Tumor – Stroma Segmentation

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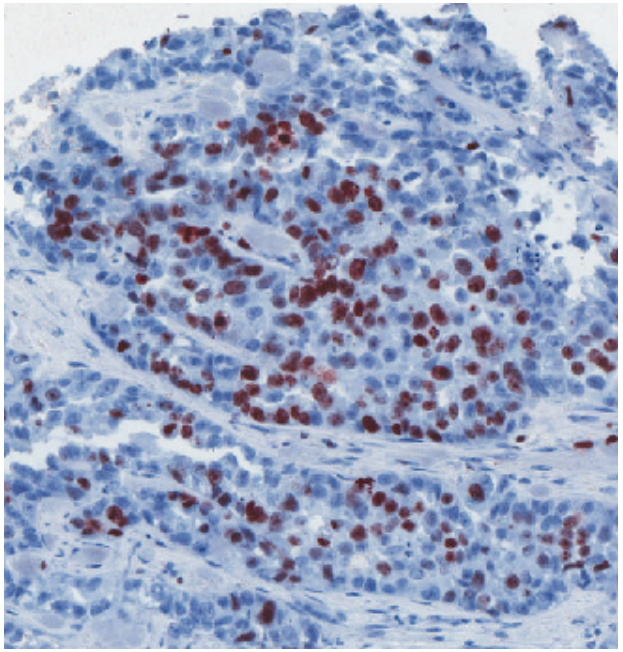
ZERTIFIZIERTES
BRUSTZENTRUM

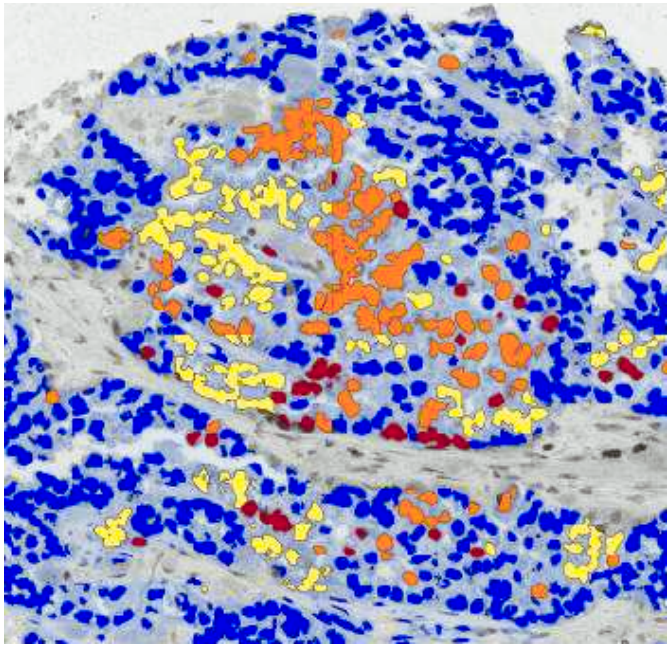
Manual versus Automated Analysis

Manual



- Subjective, time consuming
- Inherent intra-observer variability
- Semi-quantitative data
- Pathologist-based analysis remains the current standard

Automated



- Objective quantification of IHC staining
- Reproducible data
- Continuous output
- A new tool in the hand of the pathologist

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Obstacles to automatic image analysis in histopathogy (among others)

Technical:

- Variations in fixation, section quality, staining
- Artefacts (tears, bubbles, folds)

Computational:

- Separation of cells and nuclei often difficult
- Recognition of target structures (e.g. microvessels, isolated tumor cells)
- Tumor-Stroma segmentation

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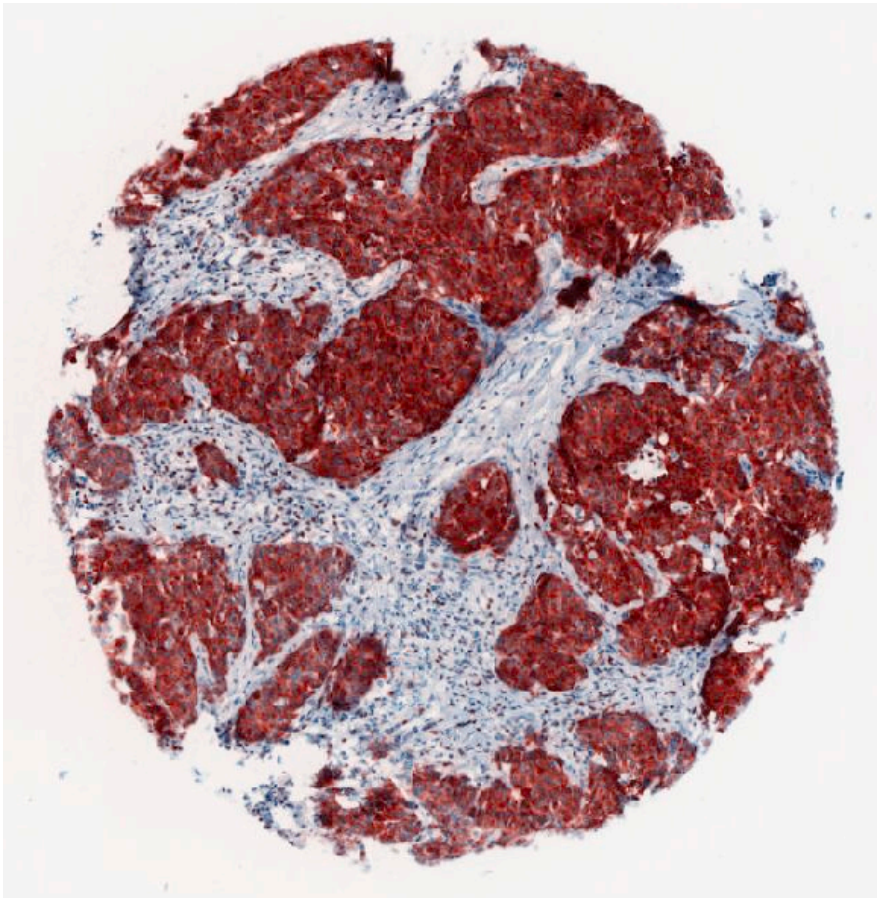
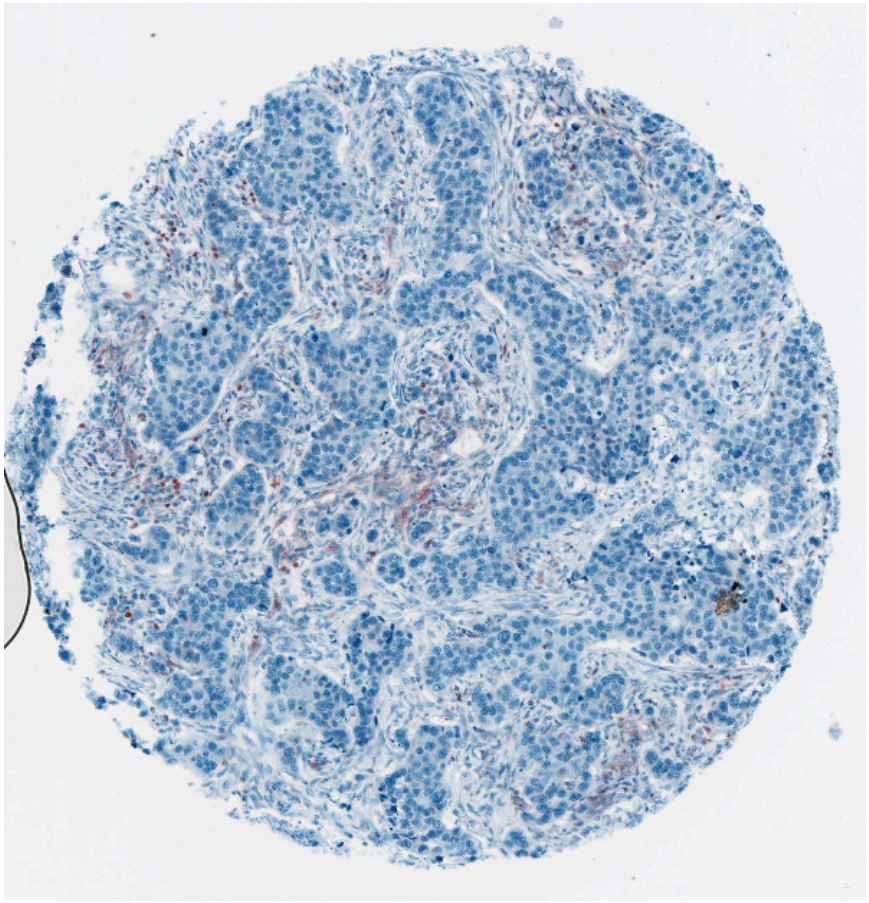
Triple-negative breast cancer
bcl-2 stain





Score: **3+** **0**

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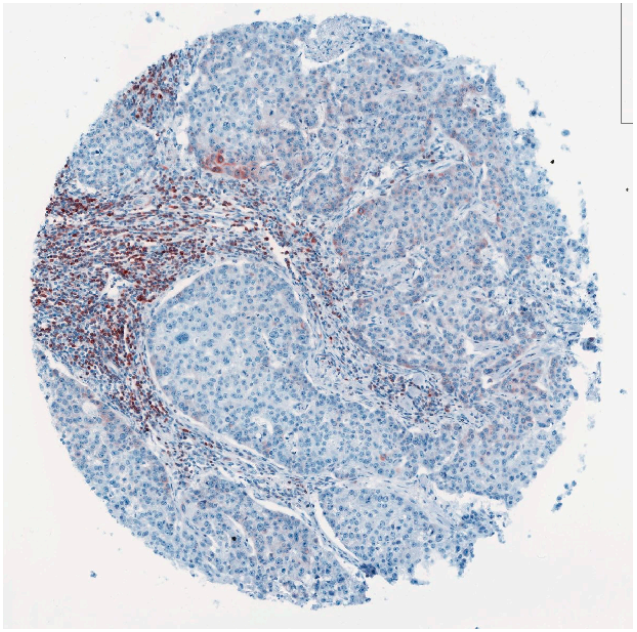
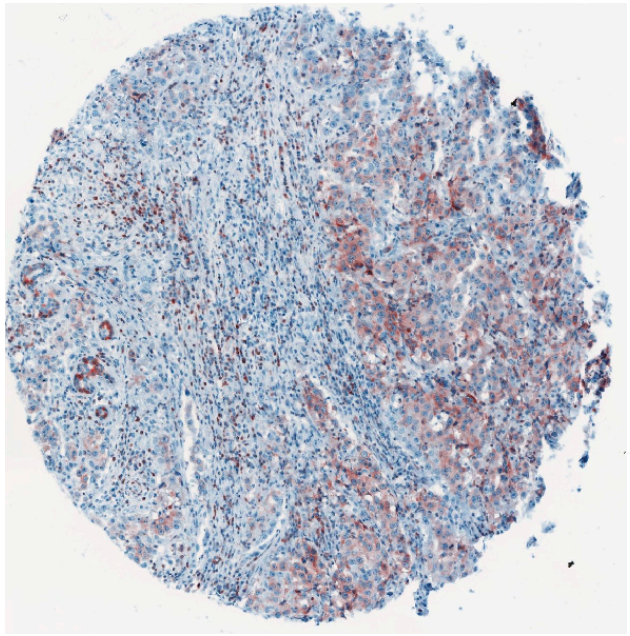
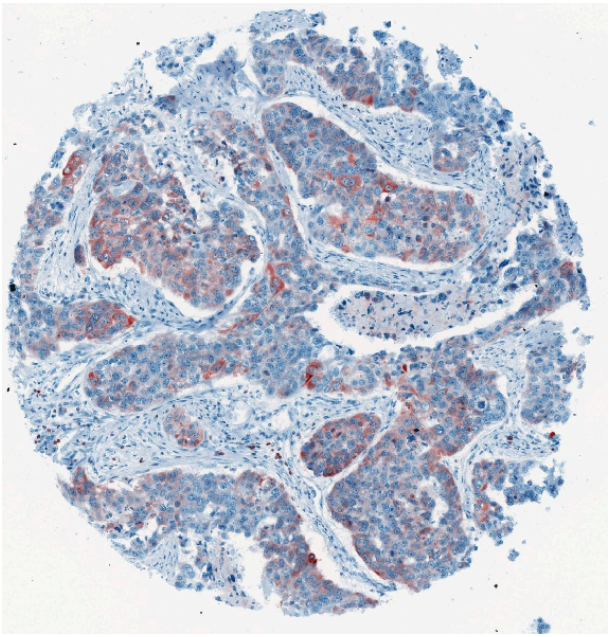
Triple-negative breast cancer
bcl-2 stain







Score: **0** **2+** **1+**

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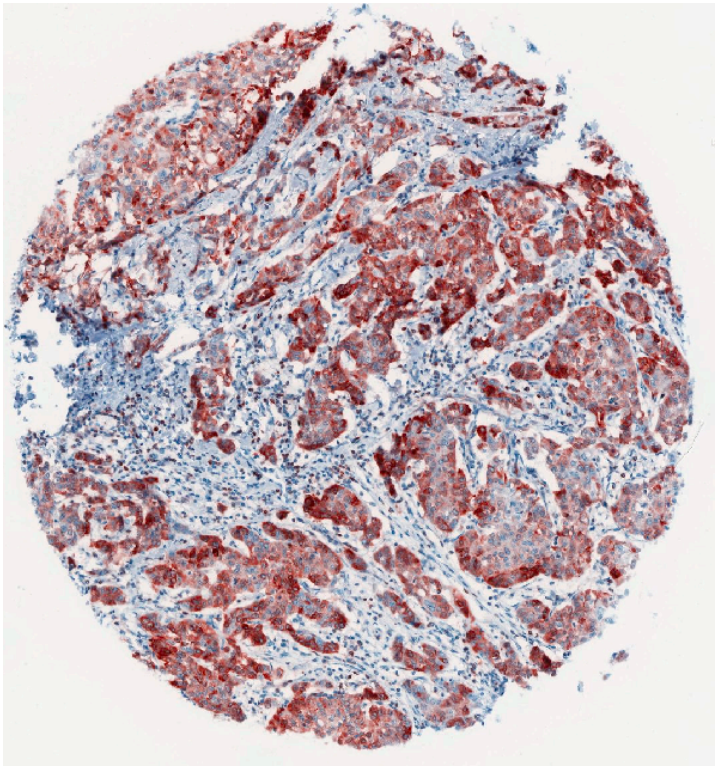
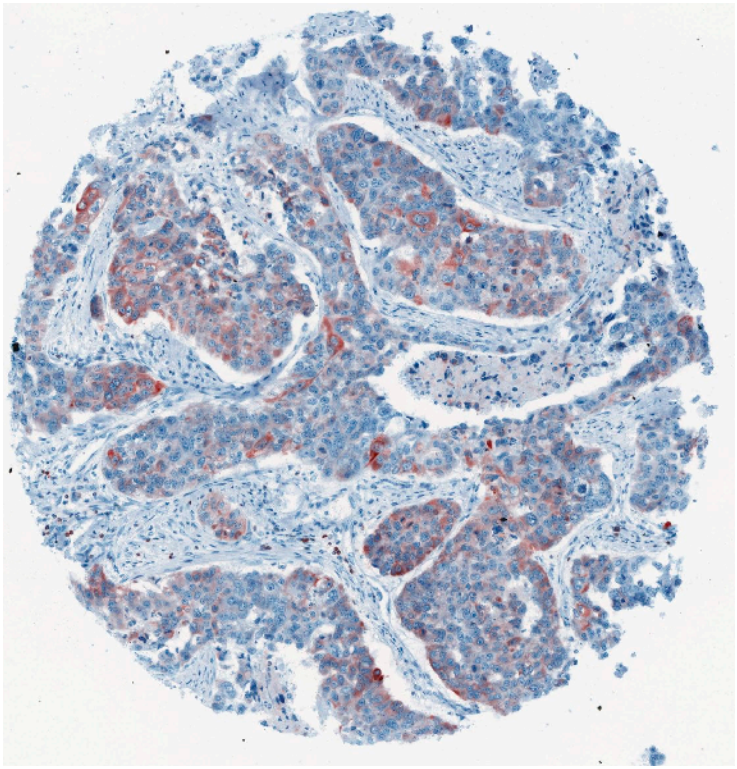
Triple-negative breast cancer
bcl-2 stain





positive: **~80%** **~5%**

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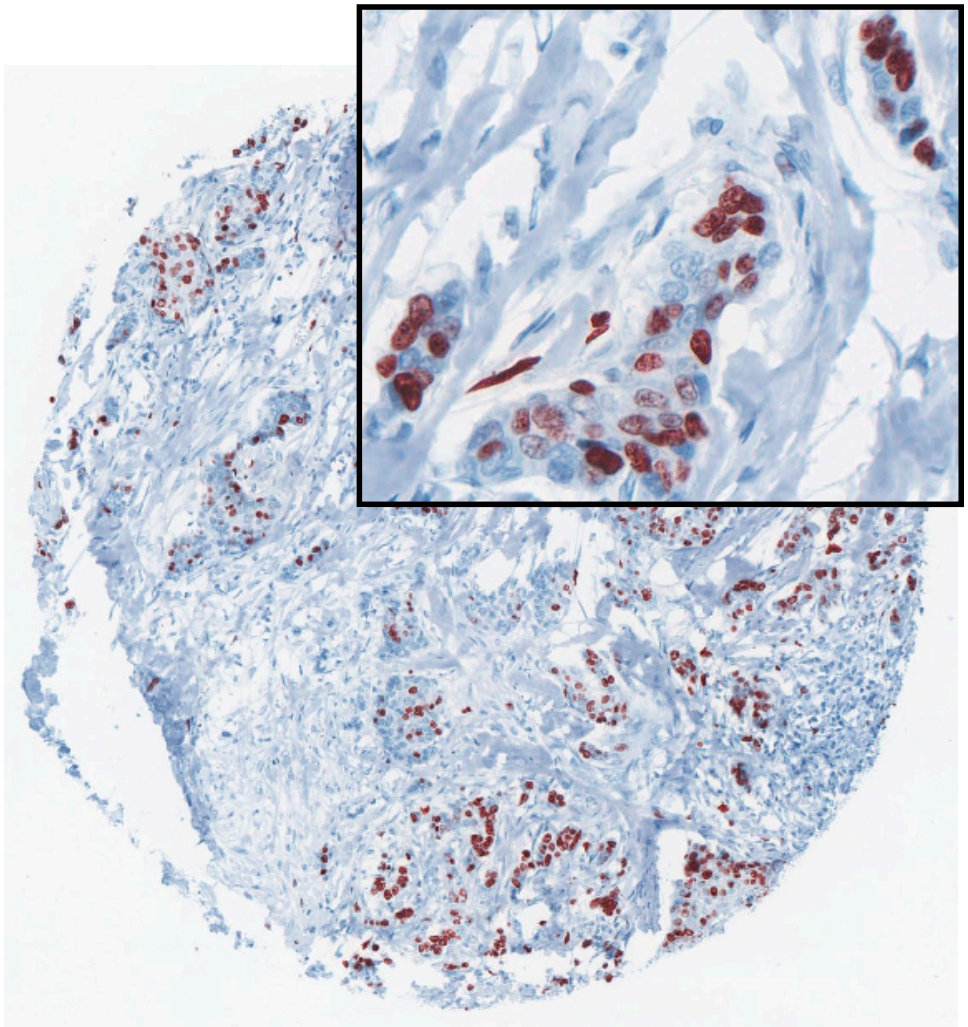
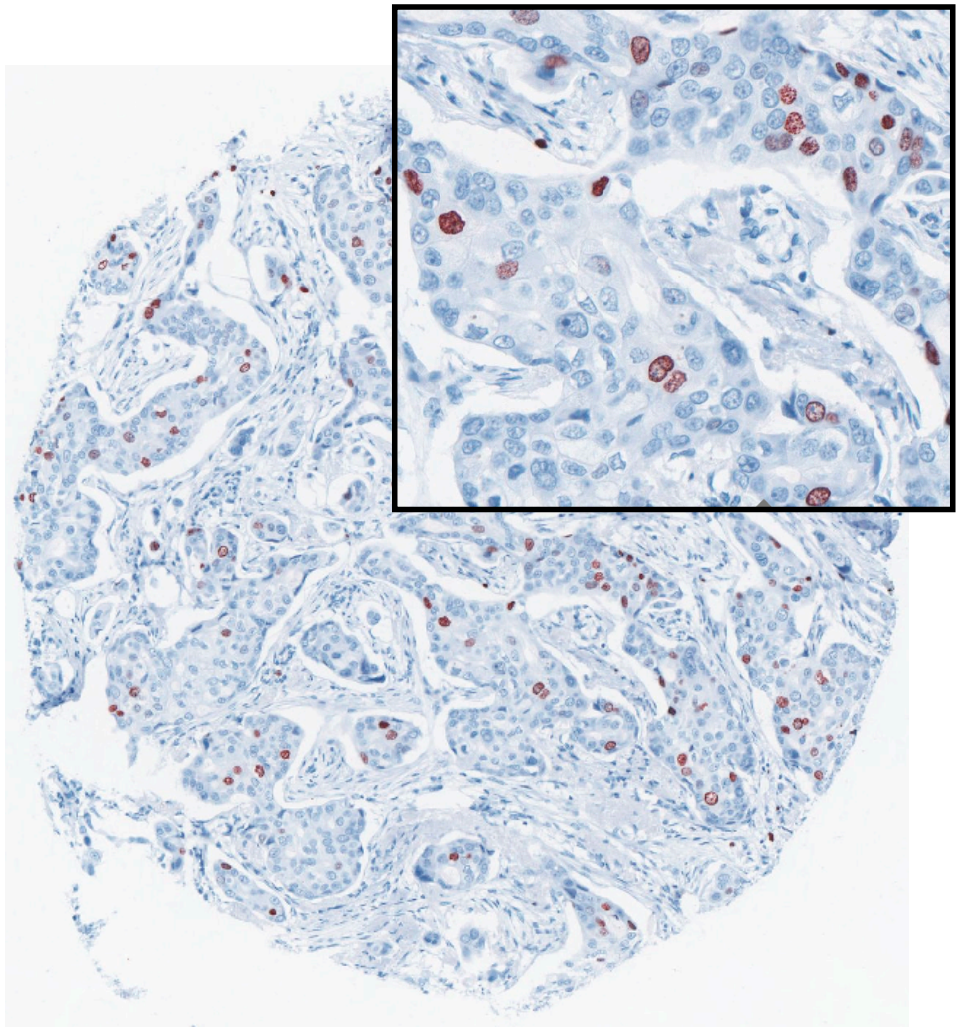
Triple-negative breast cancer
Ki-67 stain





Percent: **~90%** **~10%**

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Tumor-Stroma Segmentation

- is a prerequisite for quantitative image analysis of any tumor tissue
- is not trivial and requires pathologist‘s expertise what exactly is tumor cancerous tissue
- is dependent on tumor type, and therefore is specific to every organ tumor / project

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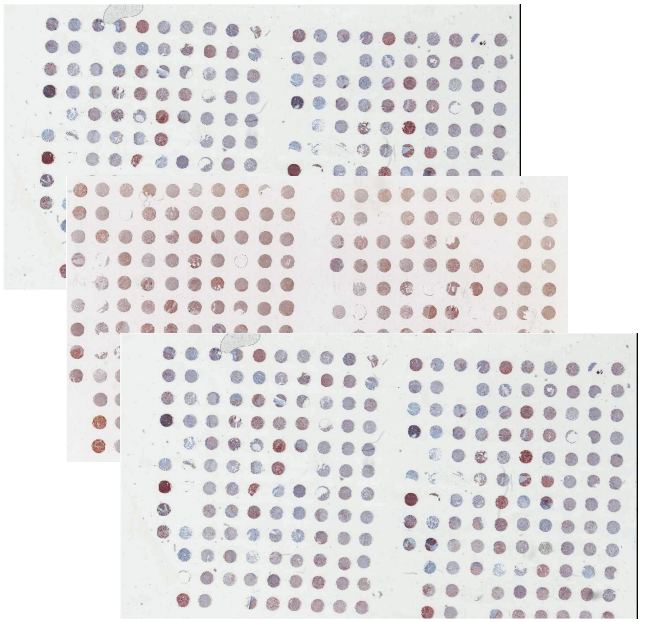
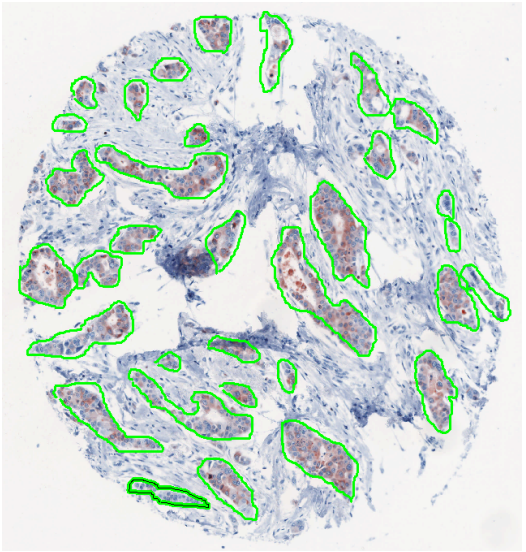
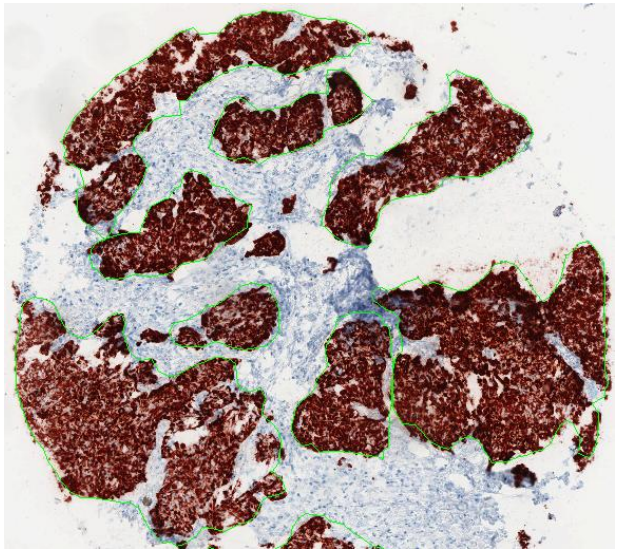
Manual Segmentation

- Very time consuming
  (2 min/core -> 400 min/TMA
  613 hours = 26d / project / 92 TMA)
- tedious and inexact
- Only areas, not cells can be marked





Aperio



Definions

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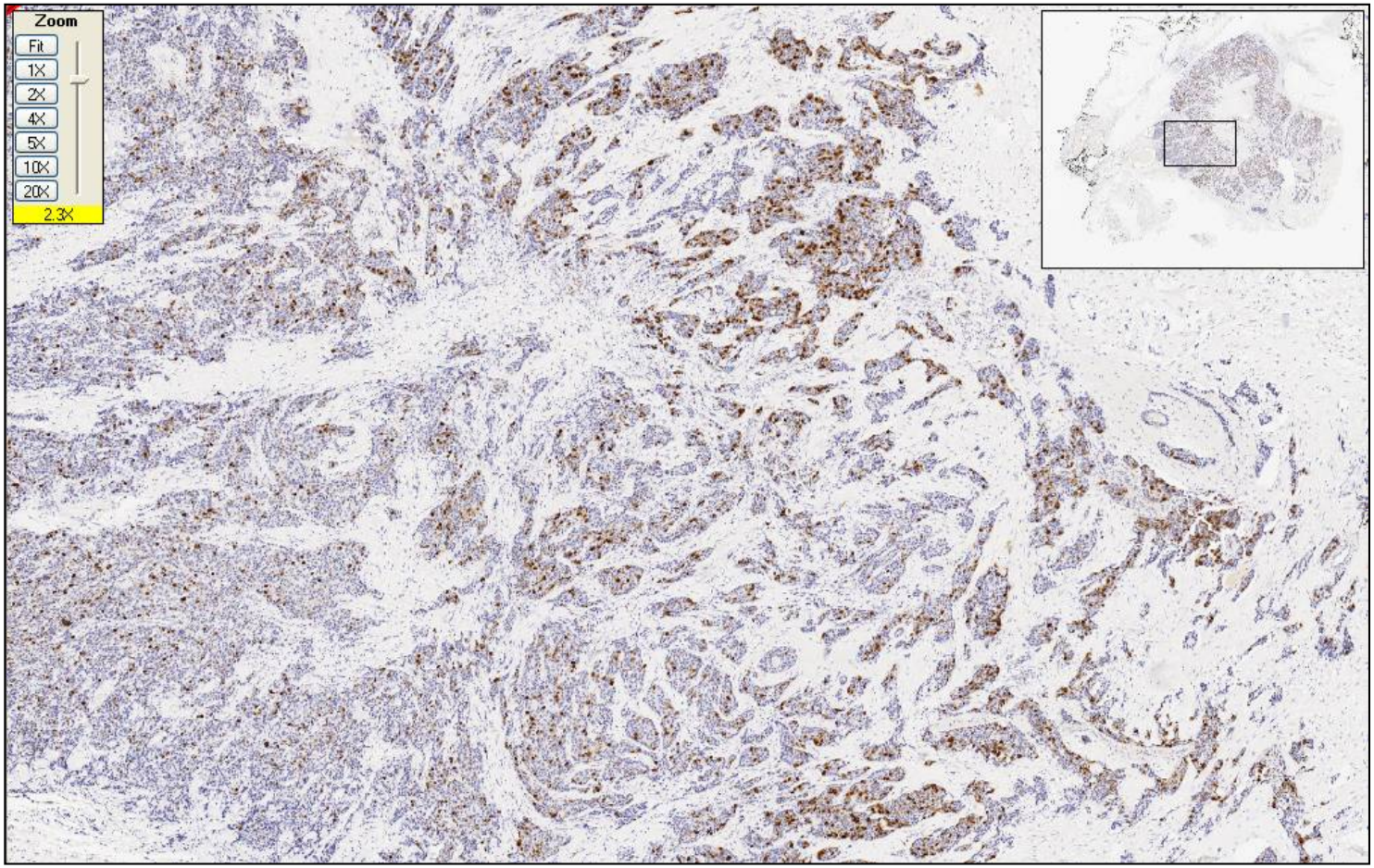
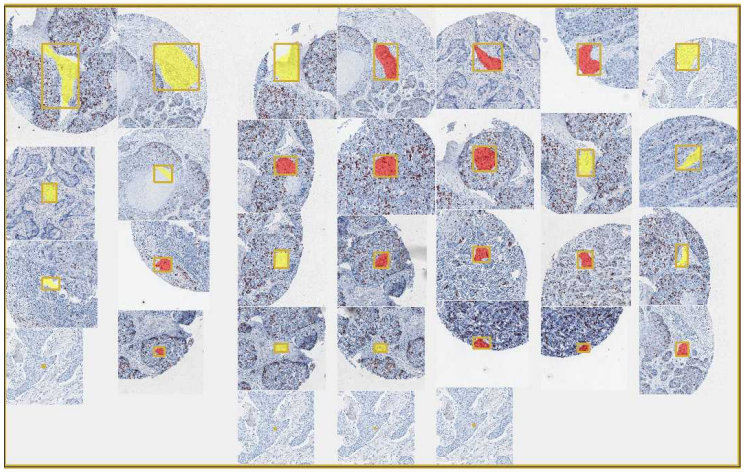
Manual Segmentation



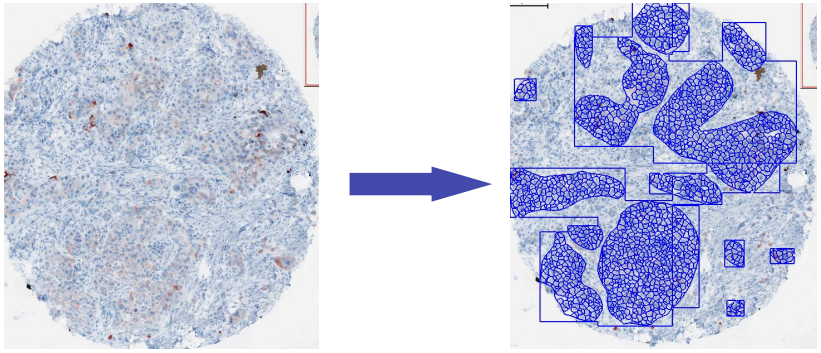
- Mission impossible for whole tissue sections

Automatic Segmentation

- Machine learning / Genetic algorithms
  - Genie (Aperio)
  - Inform (CRI Inc.)



- Feature based (nuclear size, staining characteristics)
  - Definions
  - others



Ck 5/6

Cell simulation

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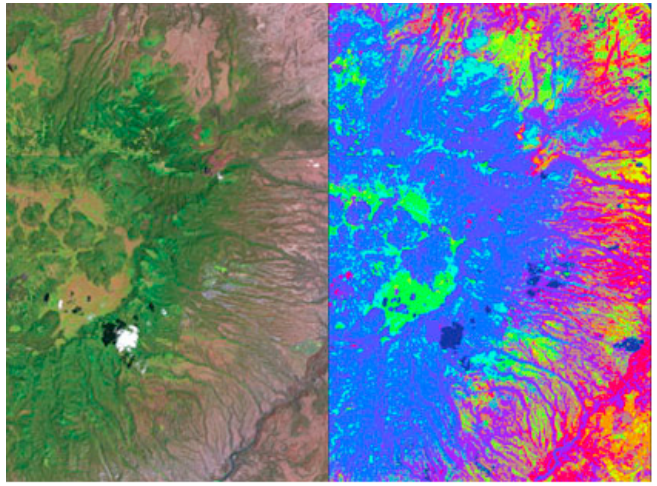
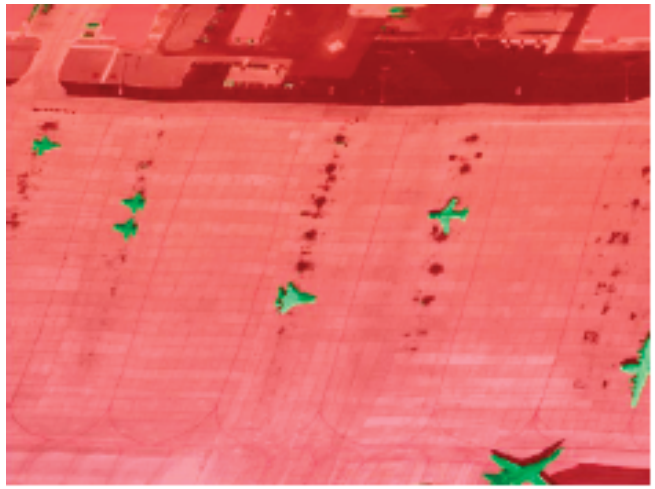
GENIE (GENetic Imaginery Exploitation) developed by Los Alamos Laboratories (LAL)



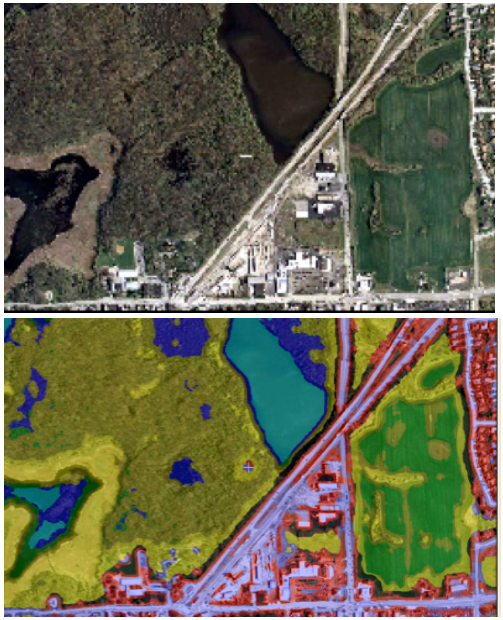
Satellite image analysis

forests: blue
grasslands: green
scrub: yellow
bare ground: red

Identification and mapping of rice fields



Aircraft on runway



Land cover map

http://www.genie.lanl.gov/

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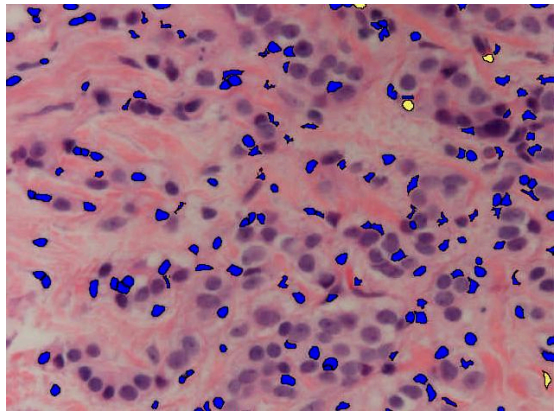
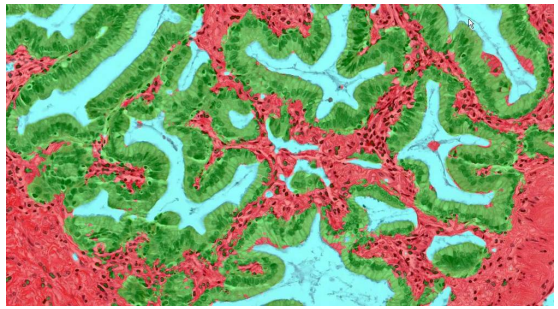
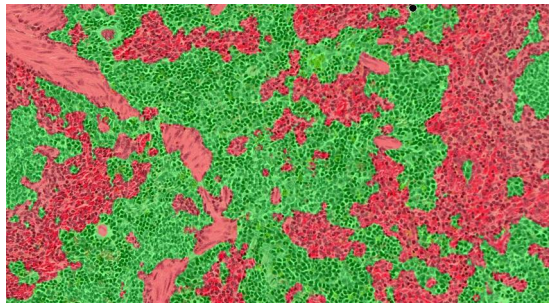
Genie Application Example Images



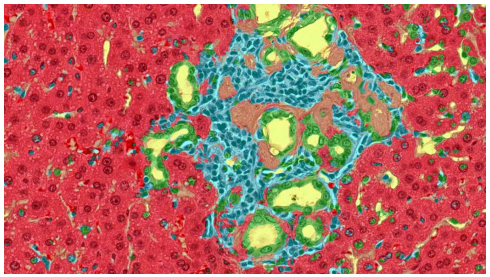
Lymphocytes (blue) and breast cancer cells (unstained)



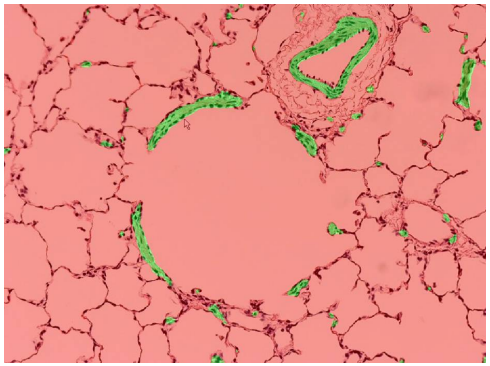
Neoplastic glands (colon cancer)



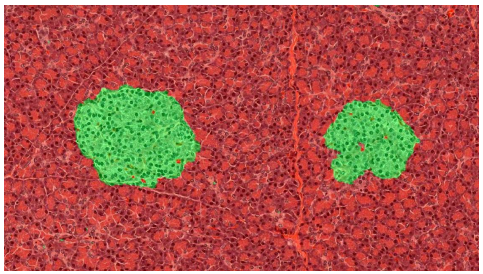
Splenic extramedullary hematopoesis (mouse)



Bile duct proliferation (rat liver)



Smooth muscle (monkey lung)



Pancreatic islets (mouse pancreas)

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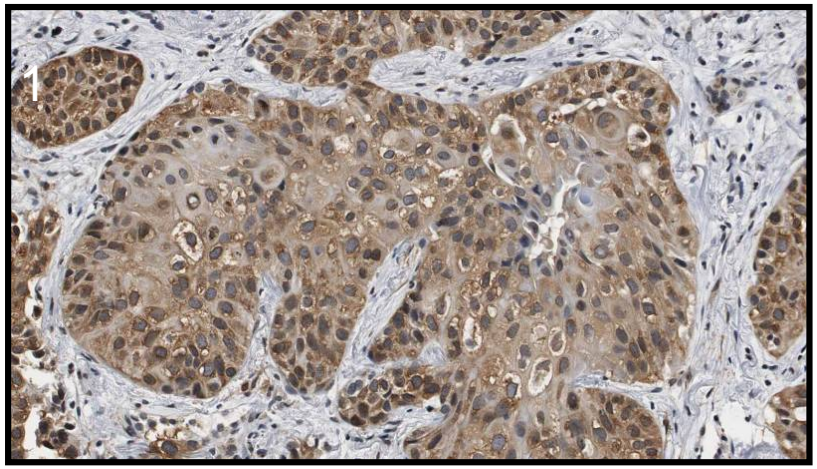
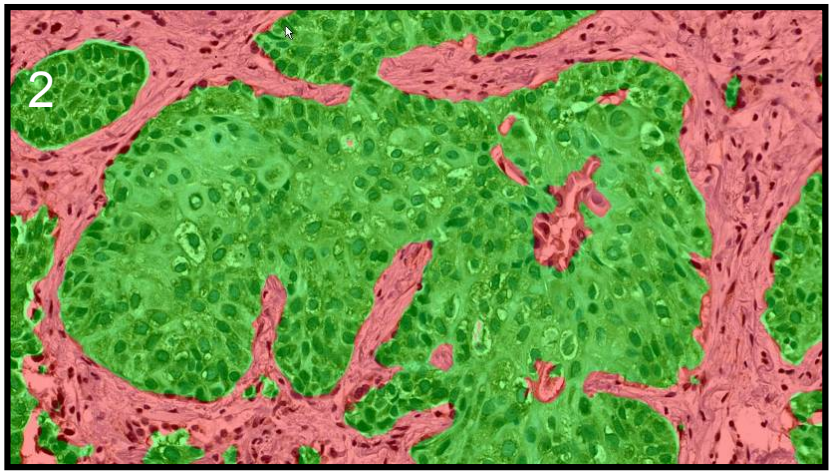
Genie Application Workflow

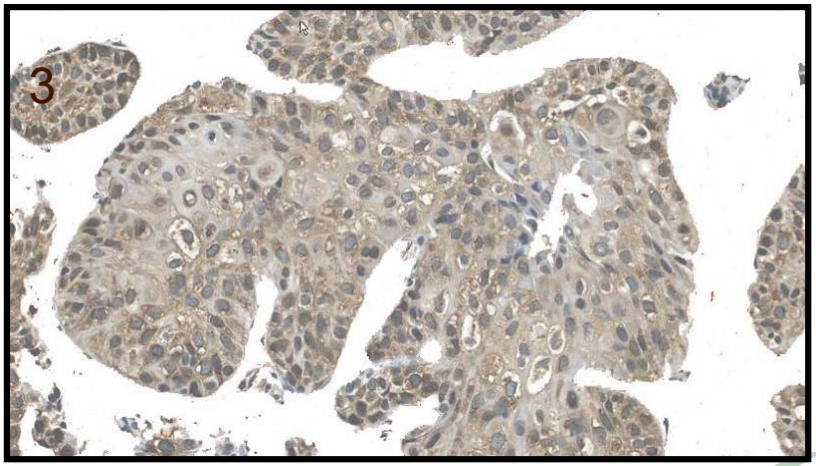
Primary IHC image



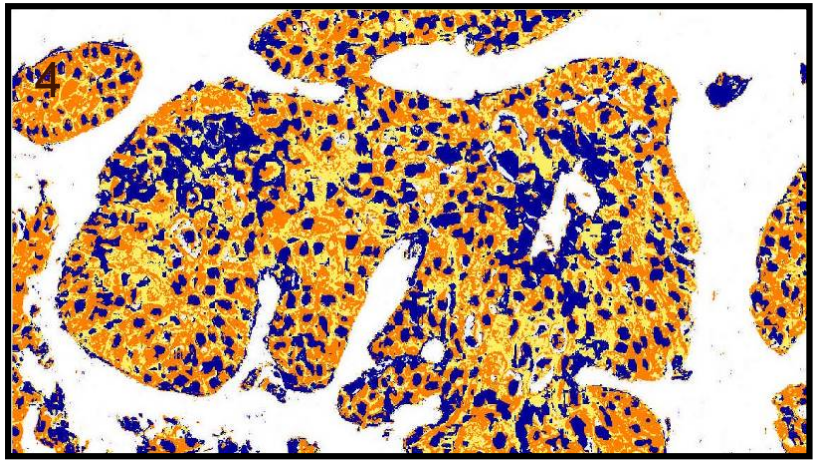
Genie™ markup with selection of neoplasm



Eliminate stroma



Final Aperio ImageScope deconvolution markup



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PathologyExperts LLC

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GeniePro - Methodology

- A supervised statistical classifier that uses training data and results to determine the decision rules for extracting the feature(s) of interest
- An adaptive spectral/textural image processing stage for learning the attributes of pixels containing the feature of interest
- An adaptive morphological image processing stage for automatic result refinement
- An adaptive thresholding algorithm that produces a raster map of the feature(s) of interest
- A vectorization algorithm designed to smoothly delineate raster regions containing the features of interest

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GeniePro - Methodology

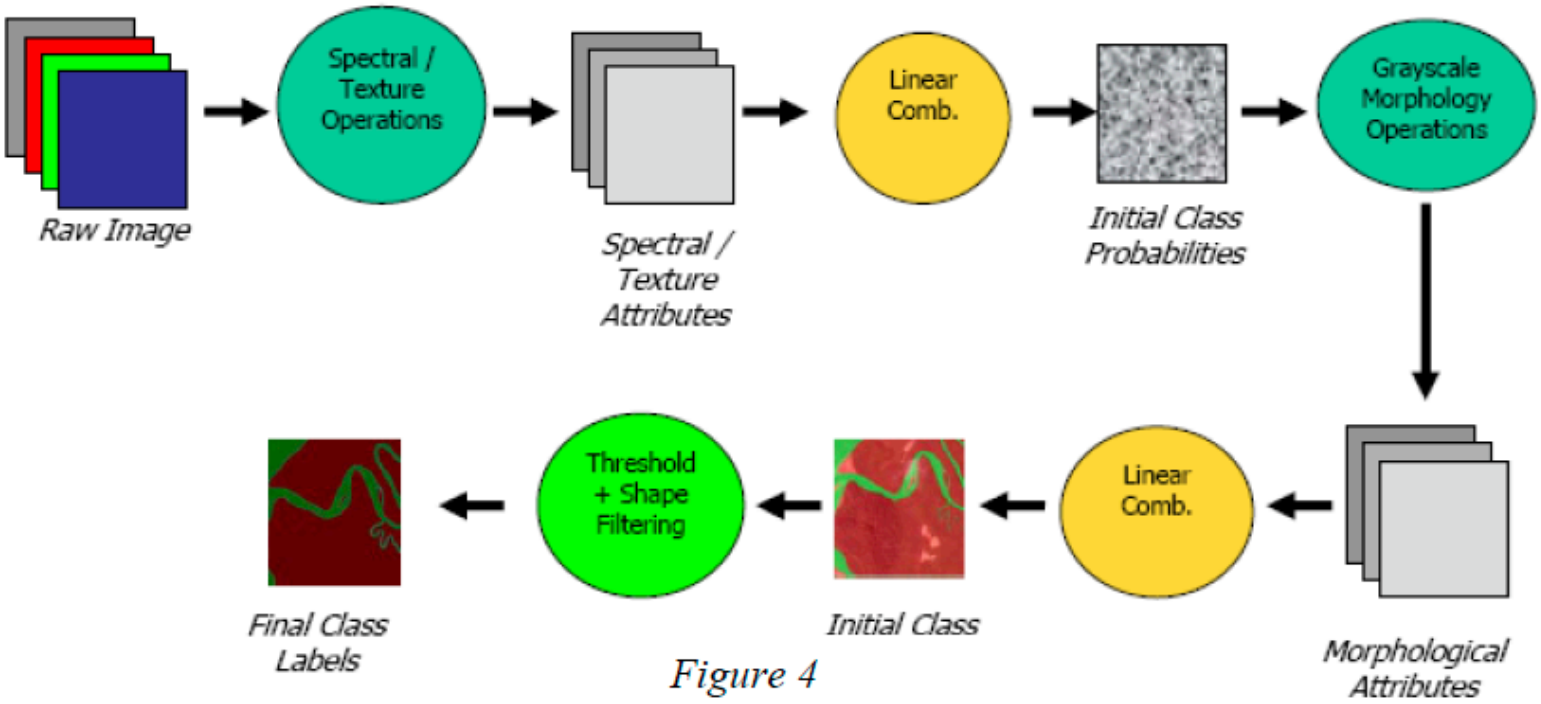

Figure 4

Development

2003: Release of GENIE Open Source version for Linux and IDL

2006: Rewrite of GENIE for Windows and Linus as GeniePro (commercial)

2007: Licence of GeniePro for Digital Pathology by Aperio

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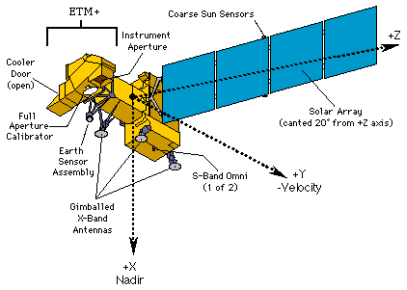
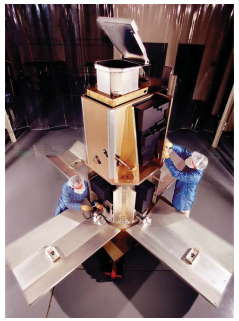
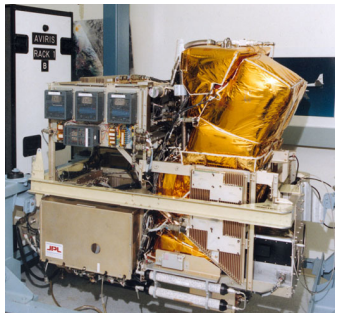
GeniePro - Applications



Landsat 7 ETM+

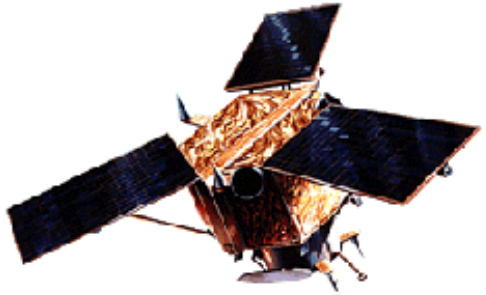


DOE/NNSA Multispectral Thermal Imager

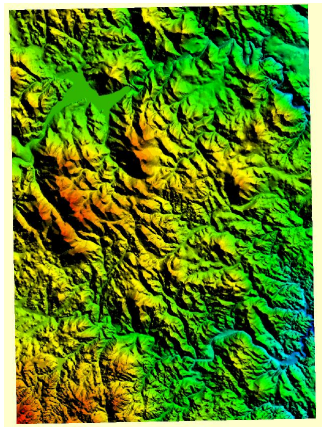


AVIRIS

Hyperion



IKONOS



Digital Elevation Model

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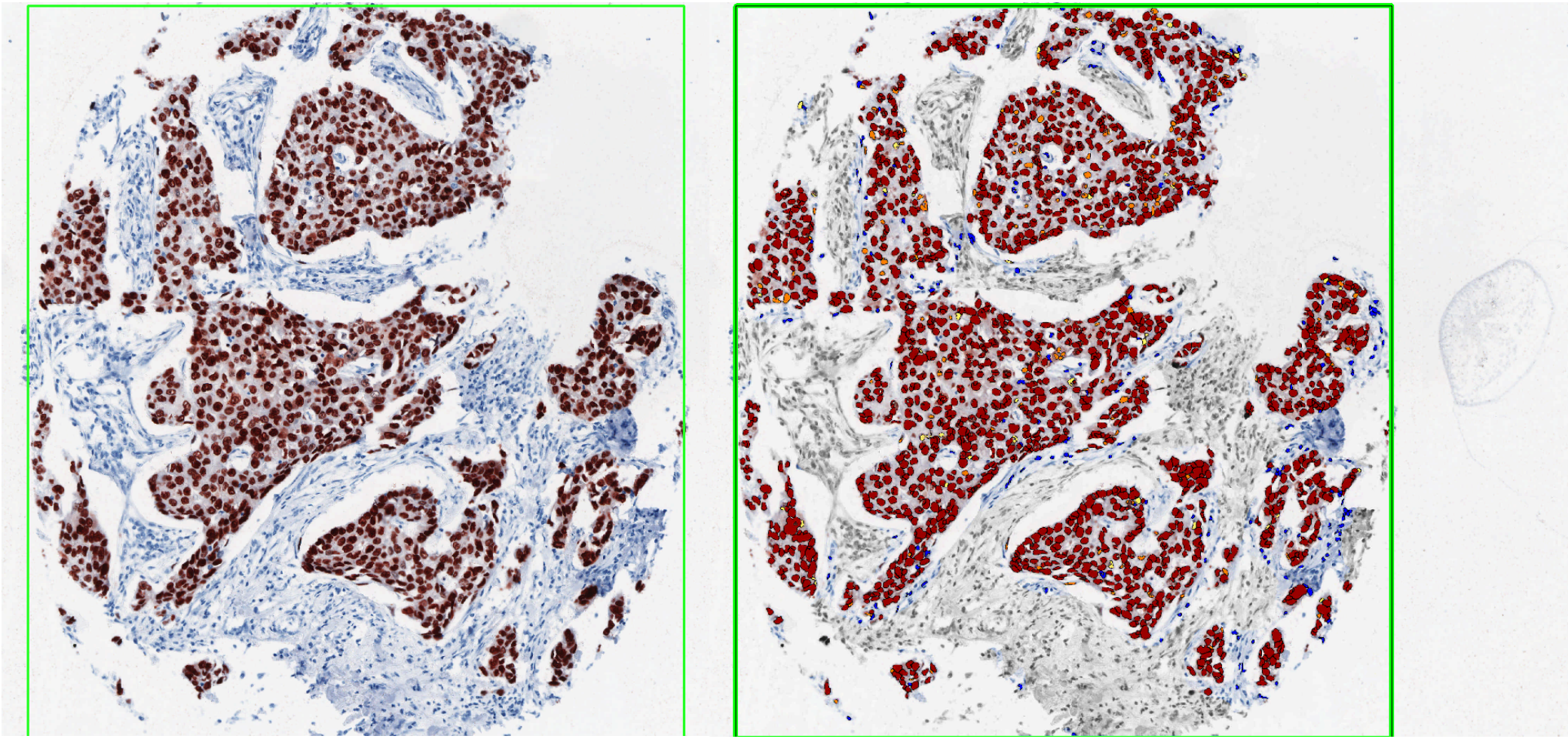
p53 in triple-negative breast cancer

Aperio - Genie histology pattern recognition



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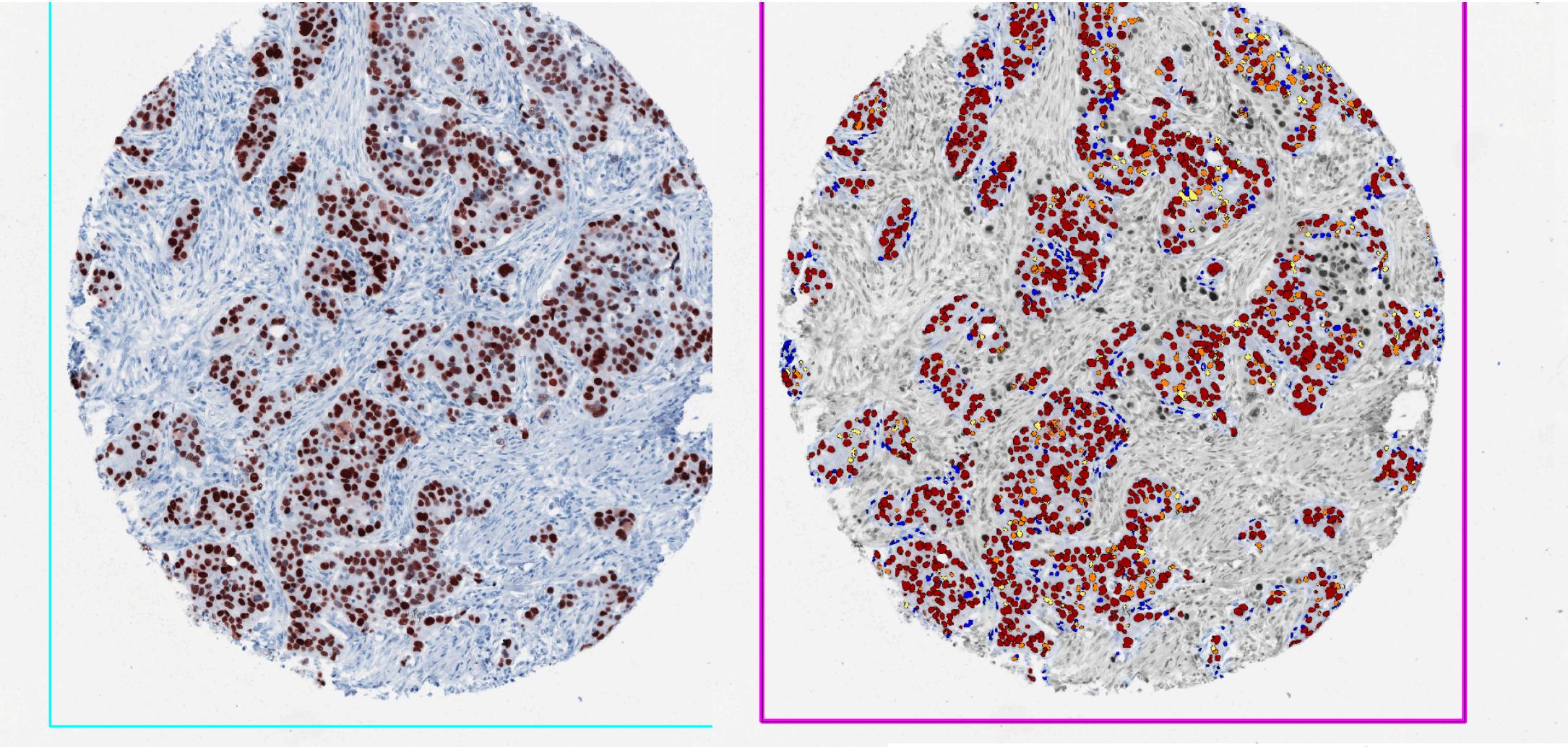
p53 in triple-negative breast cancer

Aperio - Genie histology pattern recognition



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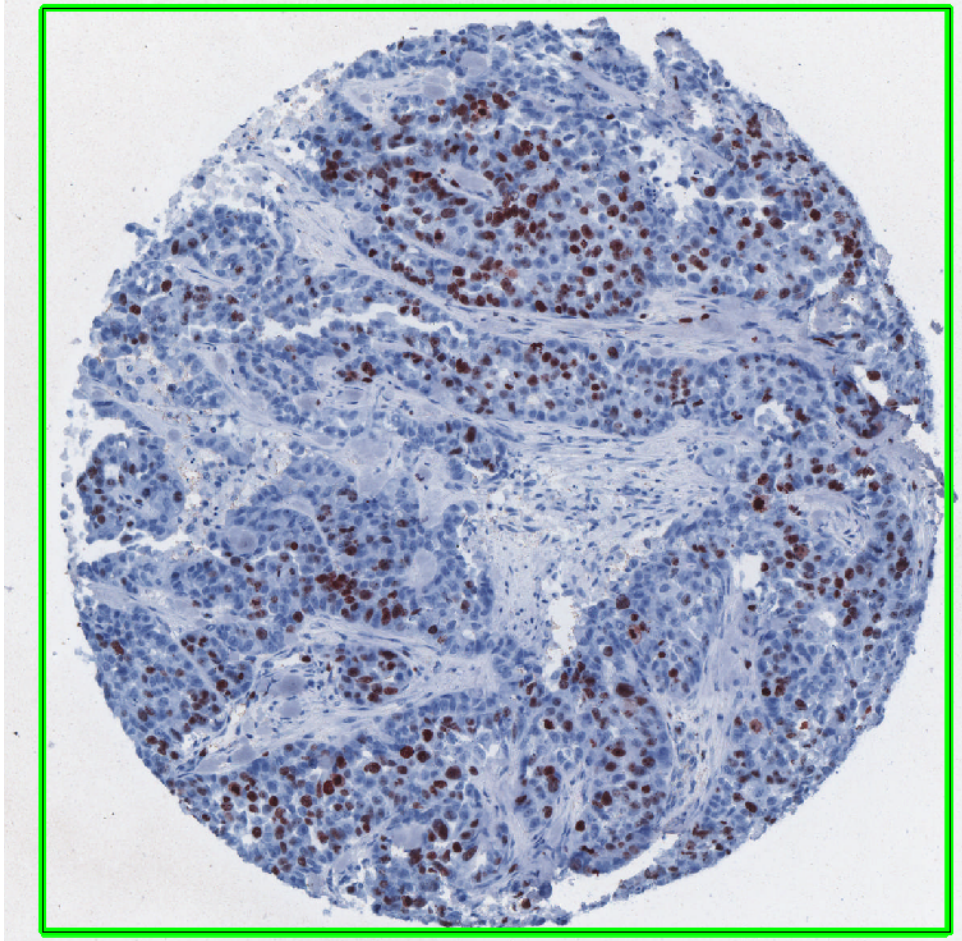
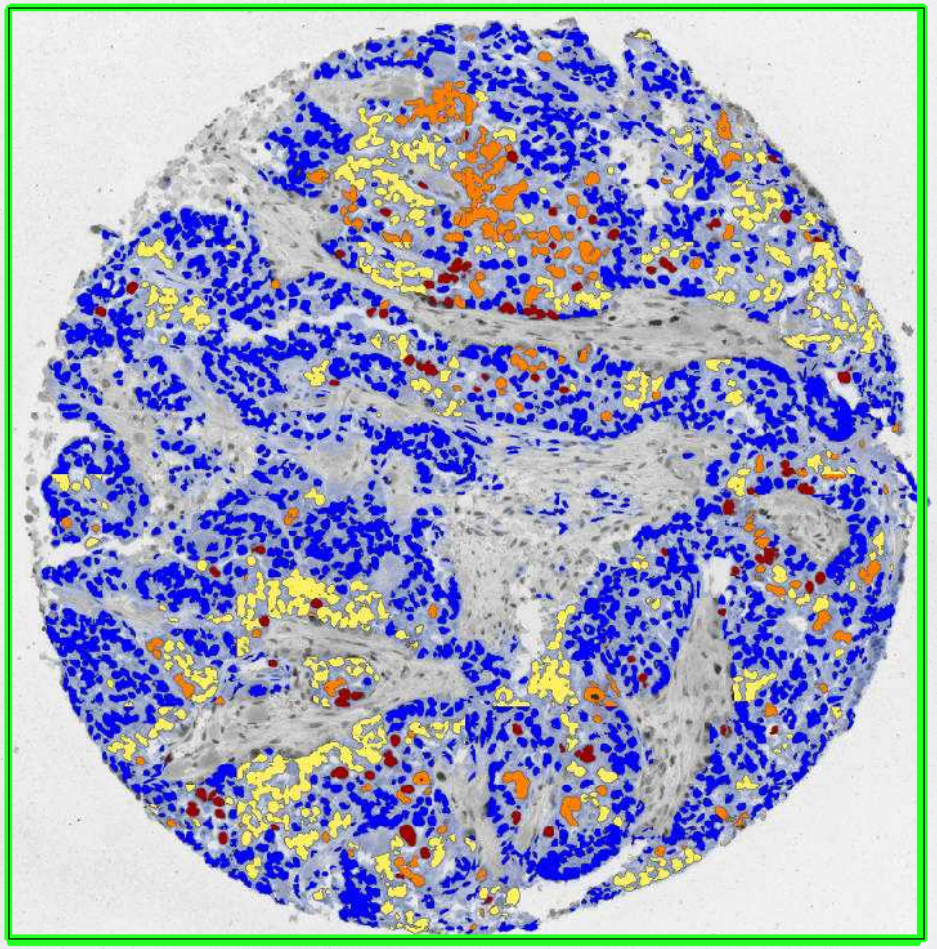
Ki-67 in triple-negative breast cancer

Aperio - Genie histology pattern recognition





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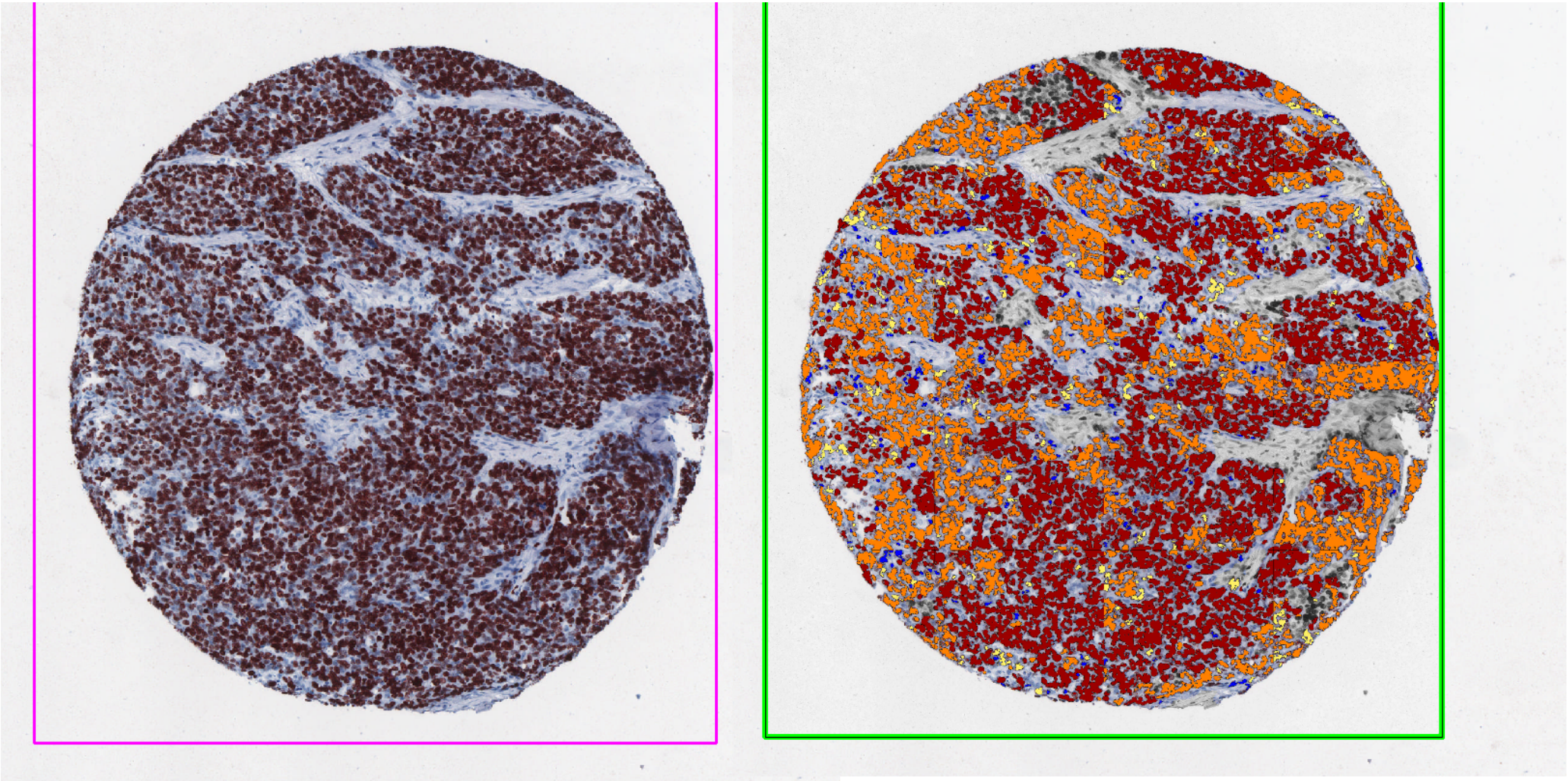
Ki-67 in triple-negative breast cancer

Aperio - Genie histology pattern recognition



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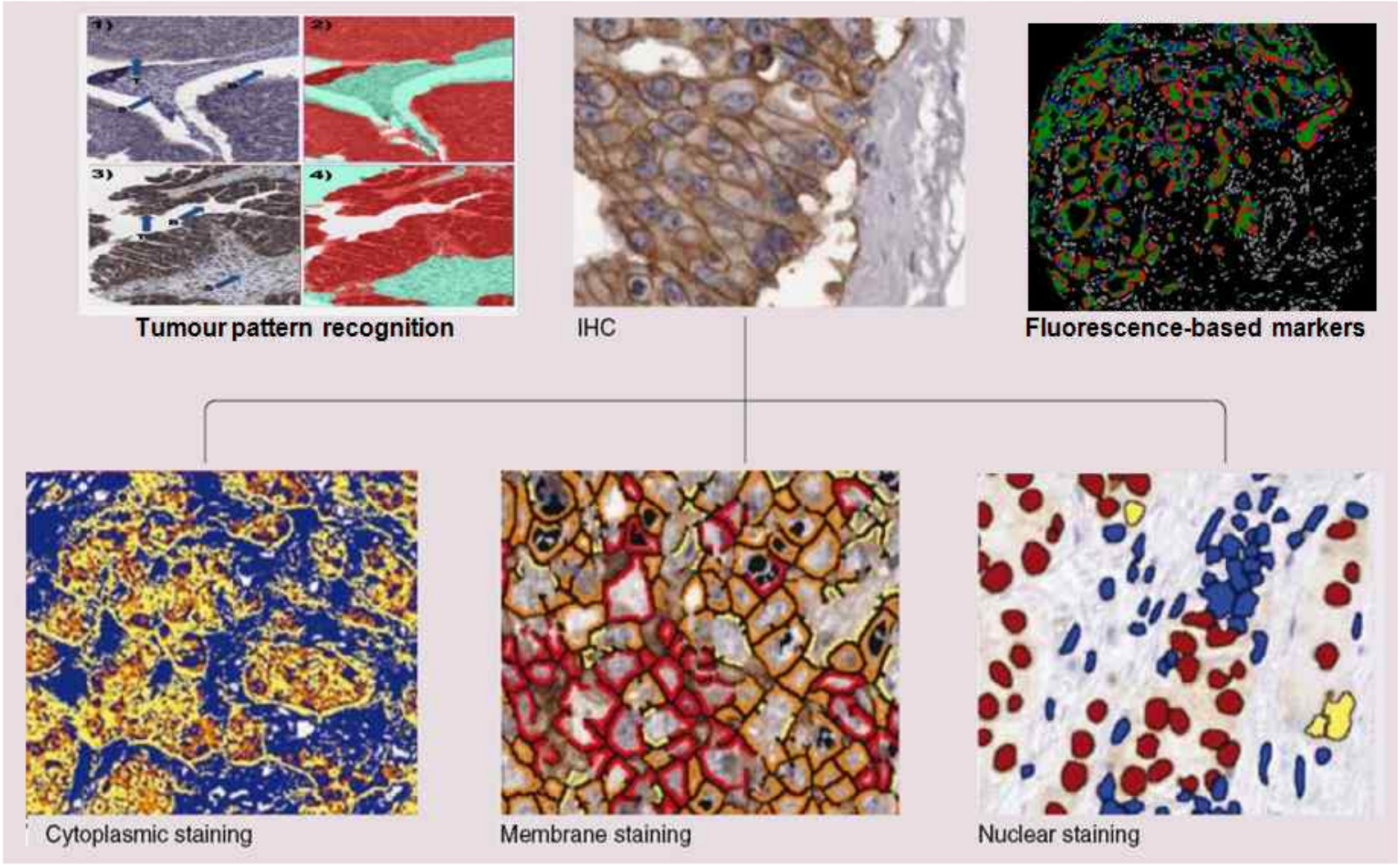
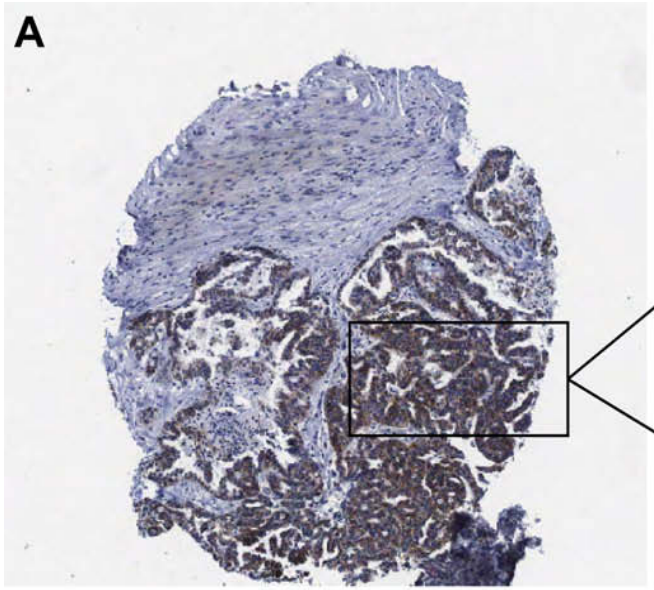
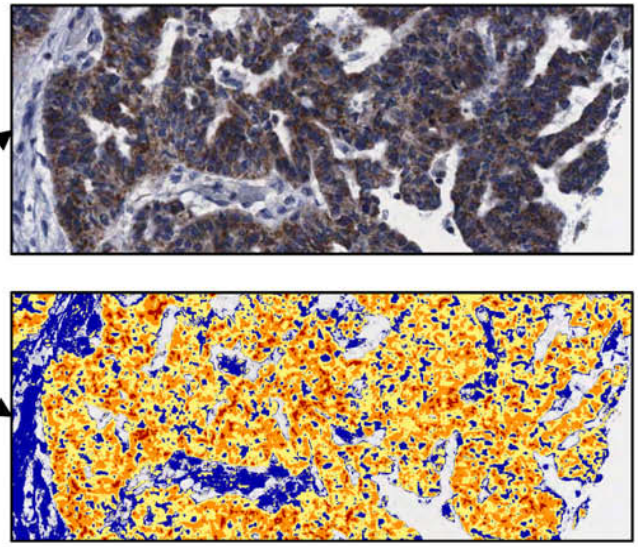
Automated Image Analysis: Oncology Case Studies

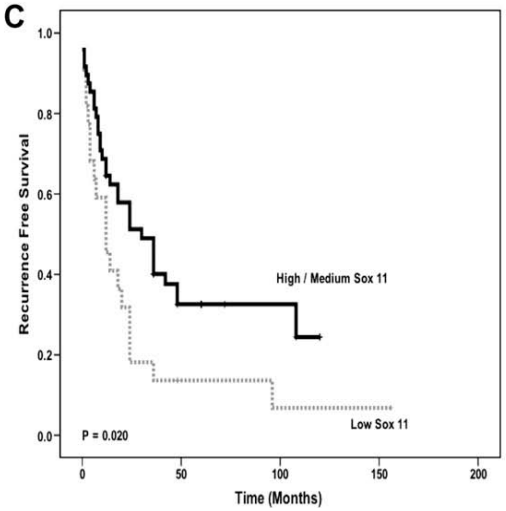


Mulrane L et al. Expert Reviews in Molecular Diagnostics (2008)

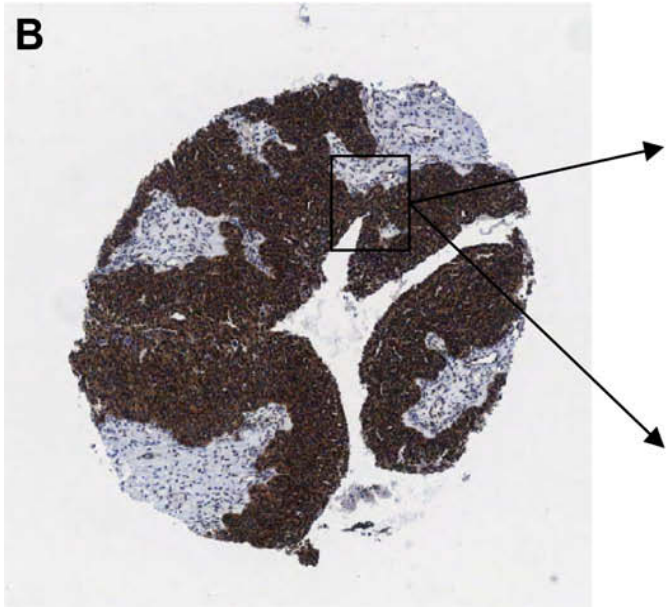
Sox11 protein expression in ovarian cancer

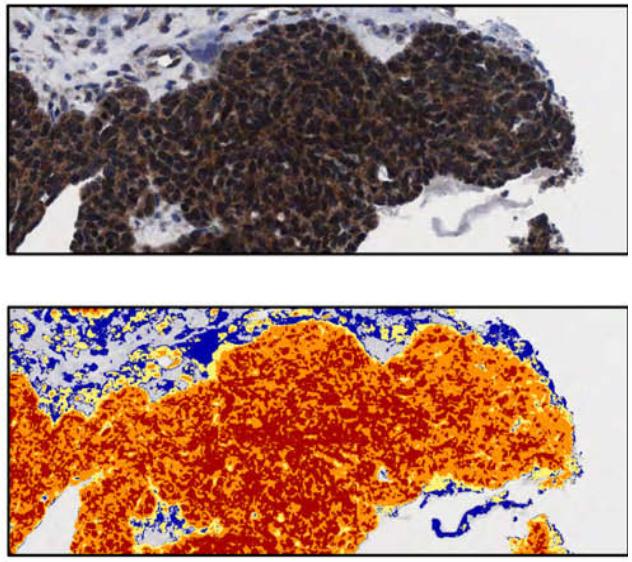






Cytplasmatic expression





Cytplasmatic and nuclear expression

Brennan et al., Eur J Cancer 2009; 45: 1510-1517

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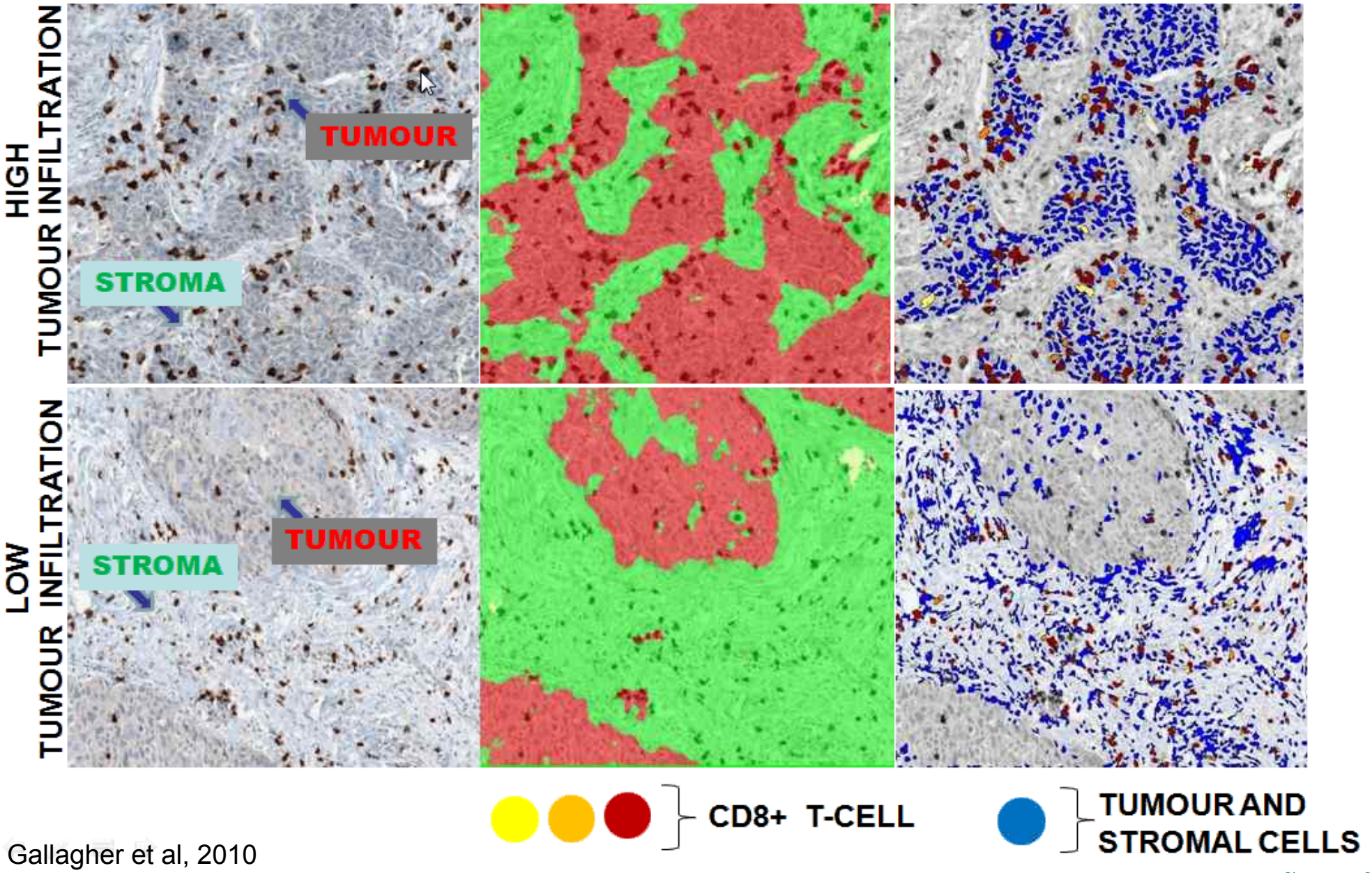
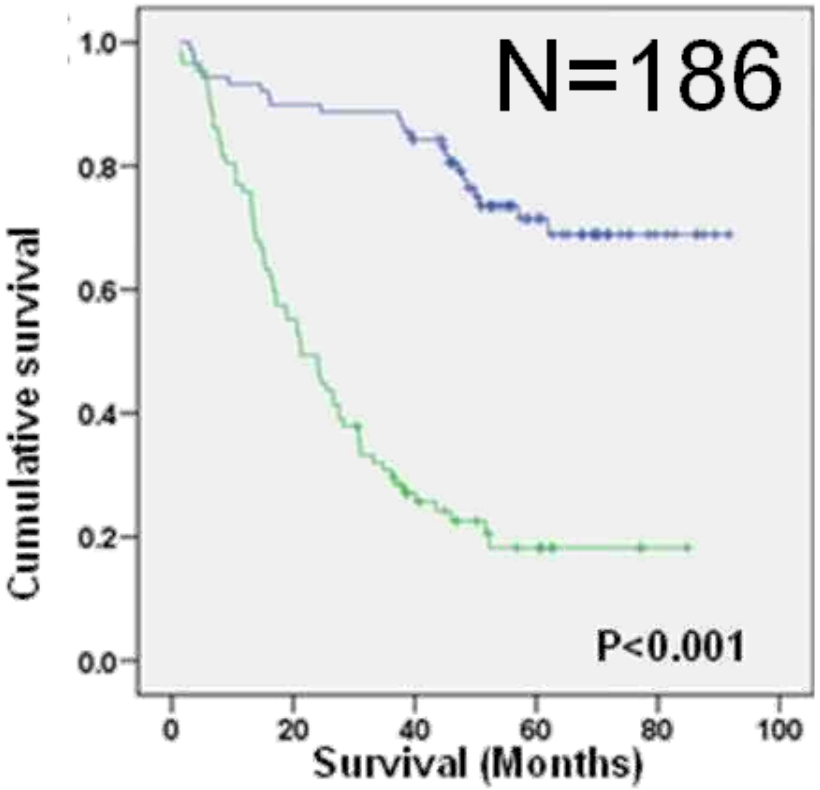
CD8+ T-lymphocyte Infiltration (Tumour versus Stroma)

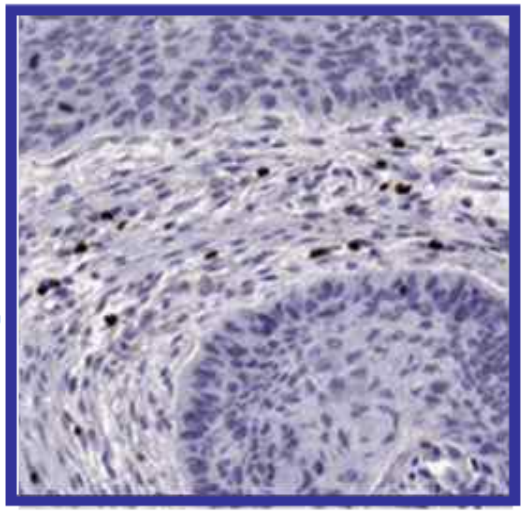


Gallagher et al, 2010

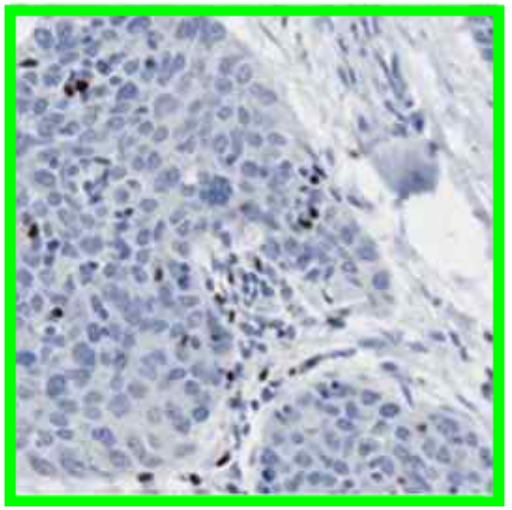
High Tumour/Stroma Ratio of FoxP3+ T-cells Linked to Decreased Patient Survival



Islet: Stroma **Foxp3+** Infiltration Ratio Below Median

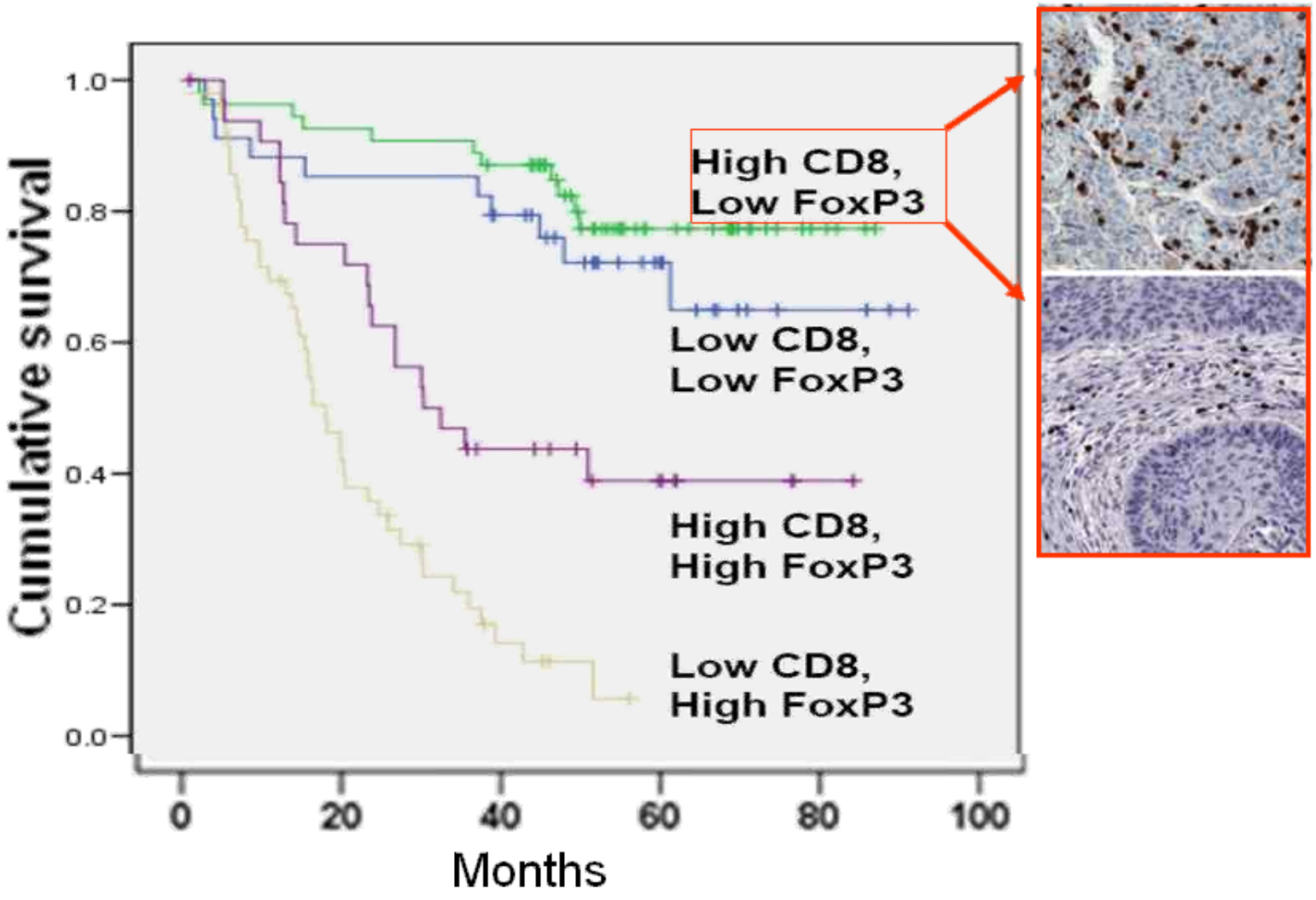


Islet: Stroma **Foxp3+** Infiltration Ratio Above Median



Gallagher et al, 2010

Intratumoural CD8+/FoxP3+ Cells is A Strong Predictor of NSCLC Patient Outcome

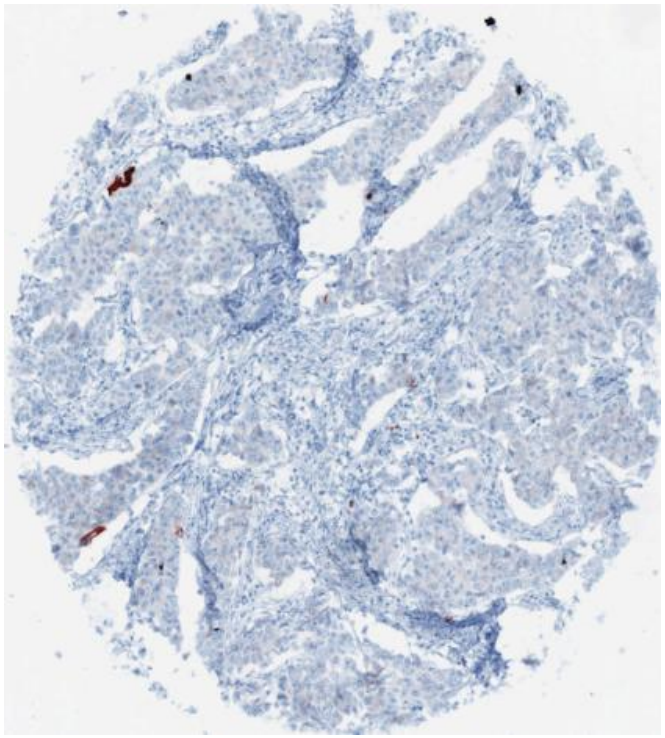


p <0.001 for all comparisons except 1 vs 2

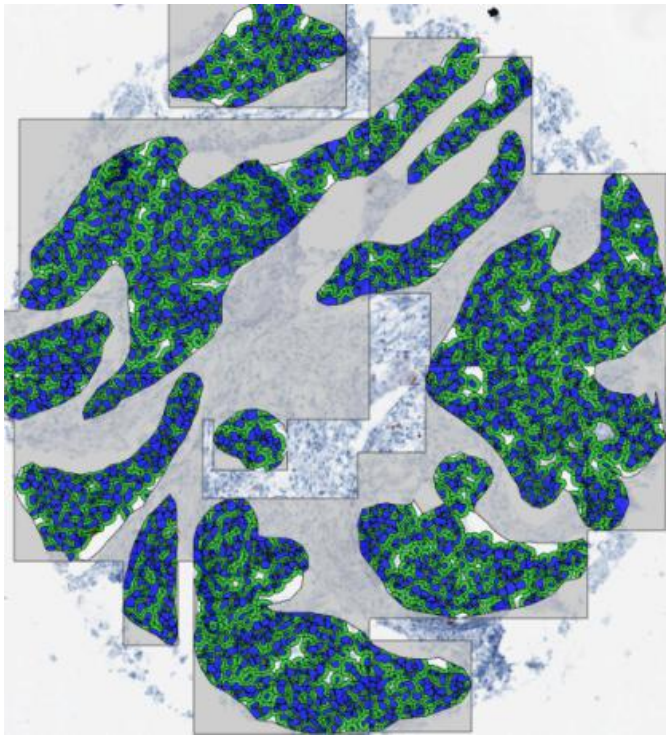
Gallagher et al, 2010

Triple-negative breast cancer

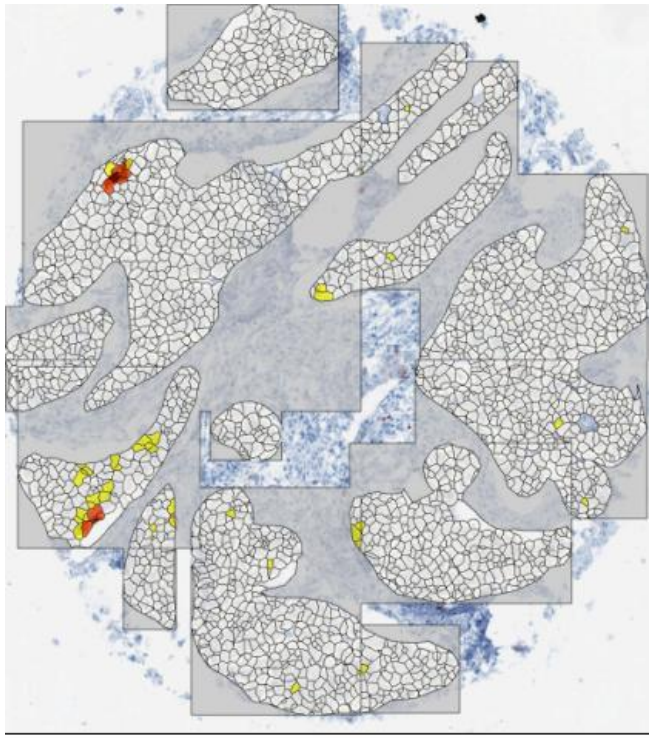
Cell simulation and classification



Ck 5/6



Nuclear detection



Cell simulation and
cell classification
2% pos. cells

Definiens algorithm

Elsawaf (2010)

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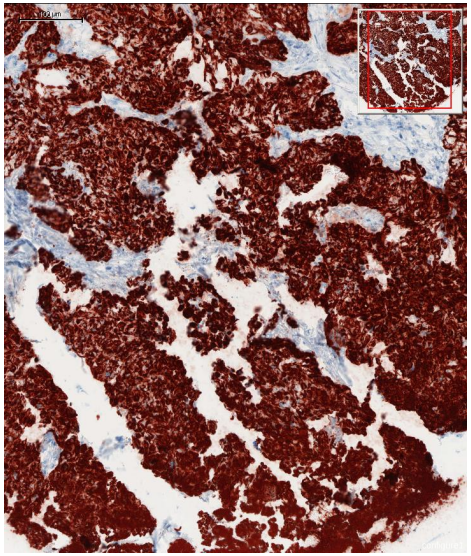
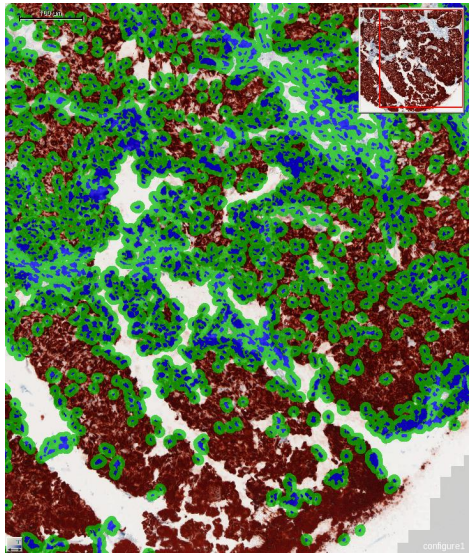
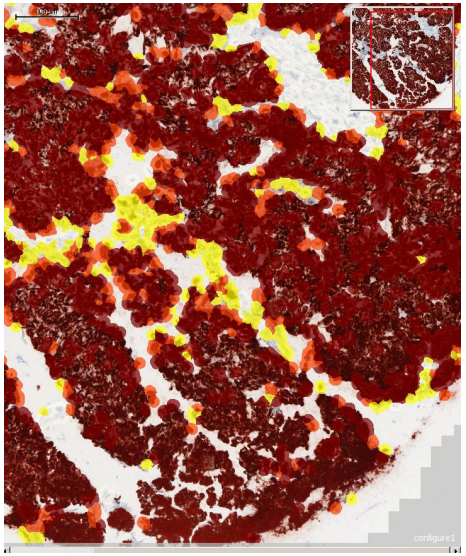
Ck 5/6

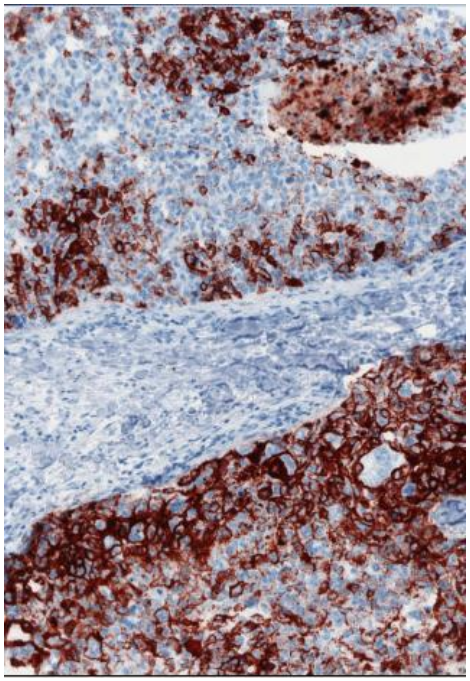
Nuclear detection

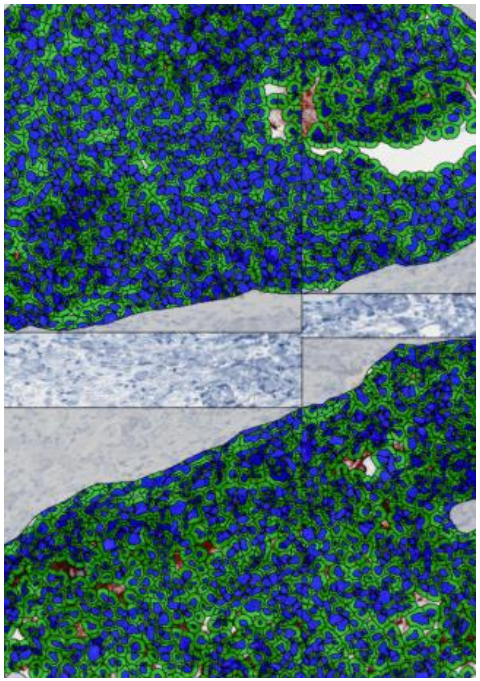
Cell classification

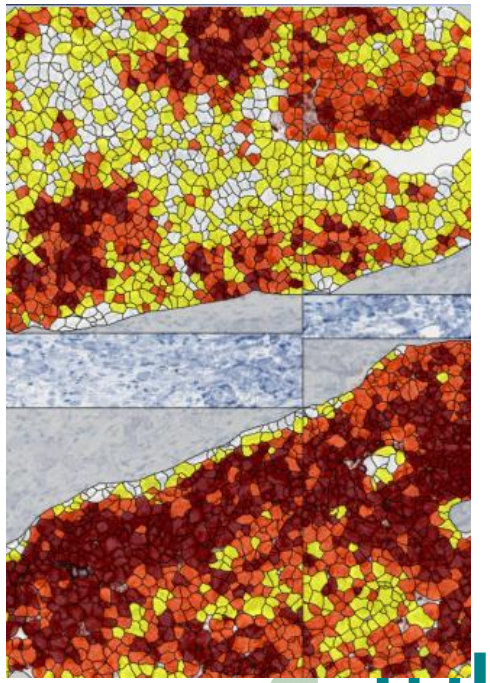












Definiens algorithm

Elsawaf (2010)

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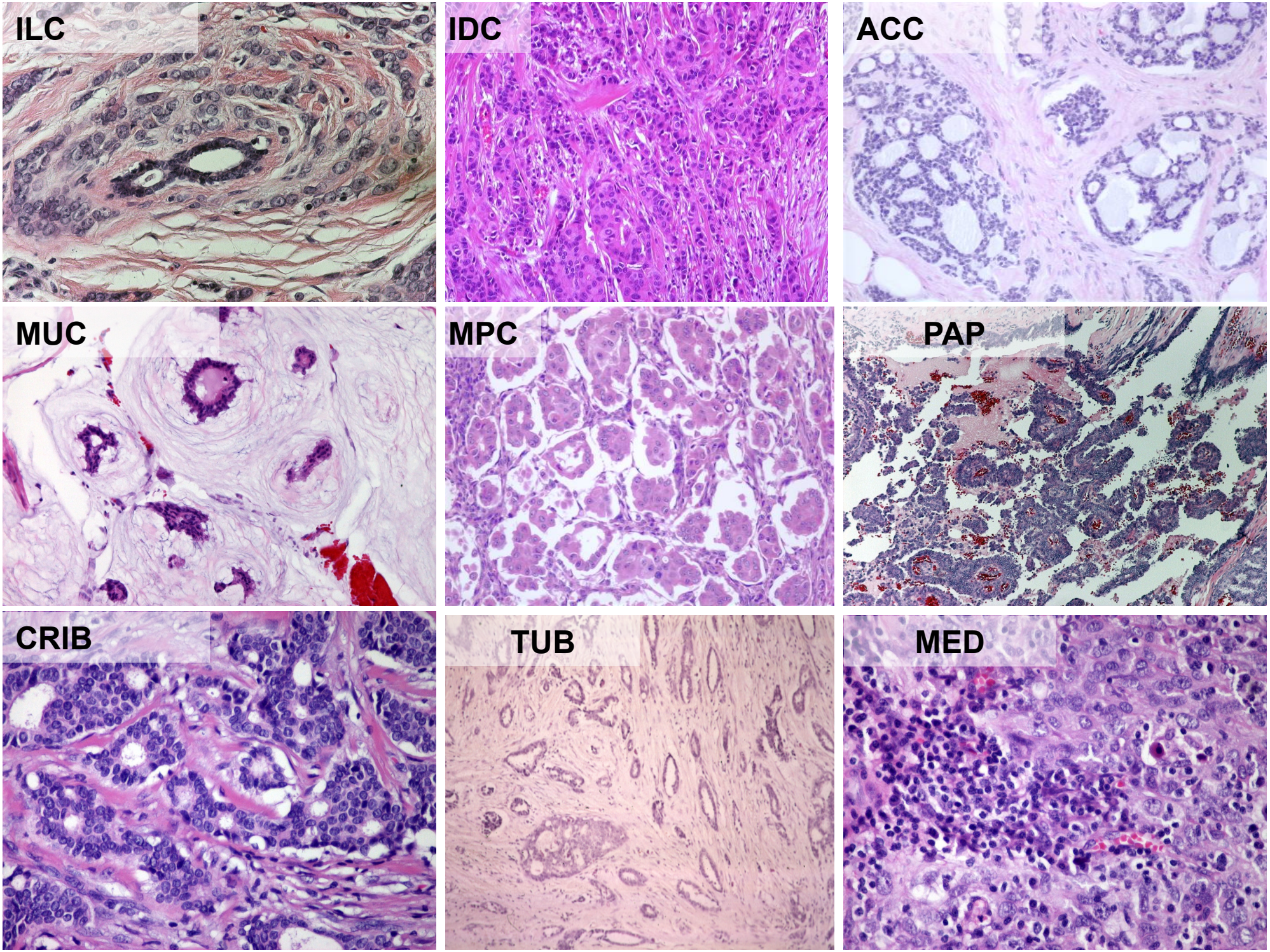
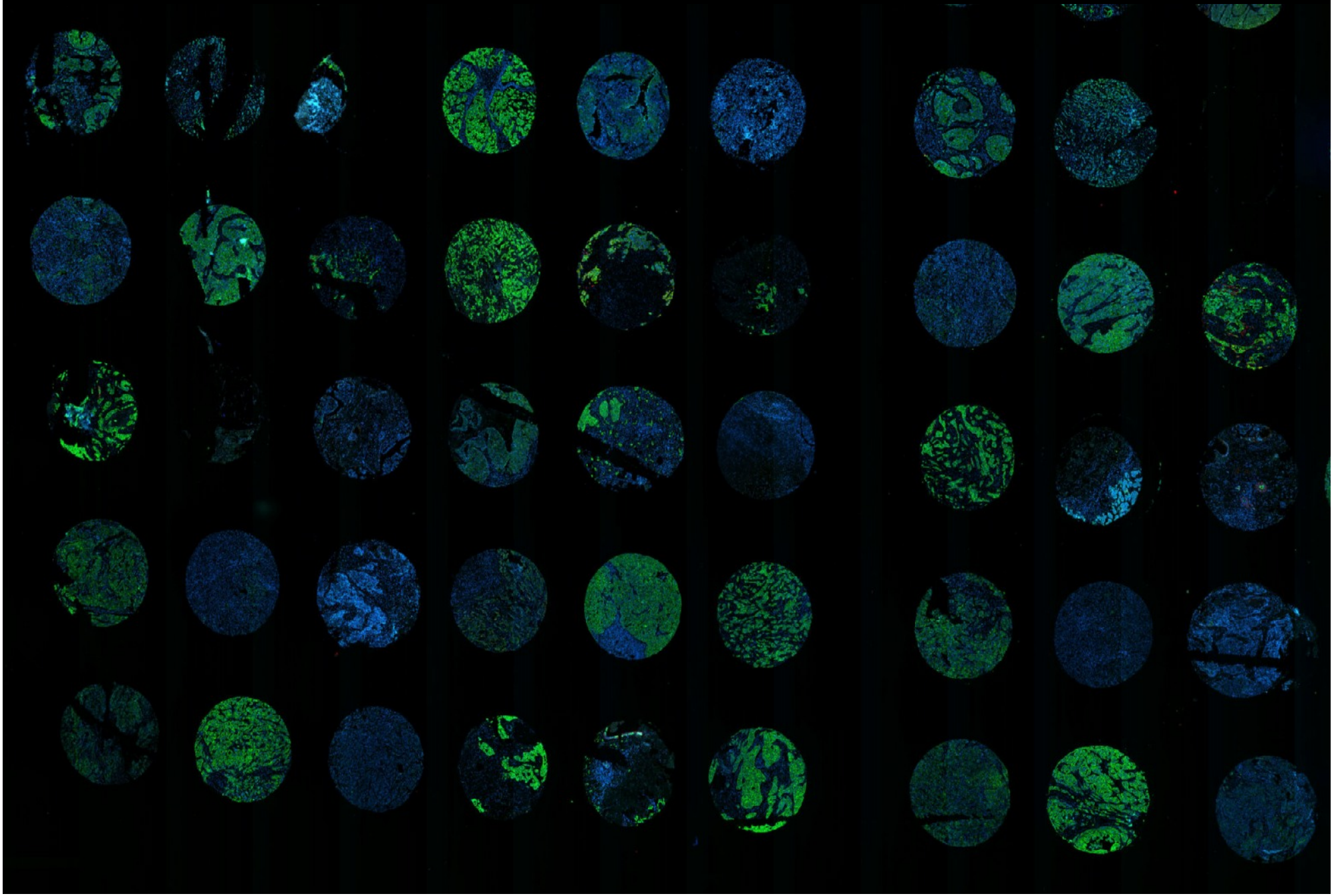
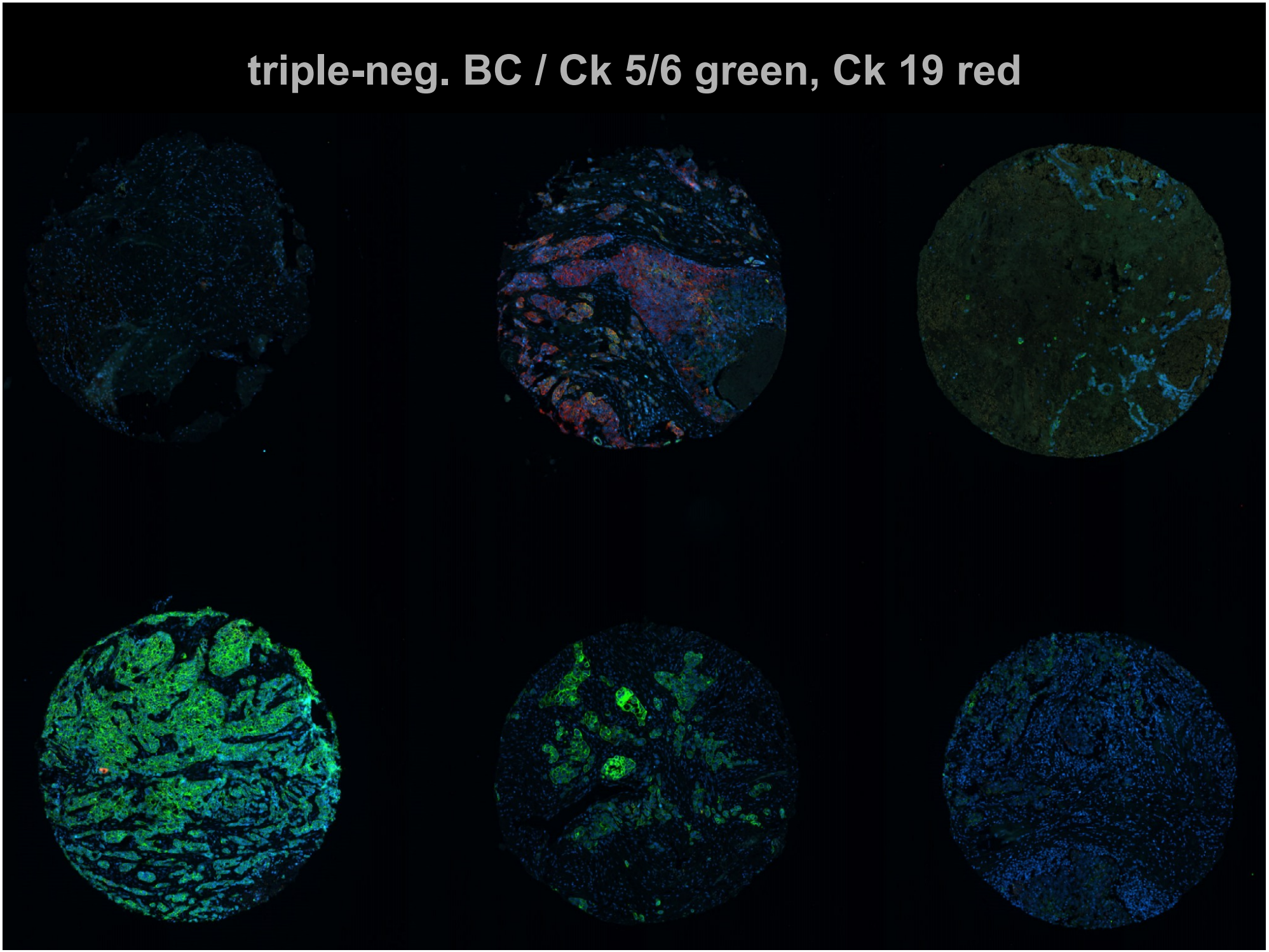

ILC
IDC
ACC
MUC
MPC
PAP
CRIB
TUB
MED

triple-neg. BC / Ck 5/6 green, Ck 19 red



triple-neg. BC / Ck 5/6 green, Ck 19 red



Limitations of digital imaging