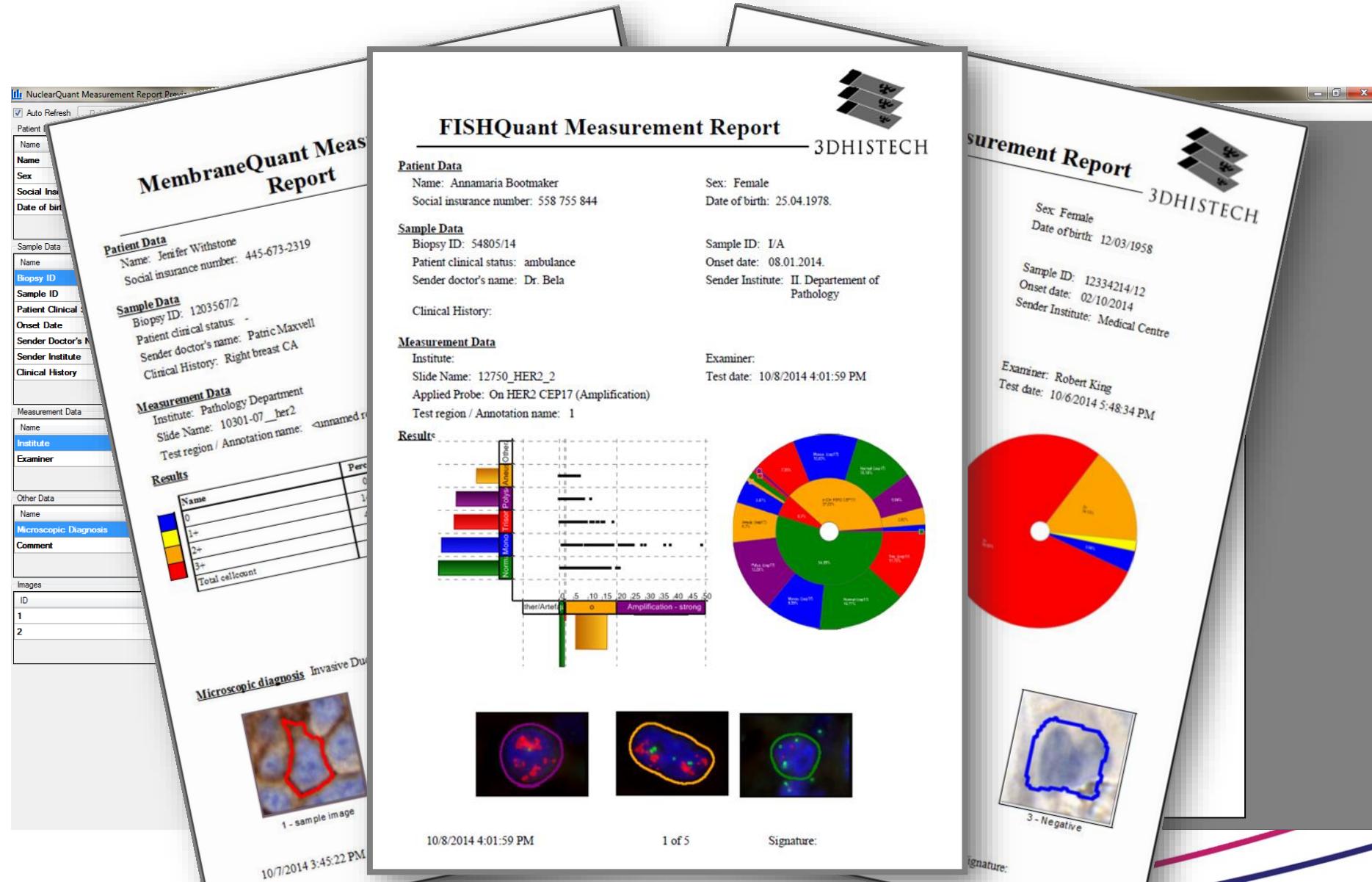
Data visualization tool

Whole slide measurement generates a huge amount of data
Data analysis and reporting

Name	Value	Unit
Annotation area	0.72	mm ²
Mask area	0.18	mm ²
Total count	2991	pcs
Relative mask area	25,53	%
Object frequency	4178.15	pcs/mm ²
Positivity Index	99,63	%
Positive mask area	99,92	%
Average positivity of Negative	221,36	
Average positivity of Weak positive	147,8	
Average positivity of Medium positive	24,83	
Average positivity of Strong positive	6,08	
Negative	11	pcs
Weak positive	17	pcs
Medium positive	879	pcs
Strong positive	2084	pcs
Negative	0,37	%
Weak positive	0,57	%
Medium positive	29,39	%
Strong positive	60,60	%



Report creation



Thank you for your kind attention!