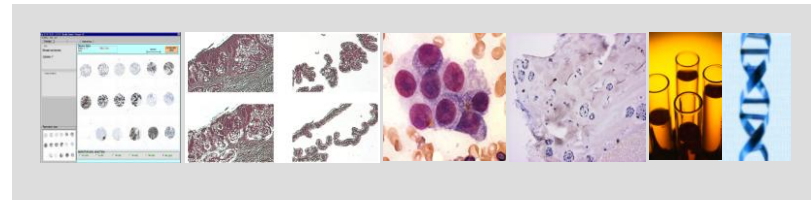


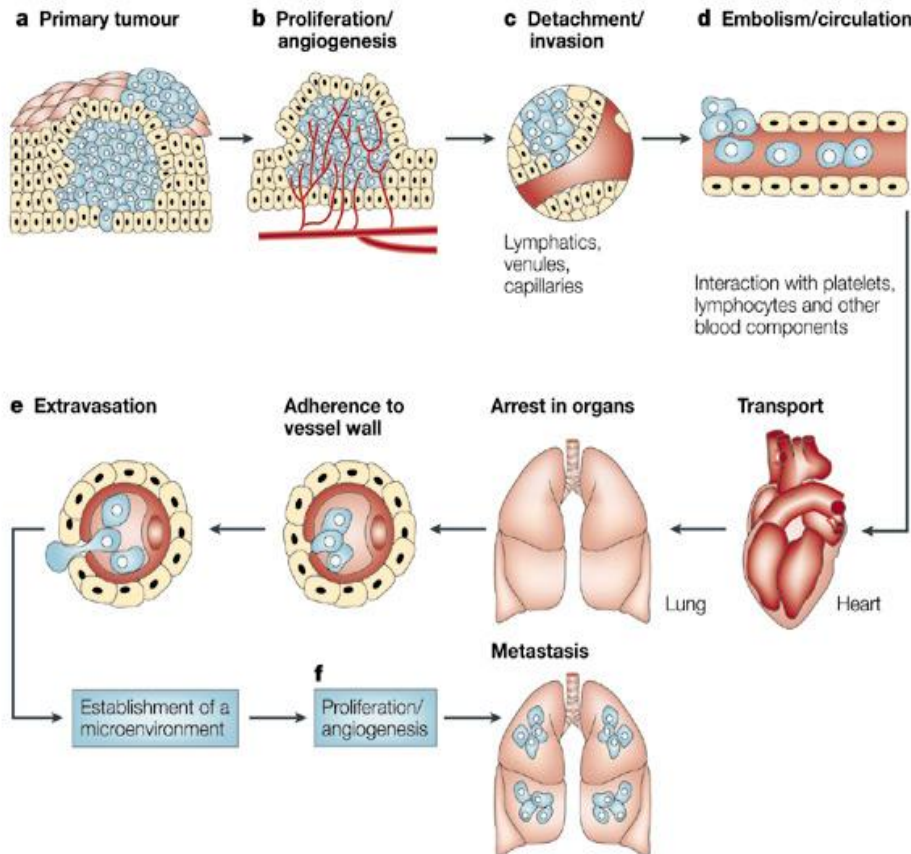
Automated tissue microarray analyses of breast cancer using the Distiller platform

Göran Landberg MD, PhD
Professor Molecular Pathology

*The Breakthrough Breast Cancer Research Unit,
University of Manchester,
Paterson Institute for Cancer Research
The Christie NHS Foundation Trust
Manchester, UK*

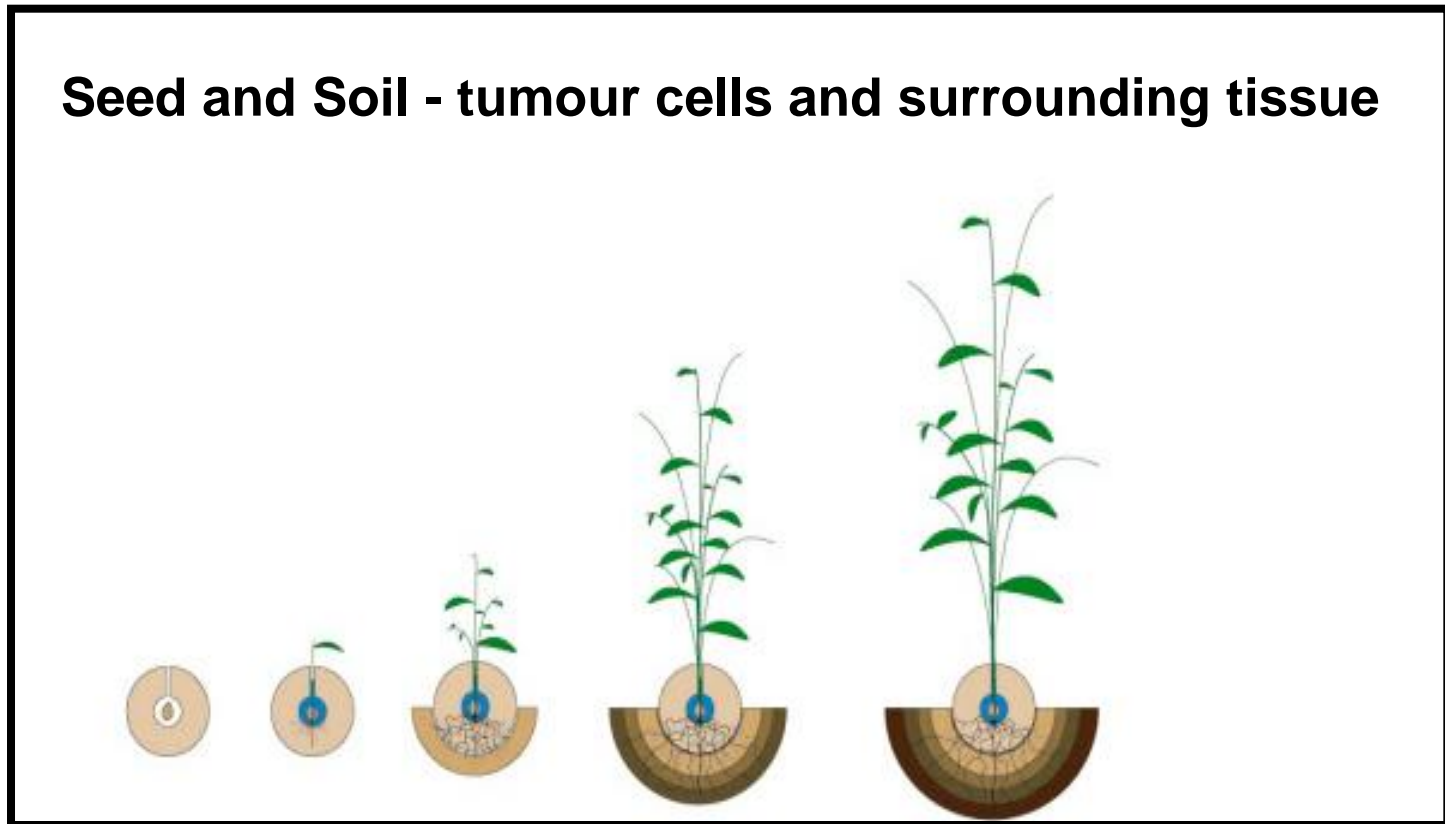


Breast cancer



- ***Breast cancer is a complex disease***
- ***Identify key events in breast cancer – potential targets for novel treatments***

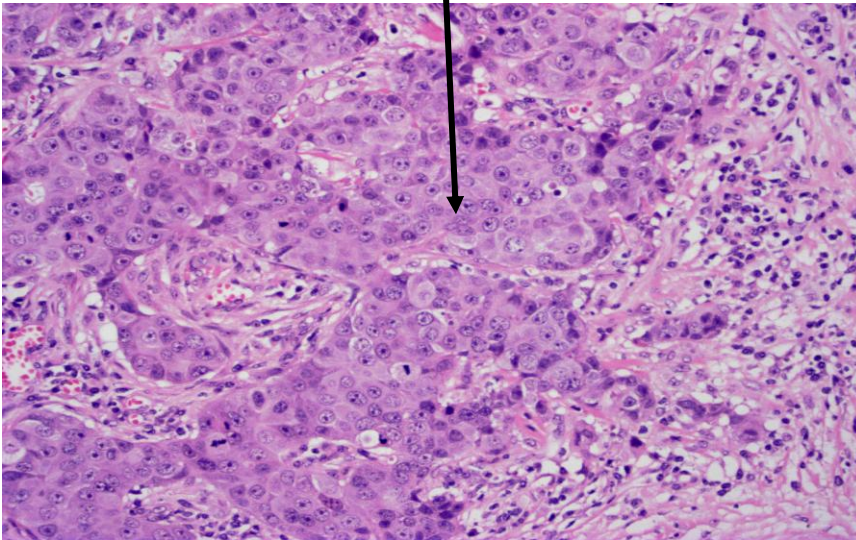
The Breakthrough Breast Cancer - Manchester Unit



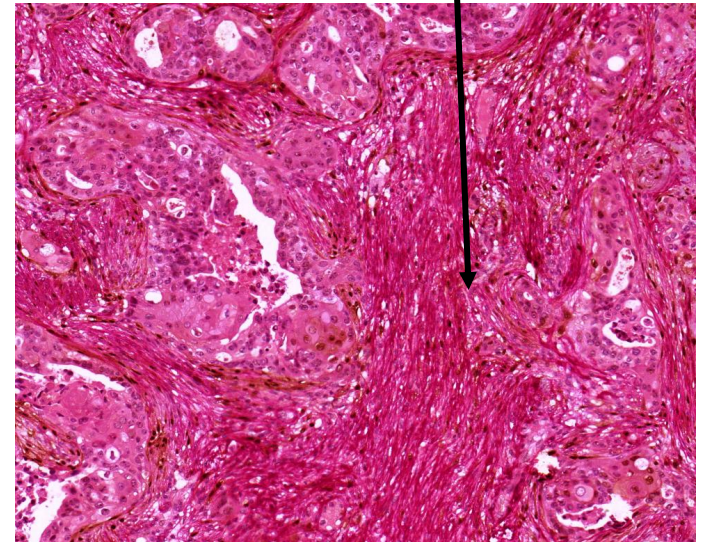
→ ***The environment is critical for the growth of breast cancer cells***

The Breakthrough Breast Cancer - Manchester Unit

Breast cancer cells
(seed)



Non-tumour cells
(soil)



Different parts of the breast cancer can be visualised and studied.

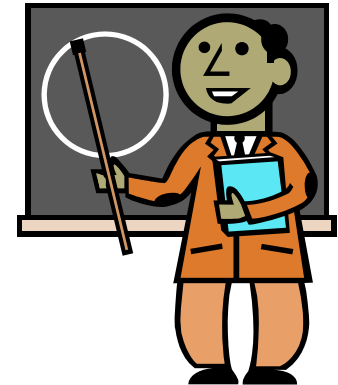
Molecular Pathology

The information is sometimes in the large context and sometimes in the details - the framework is important

Important to study cancer in its proper context

Biobank materials

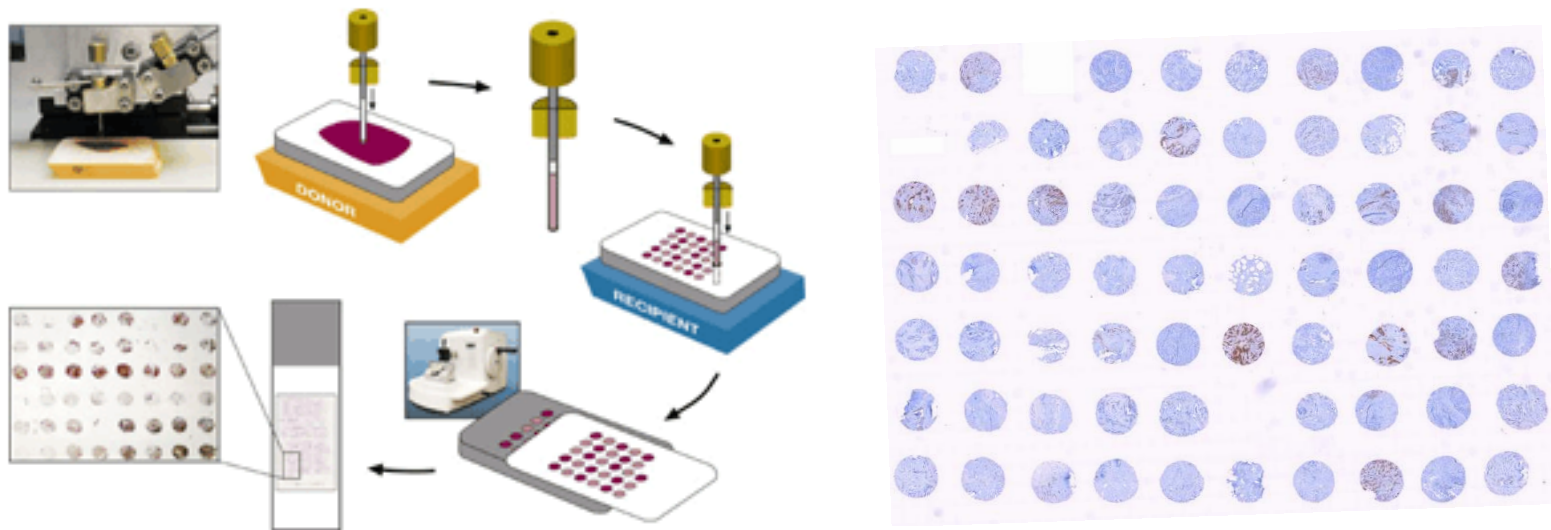
automated analyses



Automated analyses of TMAs using Distiller

- Nuclear analyses
 - ER/PR
 - cyclin D1
 - ductal breast cancer – lobular breast cancer
- Membrane analyses
 - Beta 6 integrin
- Collagen deposits
- Collagen and breast cancer

Tissue Micro Array

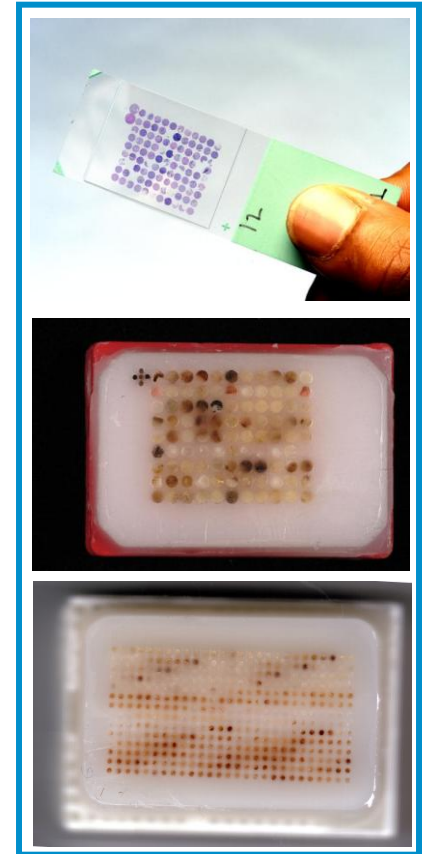


- *preserves valuable material*
- *enable high throughput analyses*

Distiller OpTMA - Slidepath

Platform for high throughput Tissue Microarray analysis.

- Automatic identification and labelling of Tissue Microarrays
- User defined scoring.
- Online automatic core navigation.
- Consolidate scores from multiple users workflow.
- Perform Random or Sequential reviews.
- Run high-throughput image analysis, and export all data.

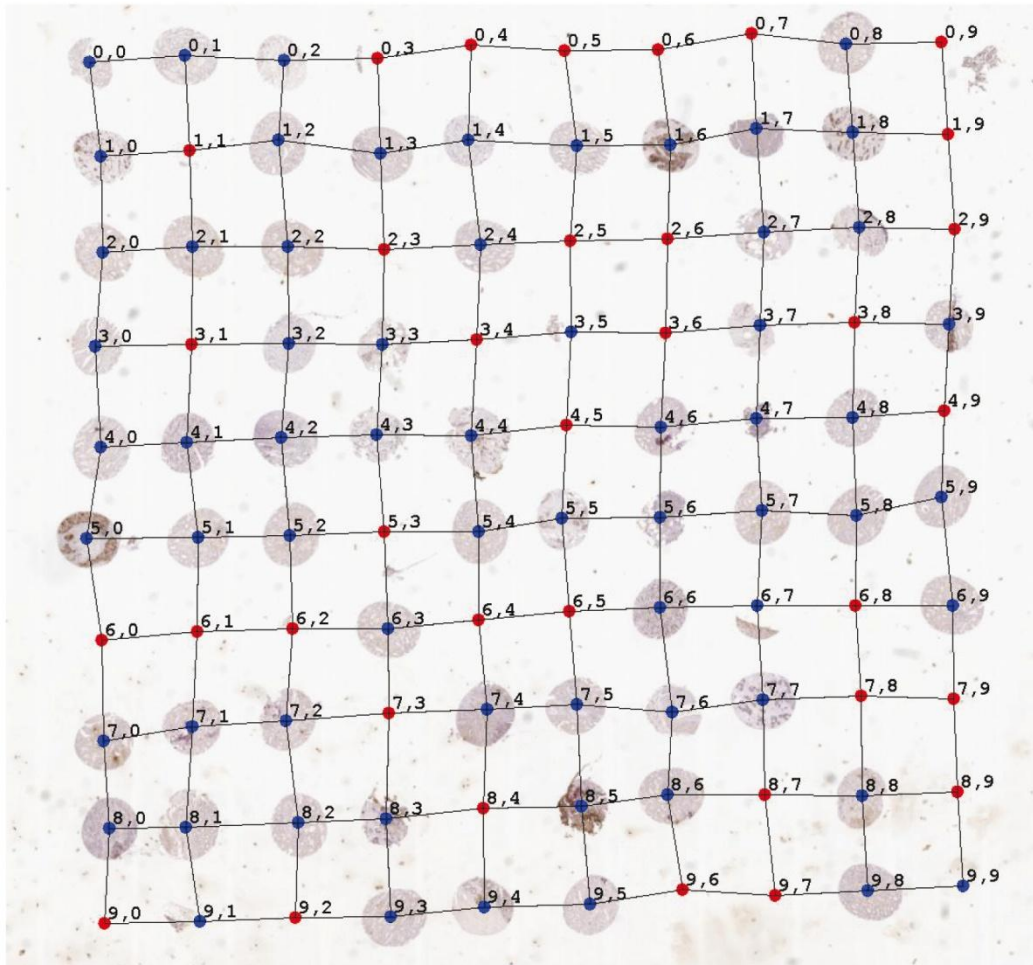


Digital Pathology – The Challenges

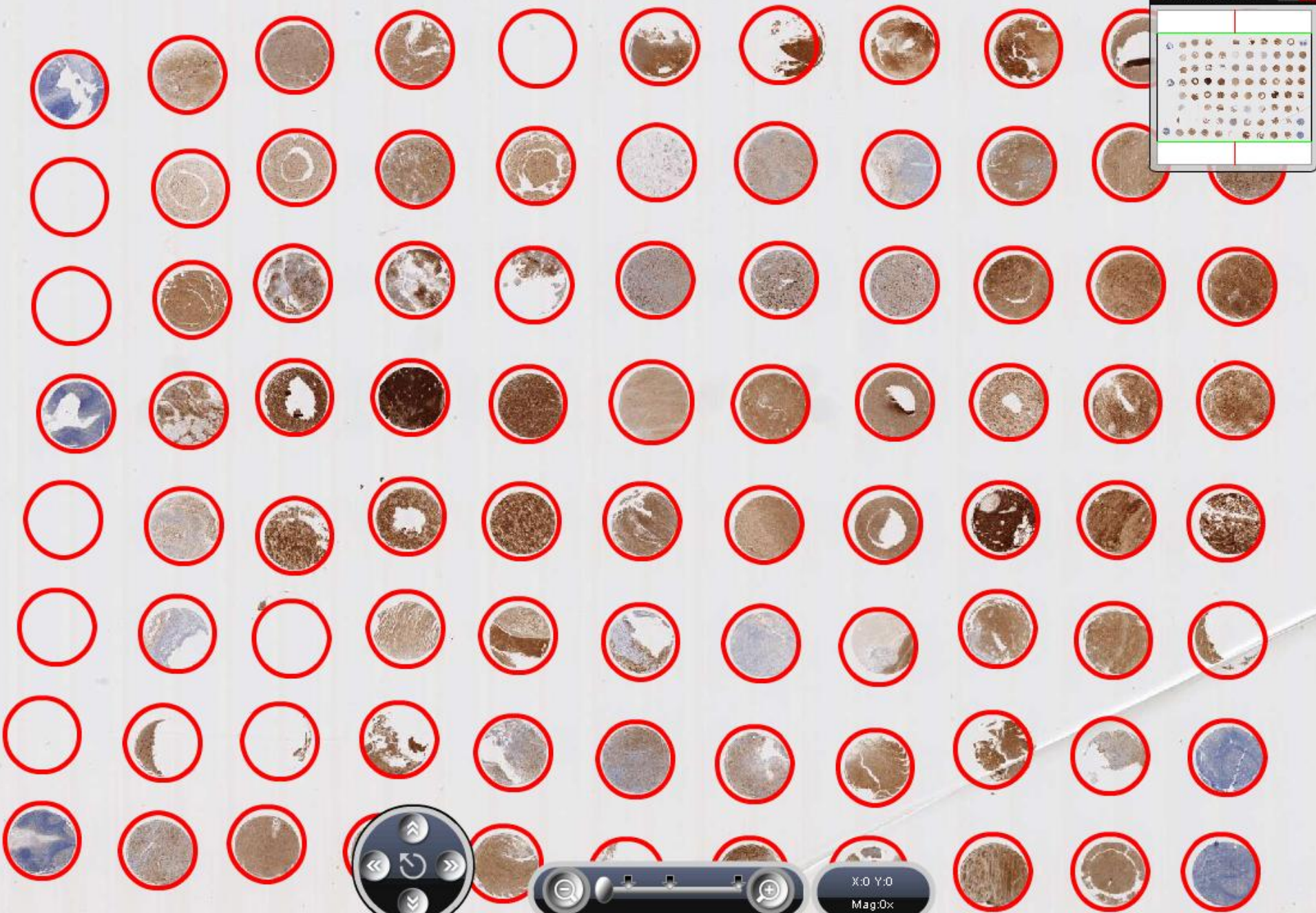
- Digital Pathology images are really large – often exceeding 10 gigapixel.
- This is the equivalent of a 1000 photos taken with a digital camera all stitched together.
- Like looking at each blade of grass in detail on a football pitch!



- Assign grid overlay to the TMA image, each coordinate links to clinical record



Slide overview



X:0 Y:0
Mag:0x

Annotation Form

select colour:



Patient Information

* HER-2 Grade

0/1+

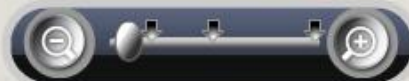
2+

3+

Previous

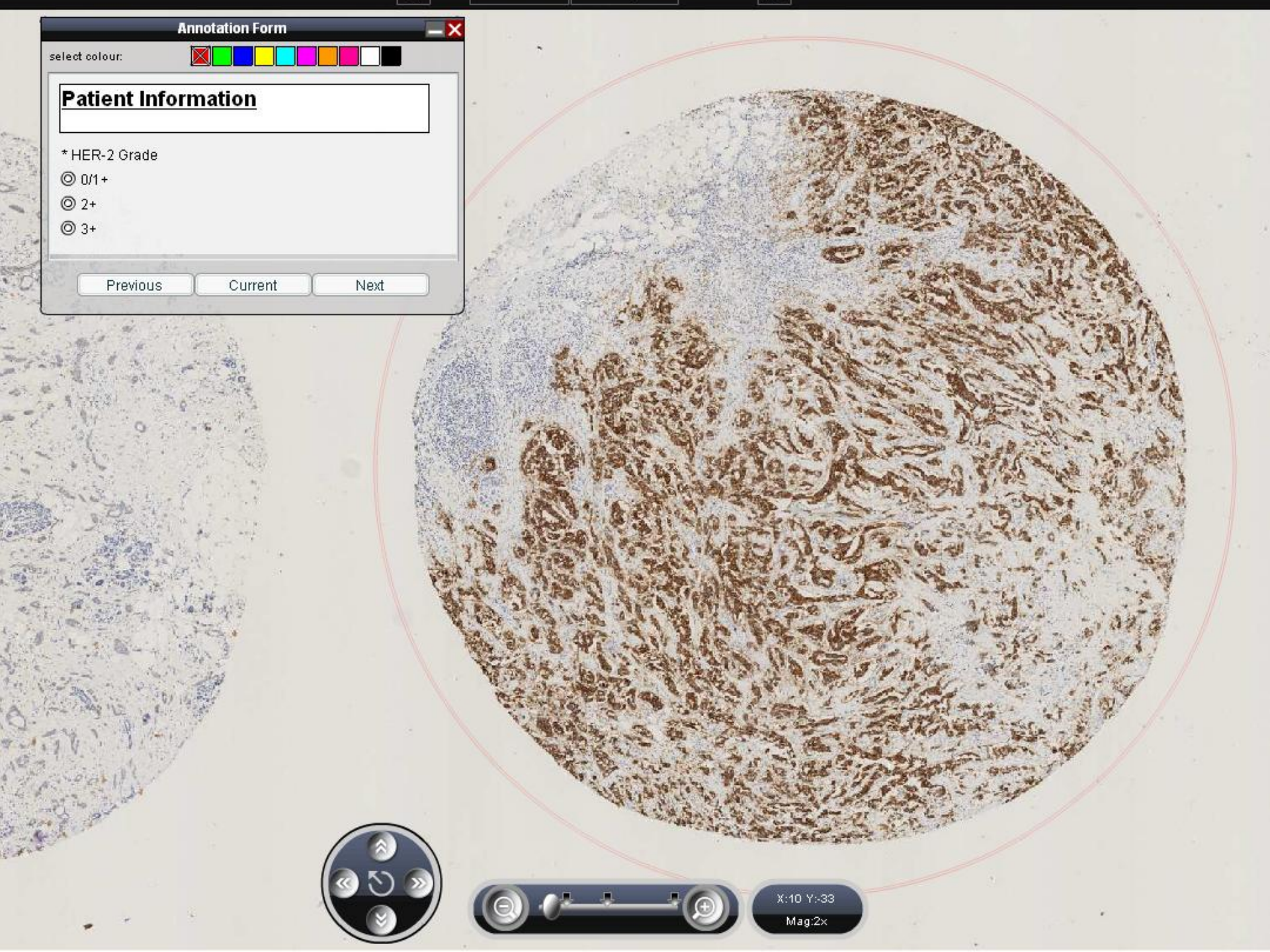
Current

Next

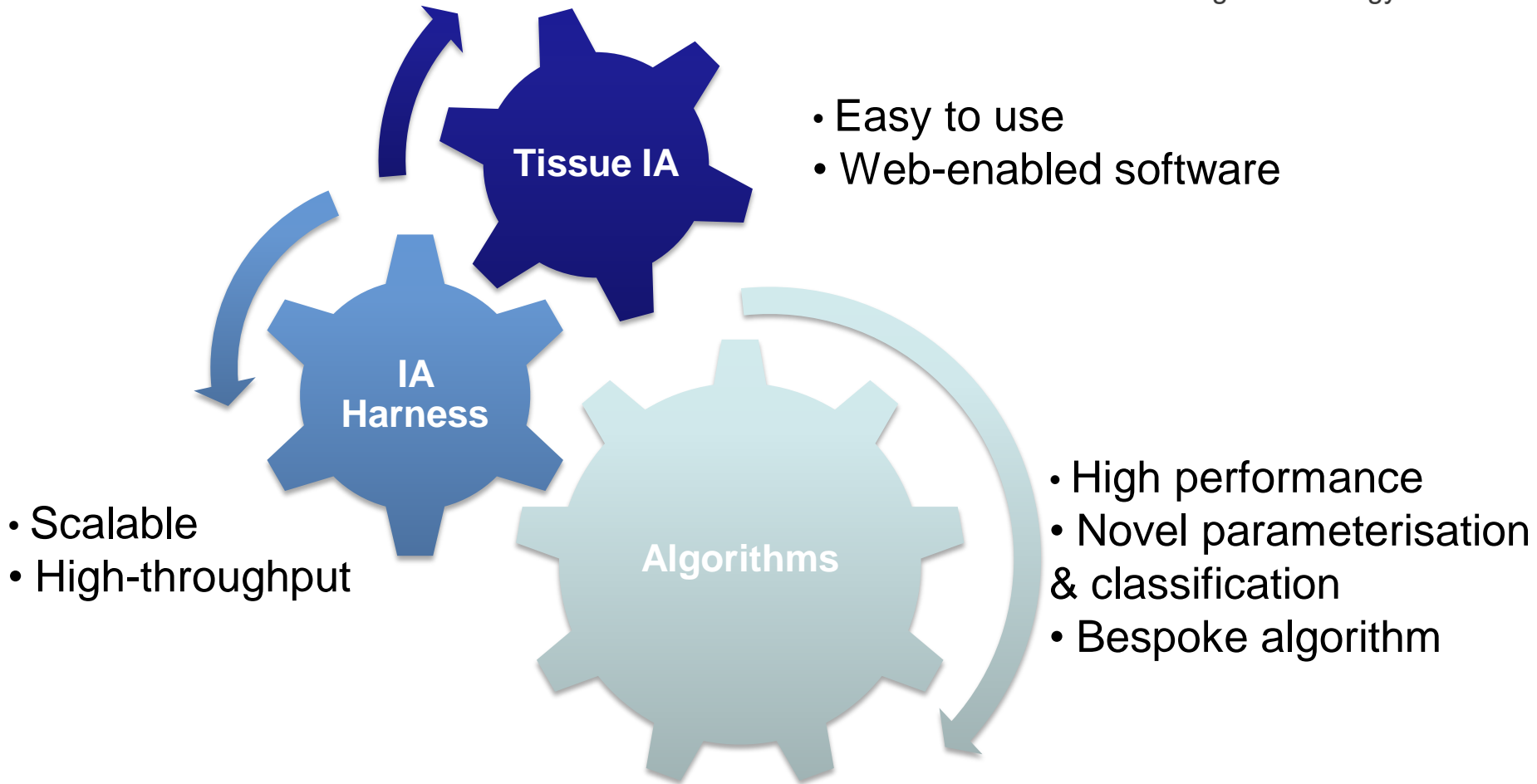


X:10 Y:33

Mag:2x



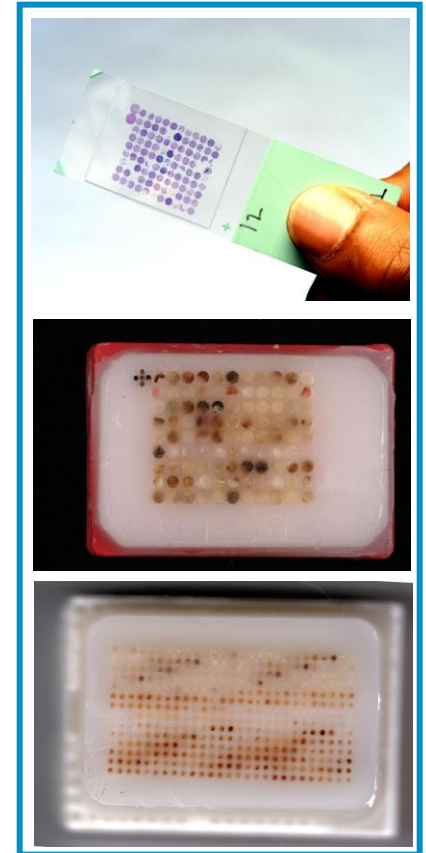
Tissue IA



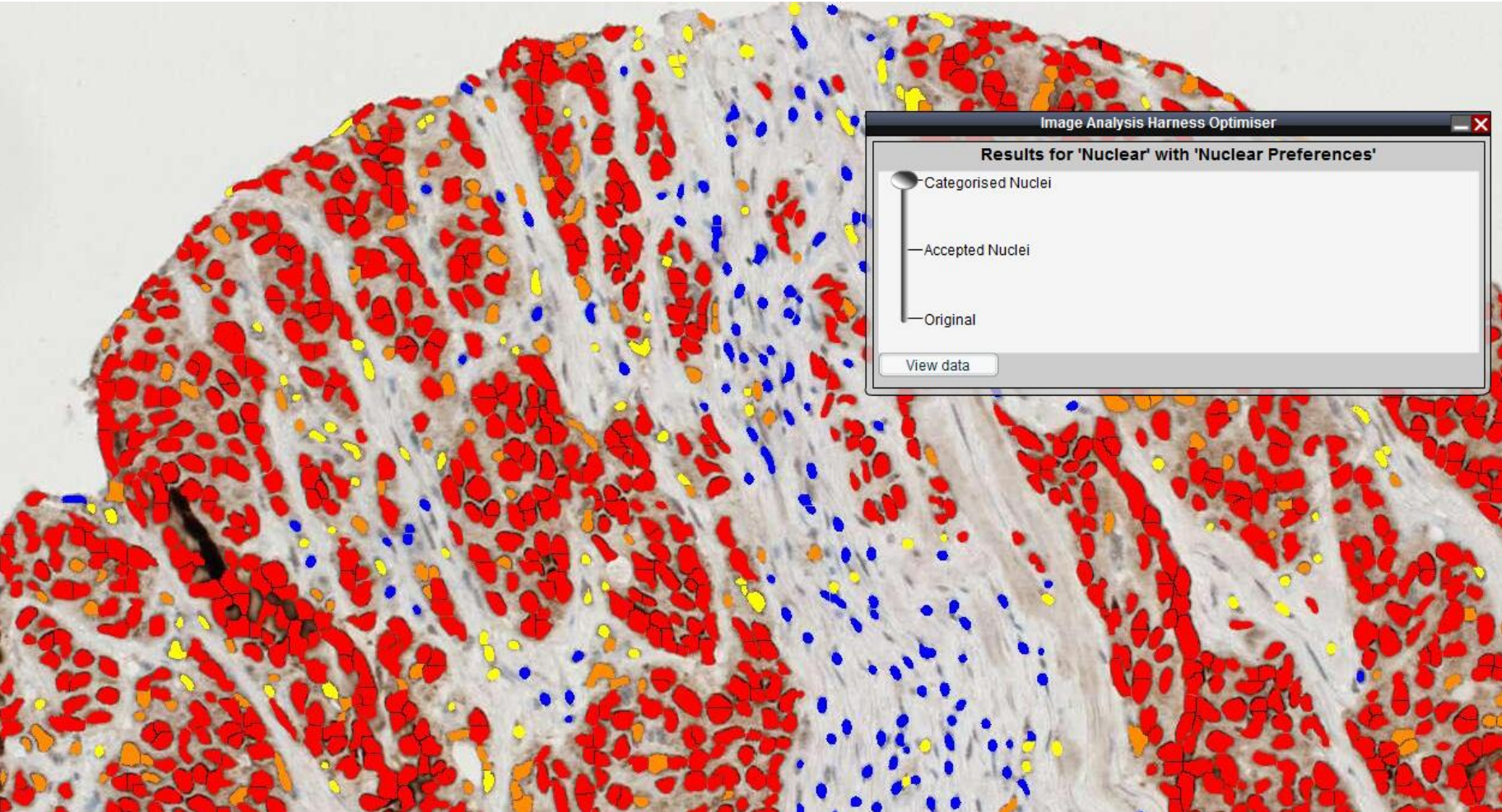
Tissue IA

Configurable, High Performance Algorithms for Quantitative Immunohistochemistry

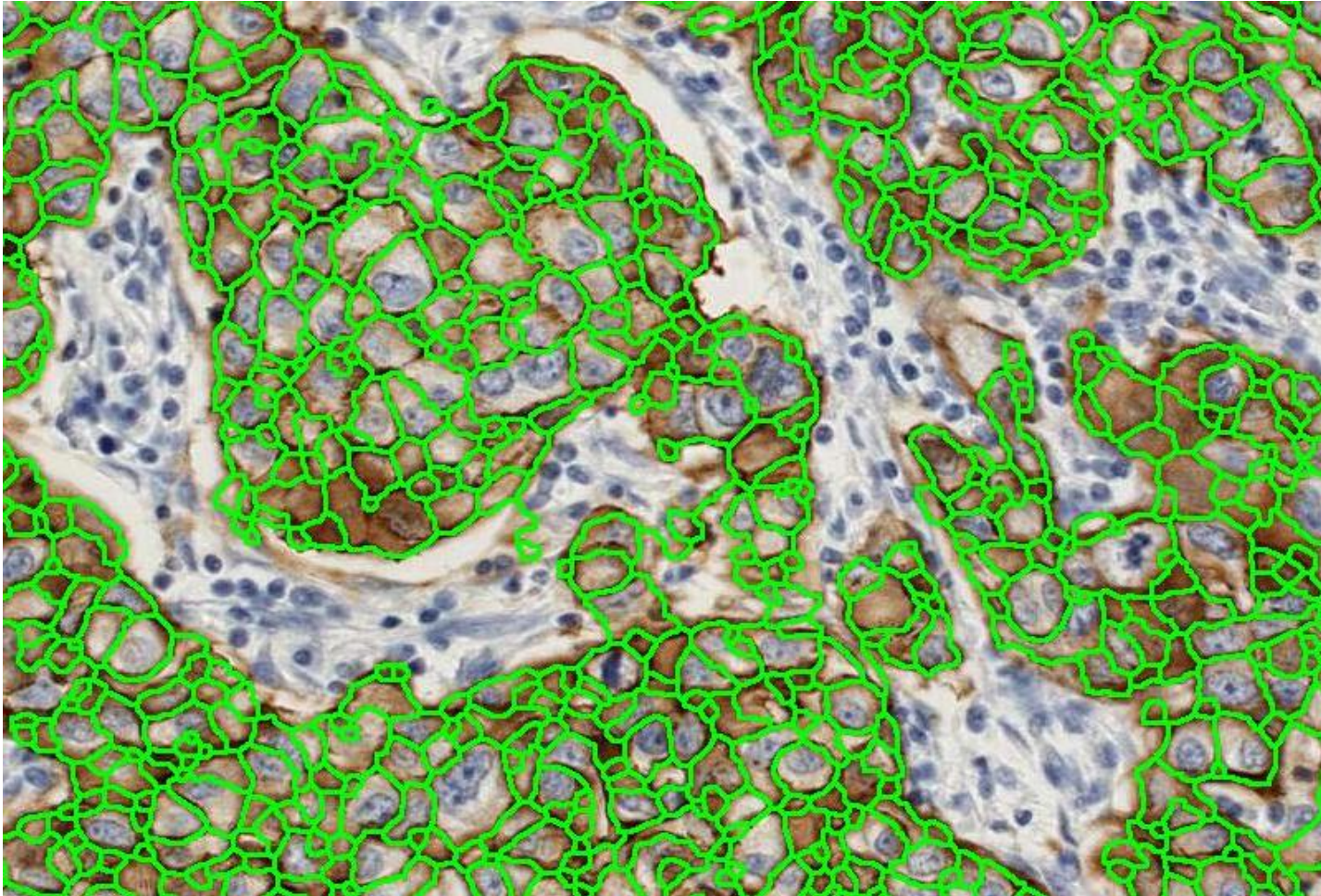
- Nuclear Algorithm
 - Accurate identification of “tumour” cell nuclei.
 - Elimination of non relevant nuclei using morphology and spatial arrangement filters
 - Accurate quantification of nuclear staining using histoscore.
- Membrane Algorithm
 - Accurate segmentation of positively stained membrane
 - Quantification of membrane staining intensity/absorbance
- Cytoplasmic Staining
 - Accurate quantification of cytoplasmic staining using colour identification system
 - Quantification of intracellular staining also possible by removal of membrane component.



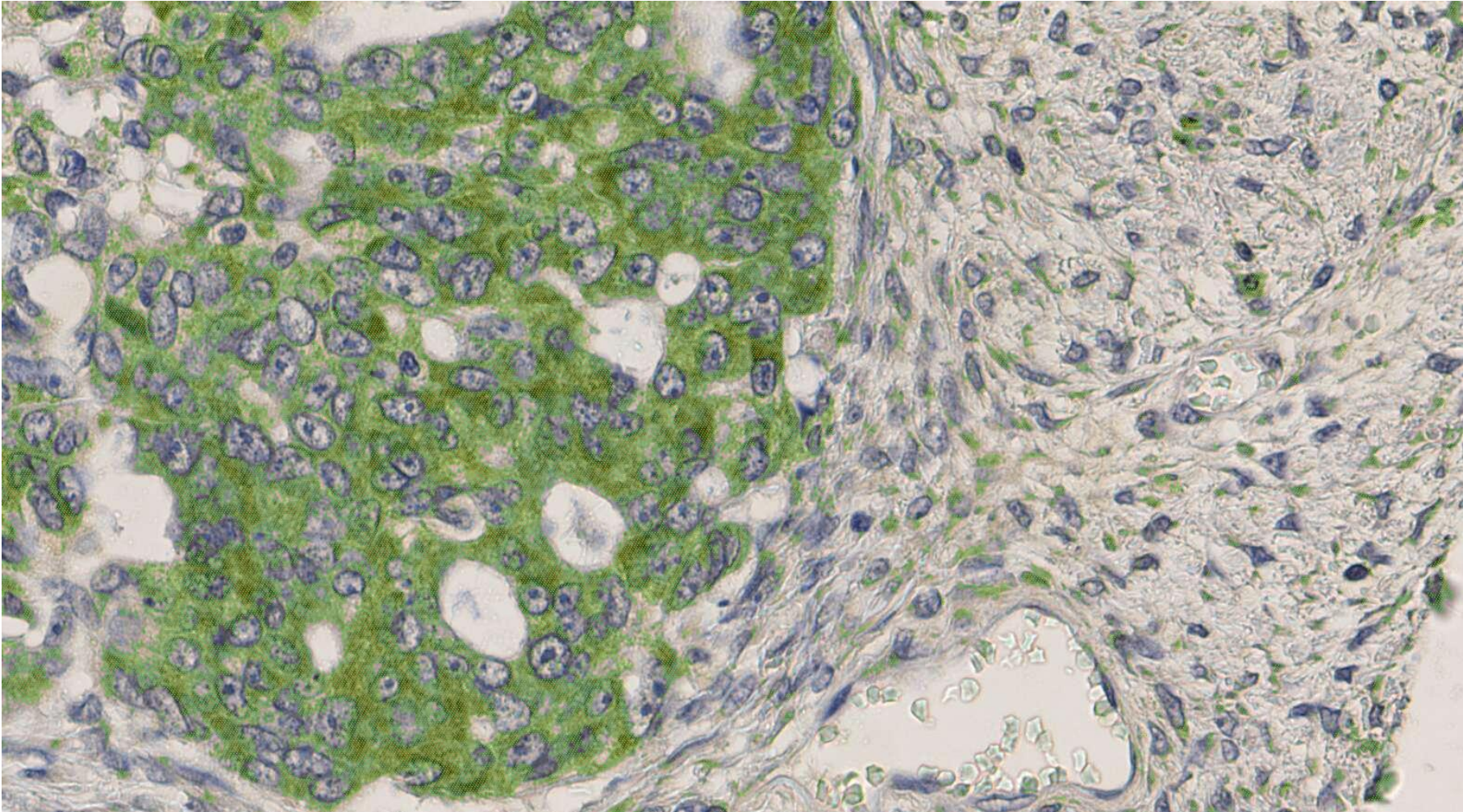
Nuclear Algorithm



Membrane Algorithm

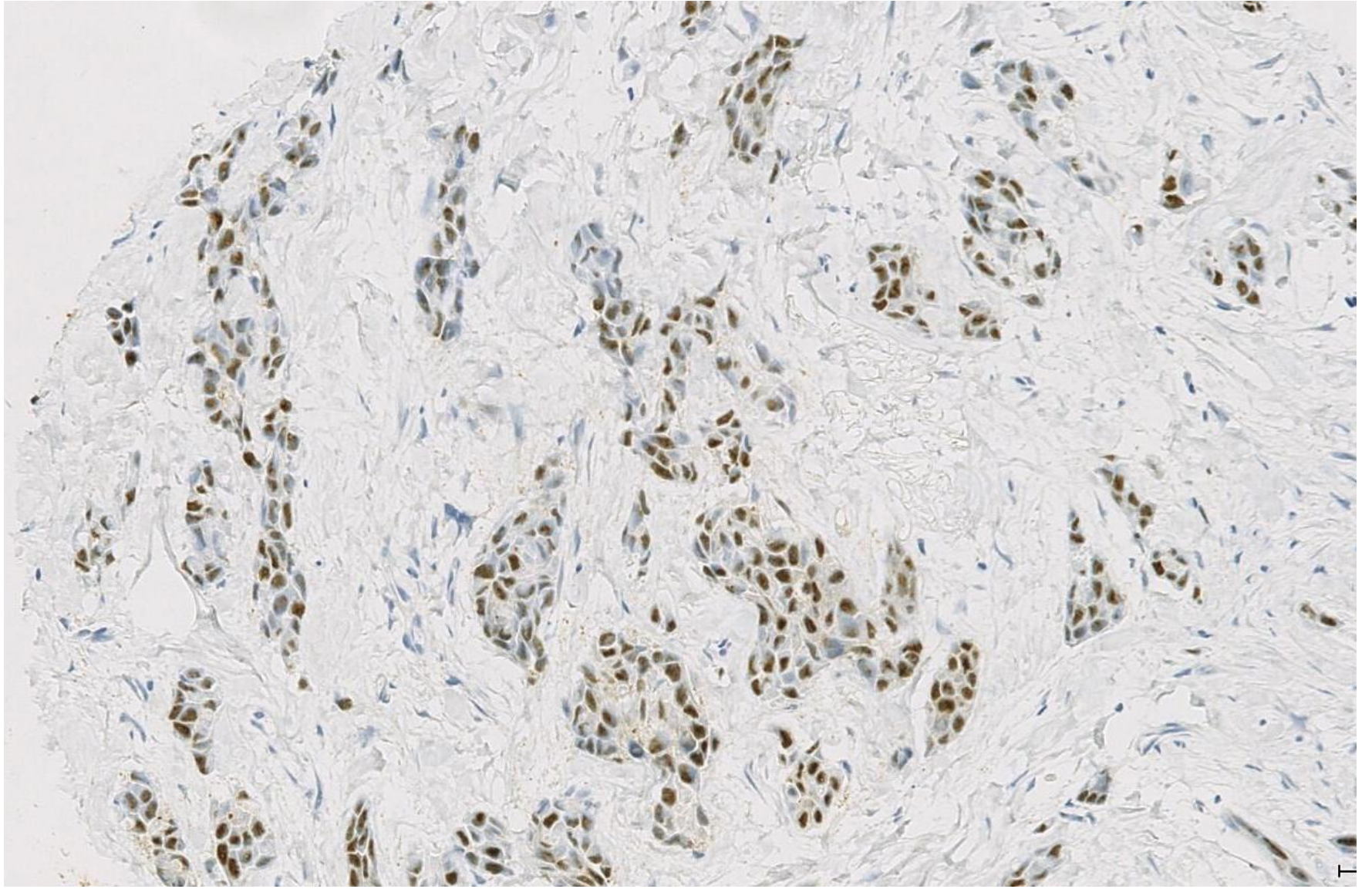


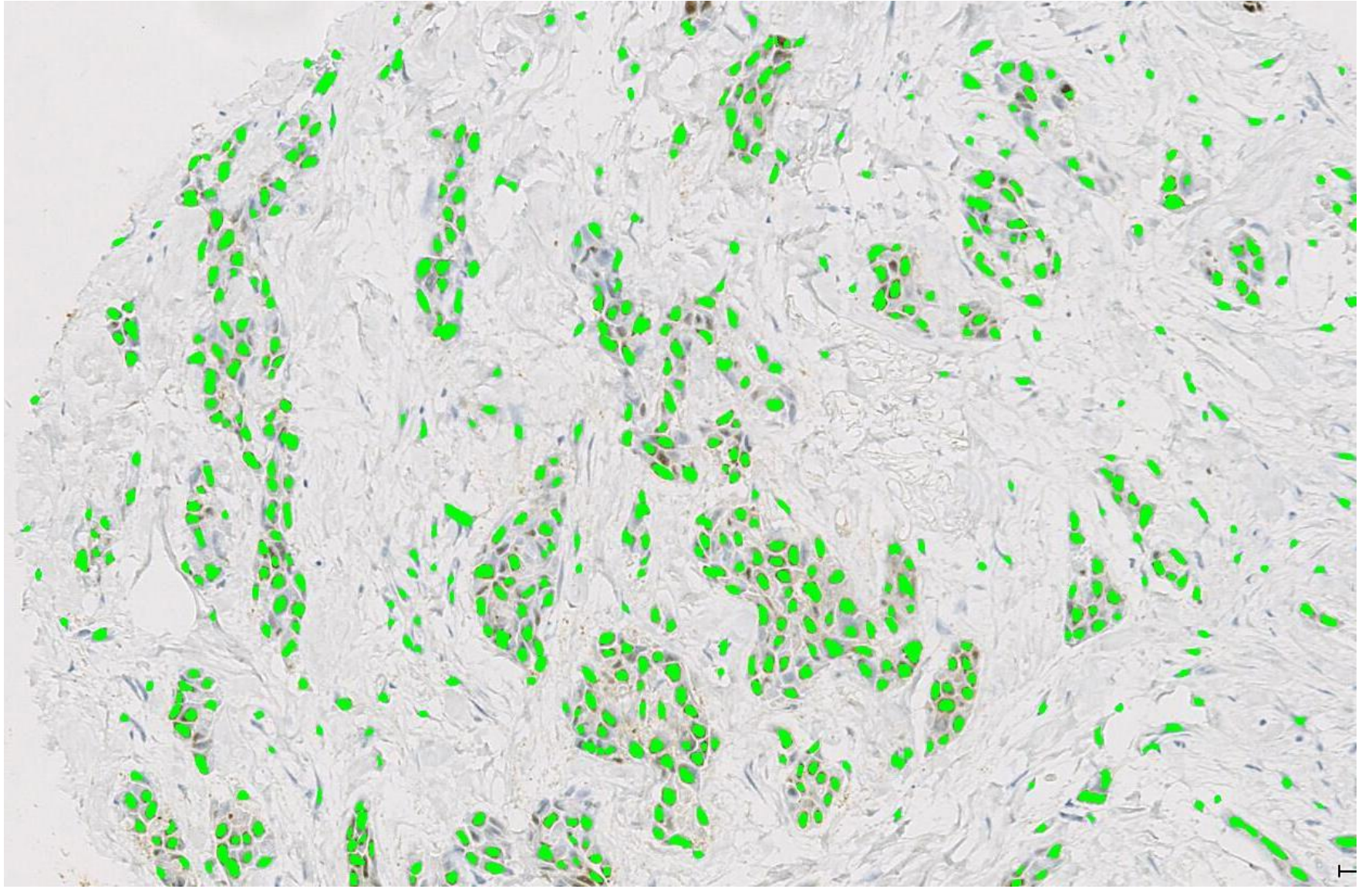
Cytoplasmic Algorithm

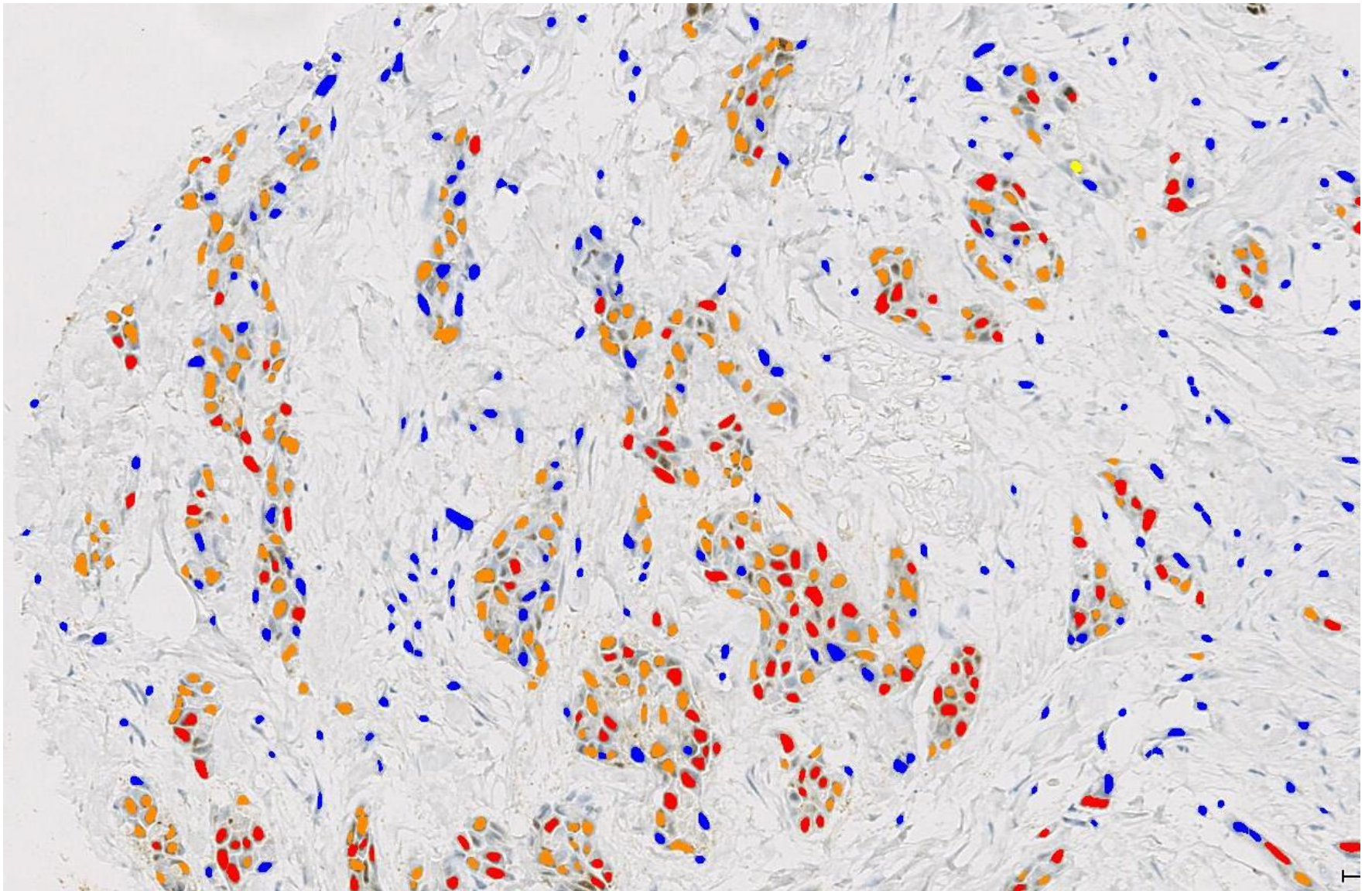


Automated analyses of ER/PR in breast cancer

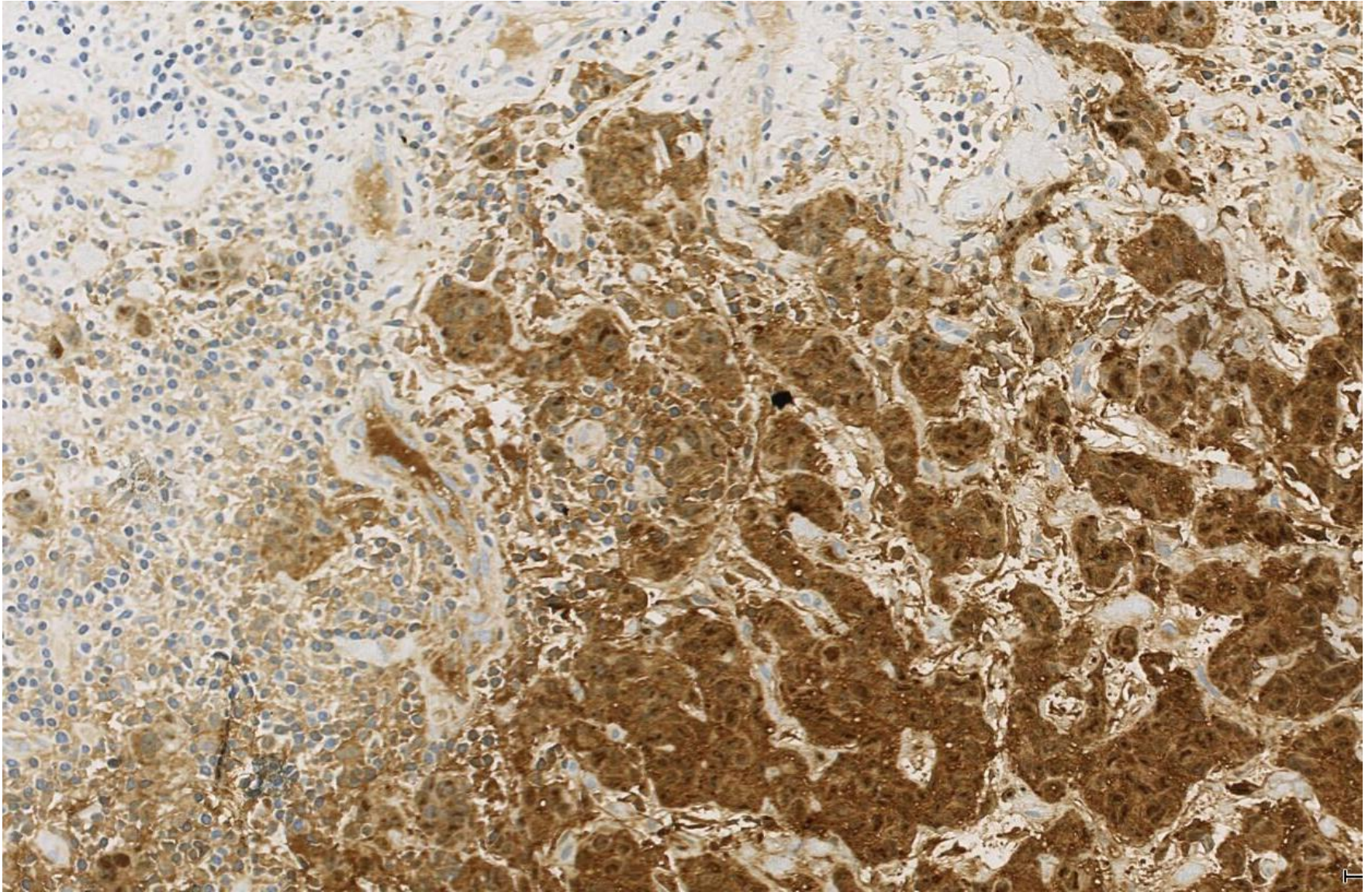
- Manual evaluation in a training set of 222 tumours
 - Allred scoring 0-8
- Algorithm optimisation using the Distiller platform
- Analyses of concordance between automated histoscore and manual Allred scoring
- Ongoing analyses of large breast cancer TMAs (>4000)
 - potential clinical relevant linear information in the ER/PR analyses



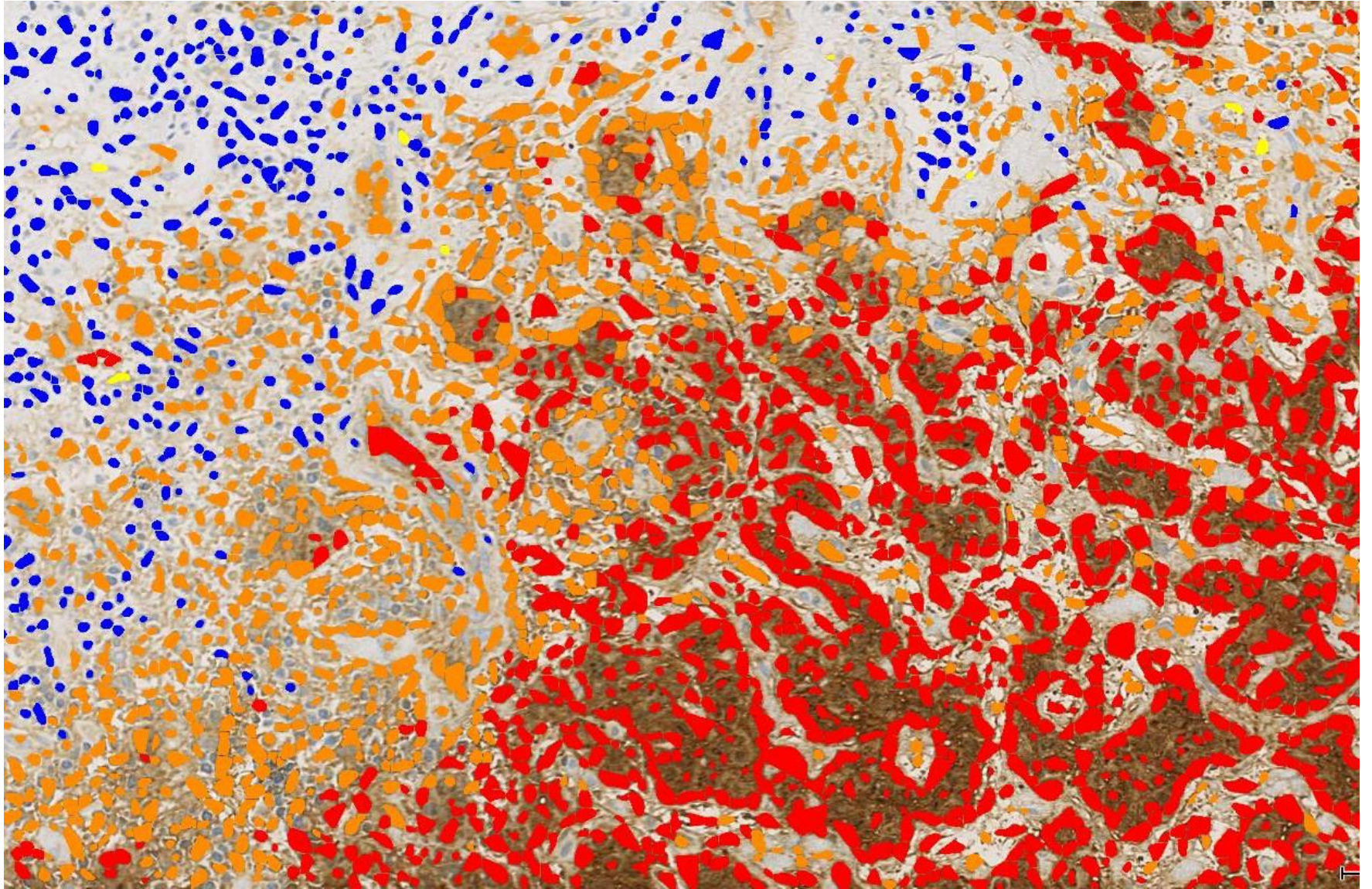




*red = strongly stained,
orange = moderate,
yellow = weak
blue = negatively stained*

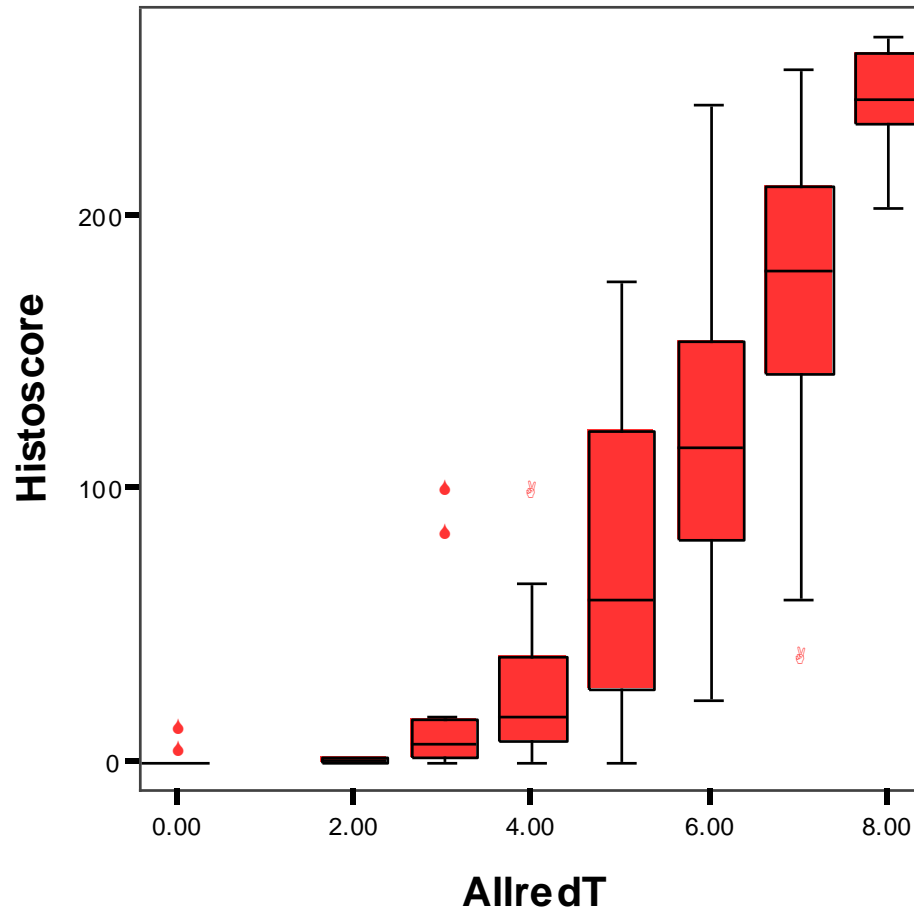


Some tumours have high general cytoplasmic and nuclear reactivity



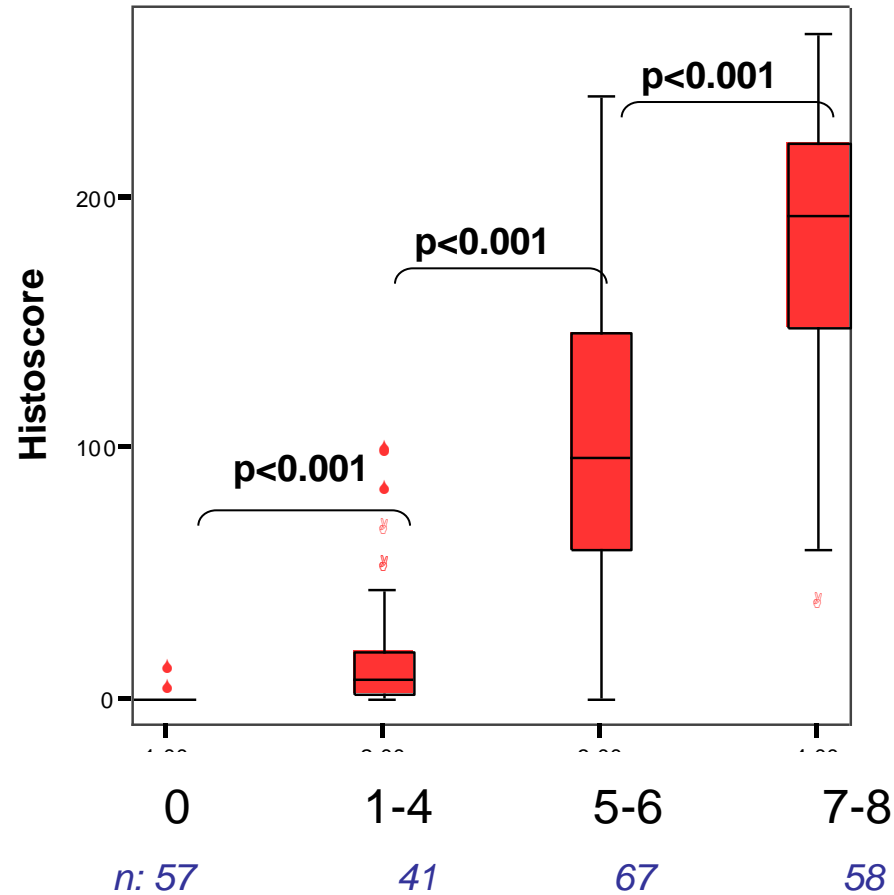
6 out of 306 samples excluded - identified with high positive pixel

Relation between automated and manual ER analyses



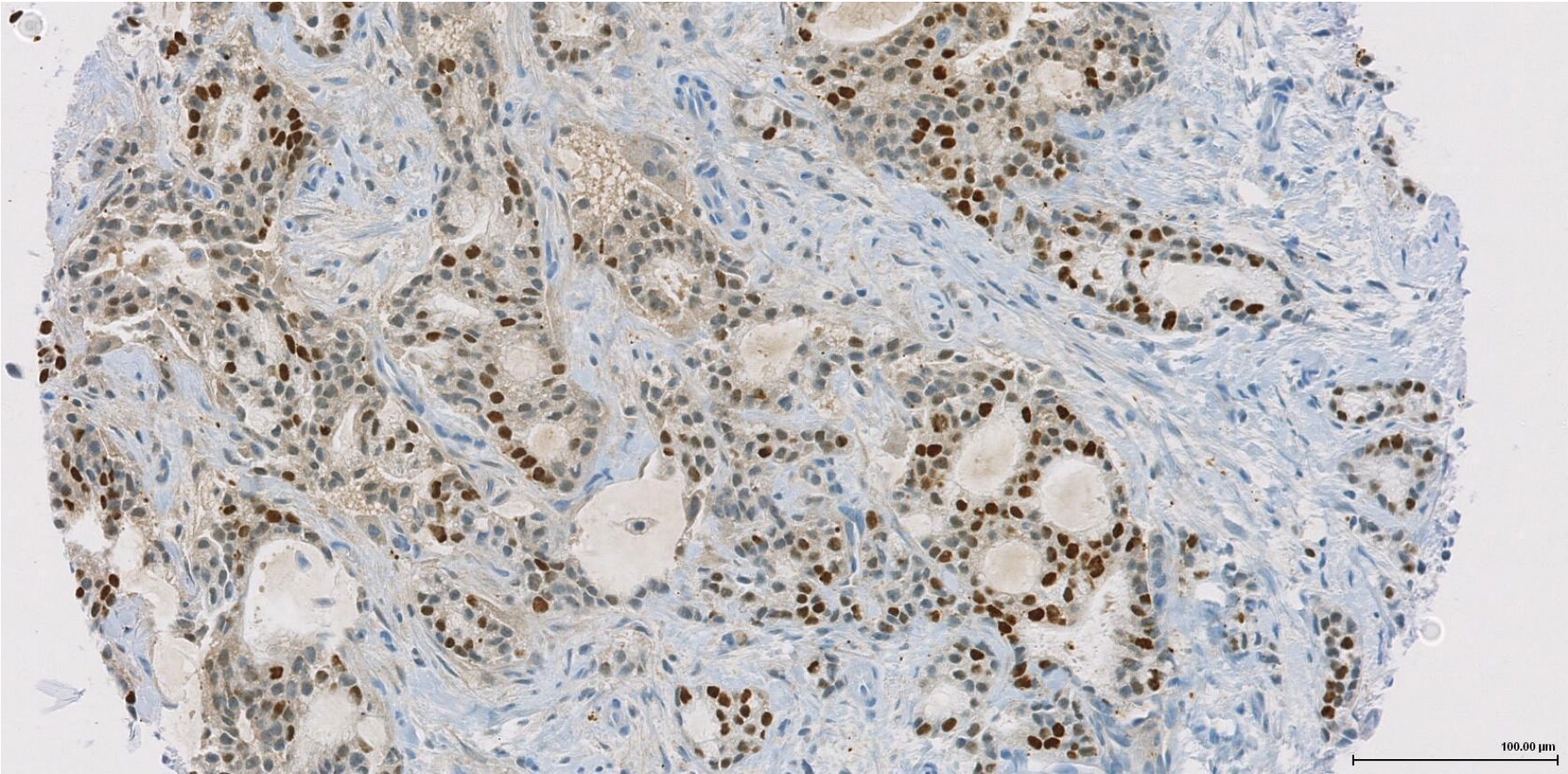
Spearman's correlation coefficient, $r = 0.917$

Relation between automated and manual ER analyses

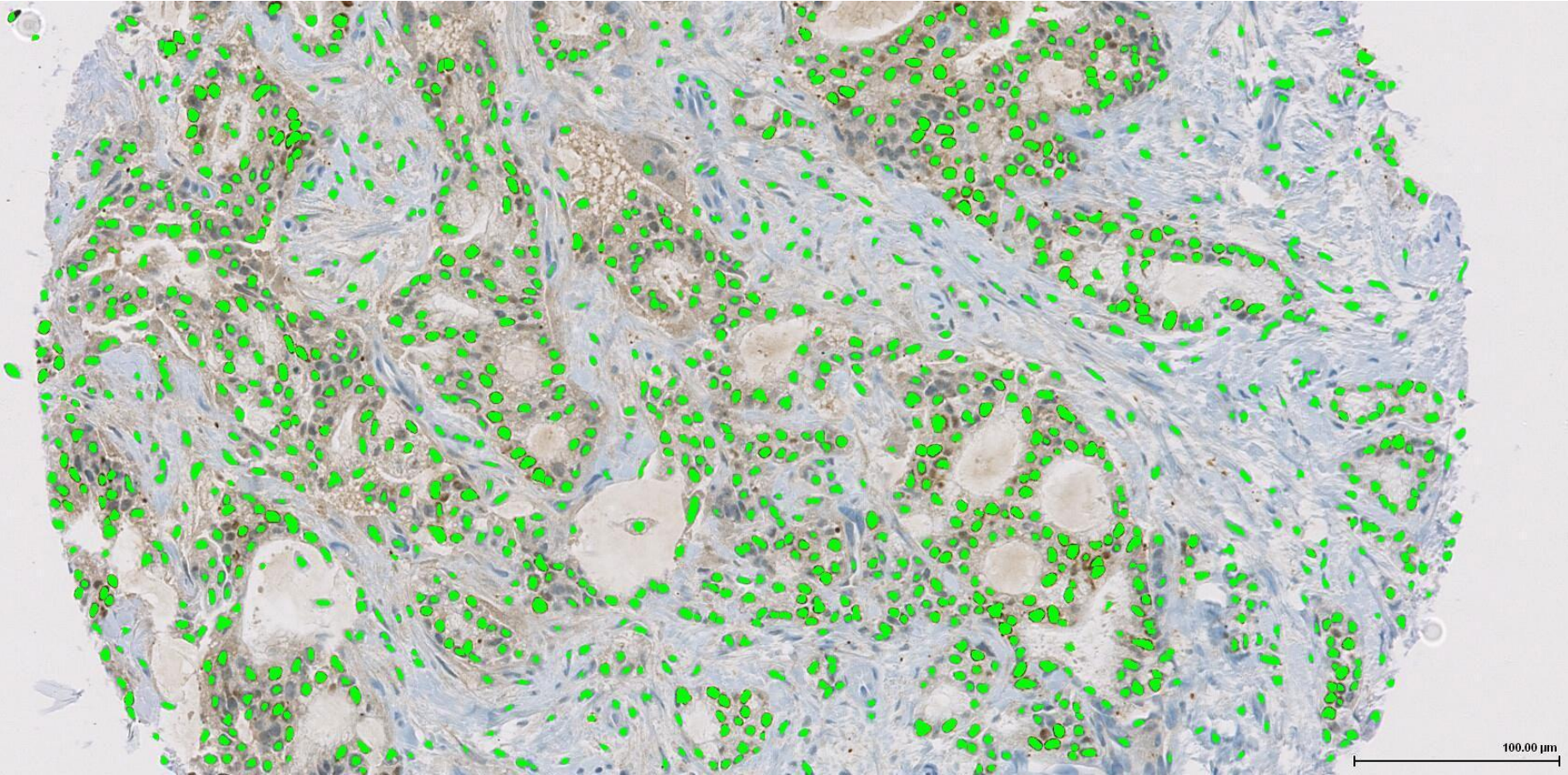


Significant difference between the different subgroups (ANOVA)

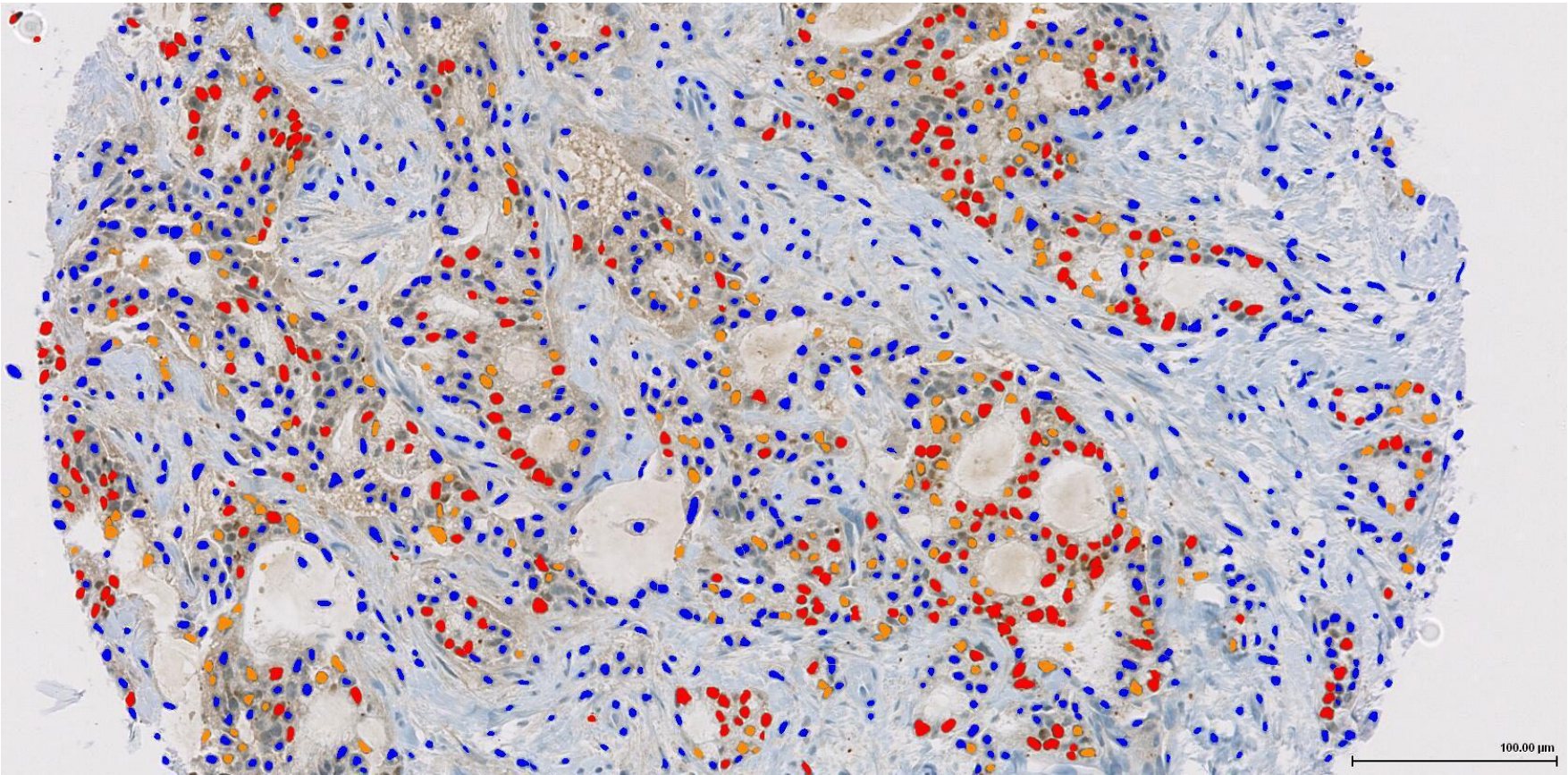
PR



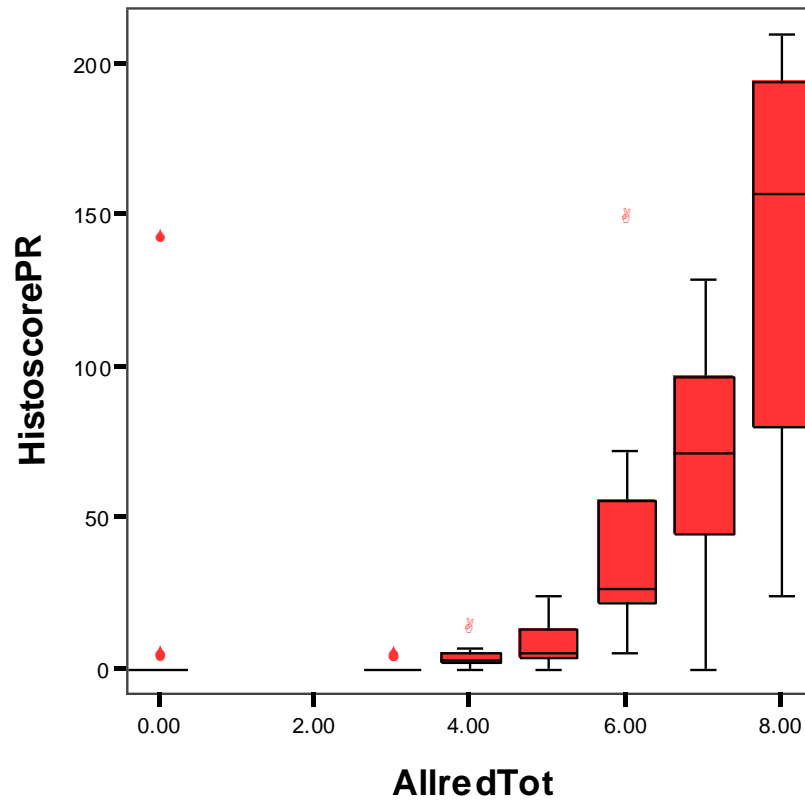
PR



PR

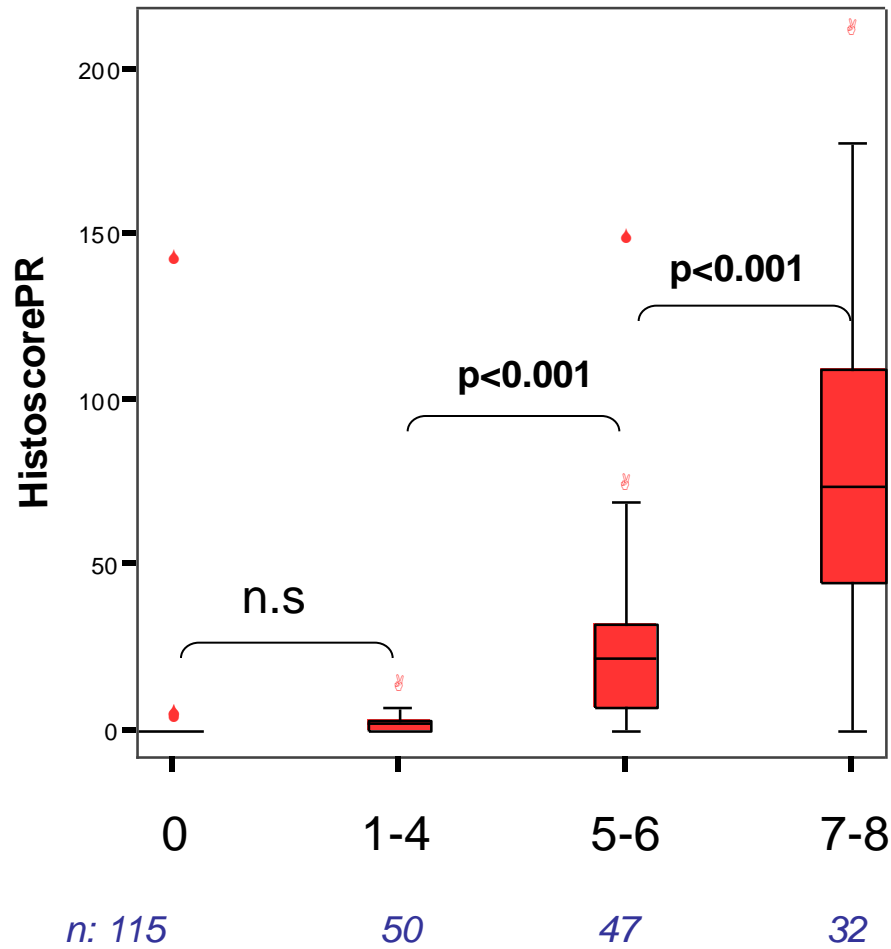


Relation between automated and manual PR analyses



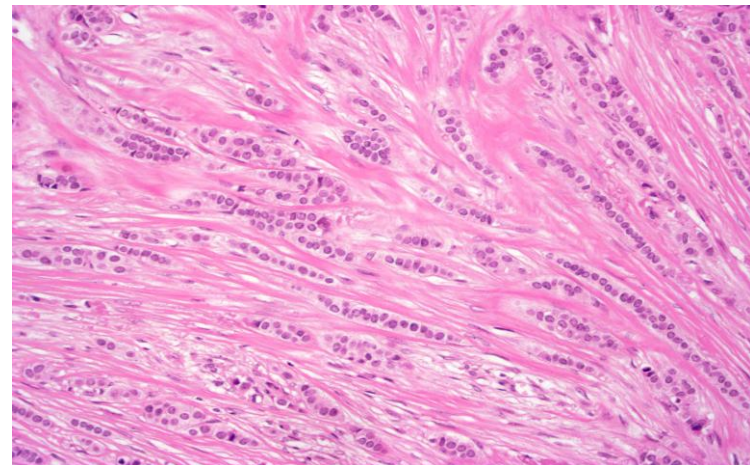
Spearman's correlation coefficient, $r = 0.869$

Relation between automated and manual PR analyses

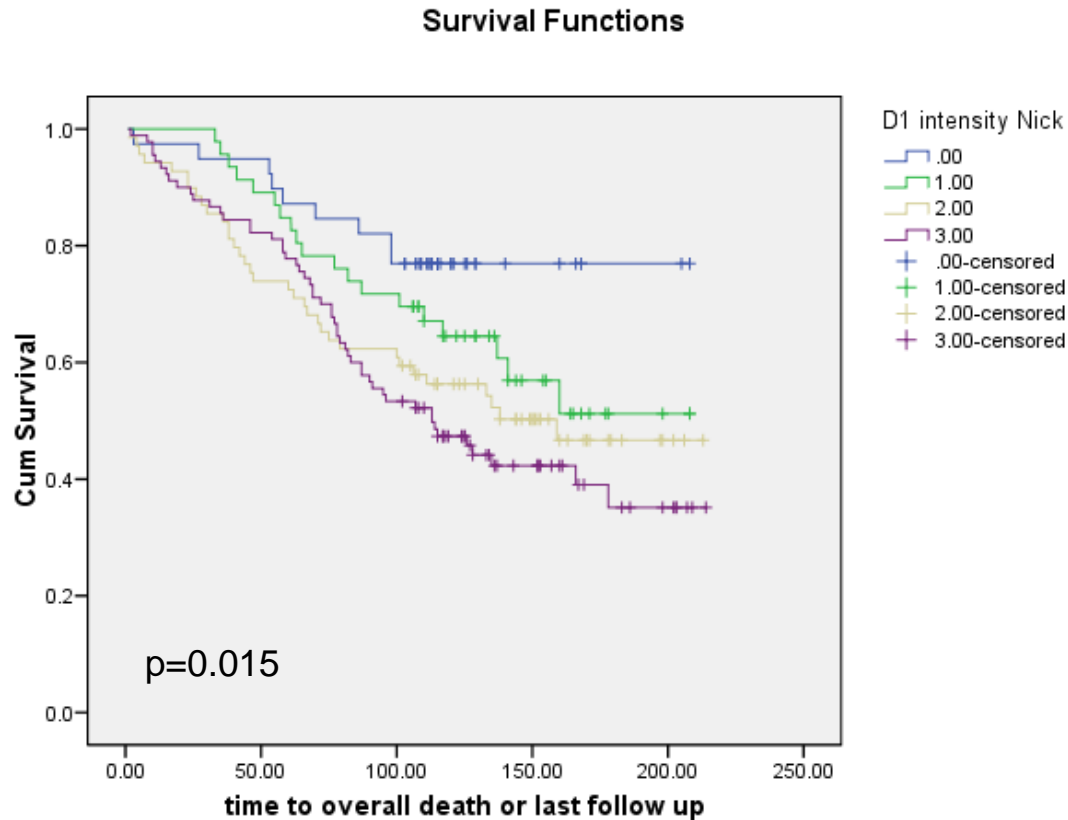


Automated analyses of cyclin D1 in lobular breast cancer

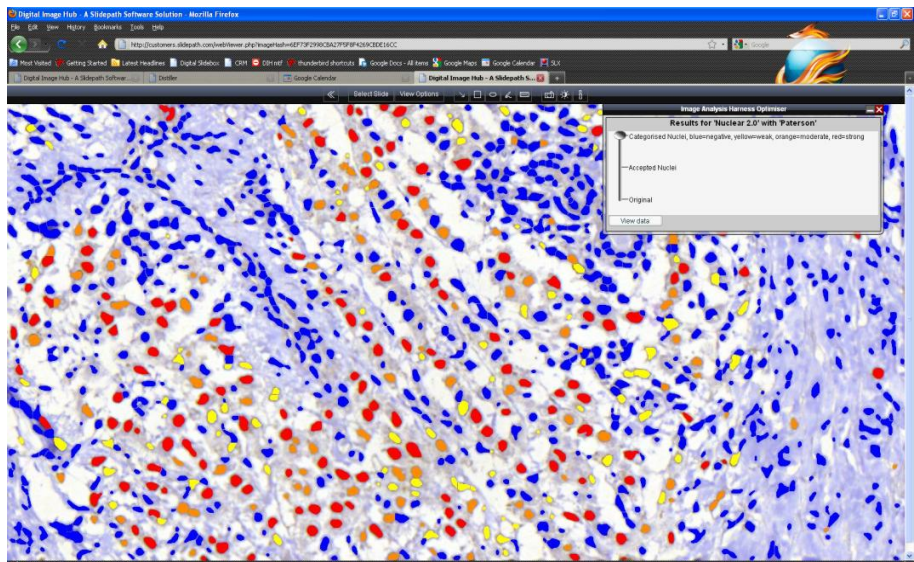
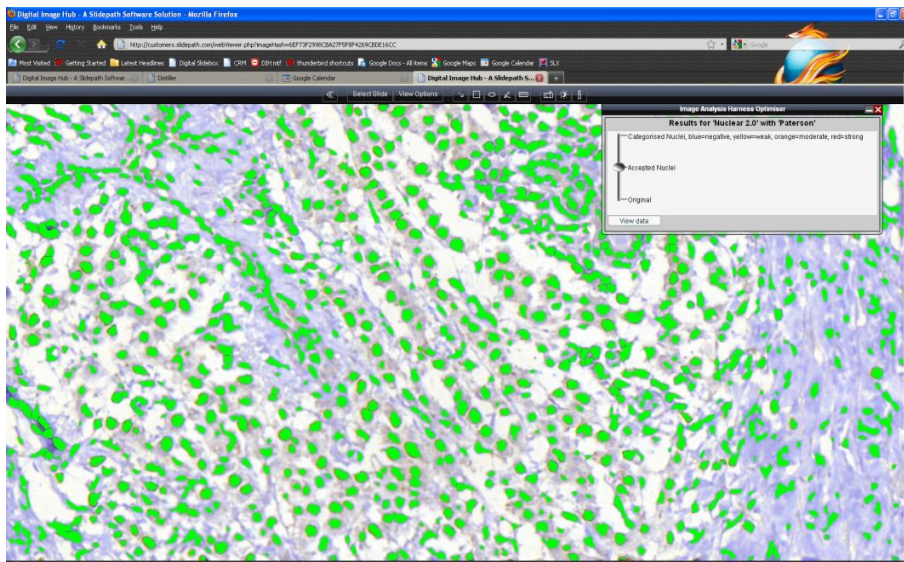
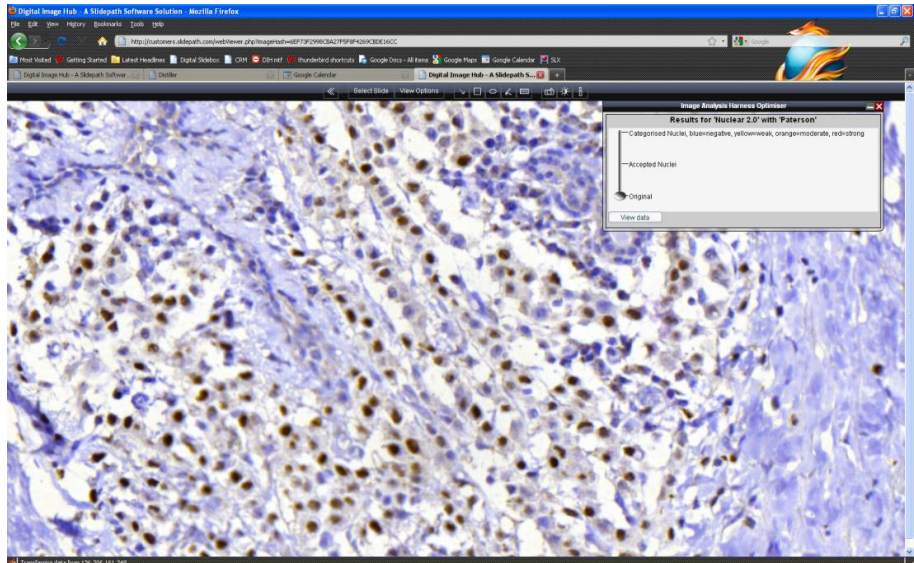
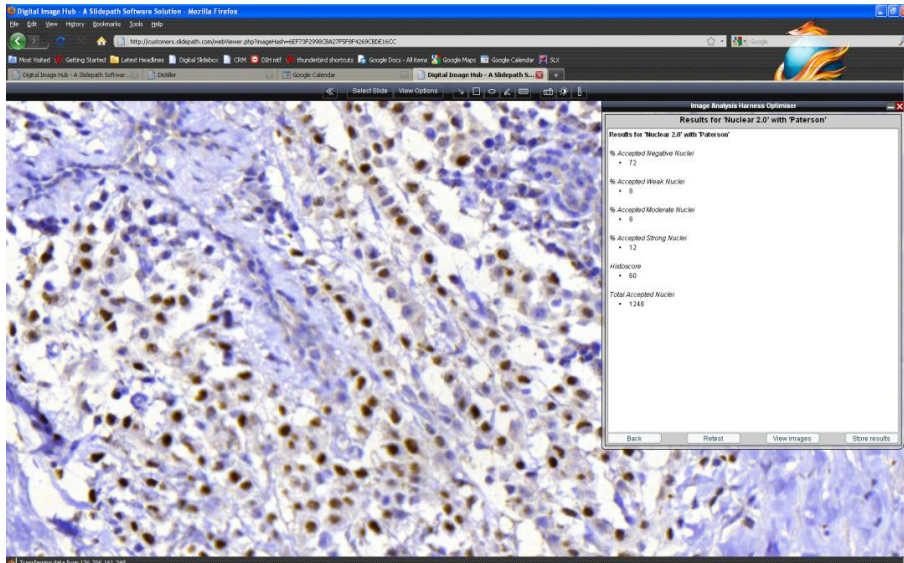
- Nuclear antigen – intensity and fraction probably relevant
- challenging subgroup of breast cancer
 - smaller tumour cells – difficult to distinguish from normal surrounding cells

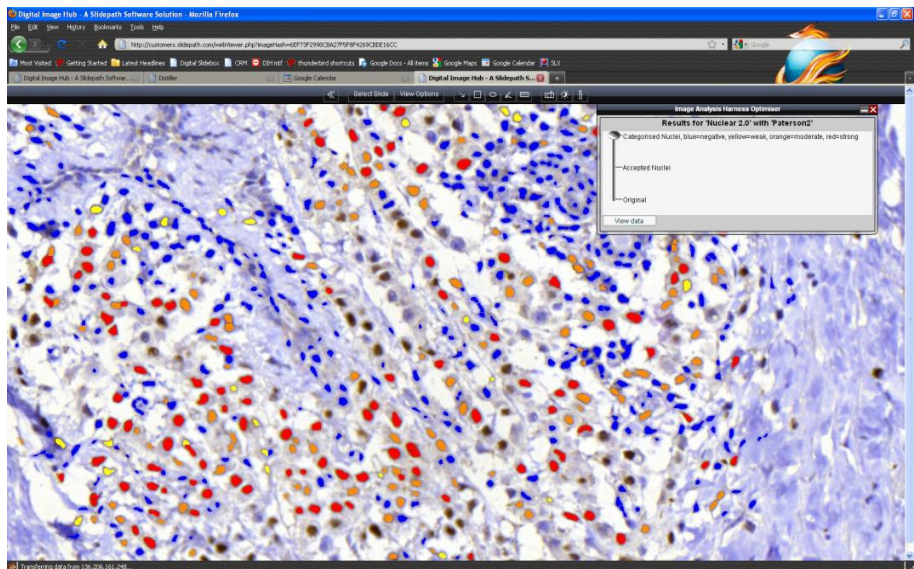
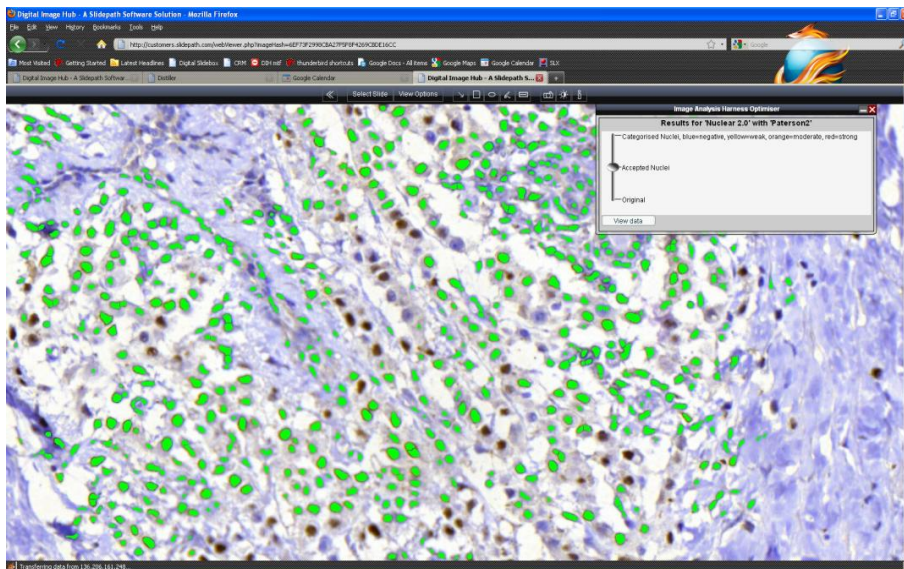
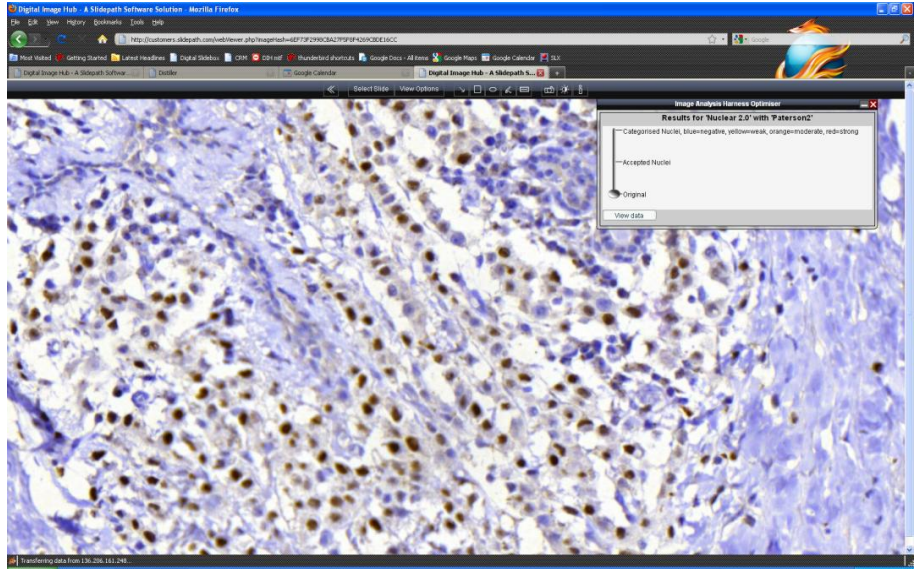
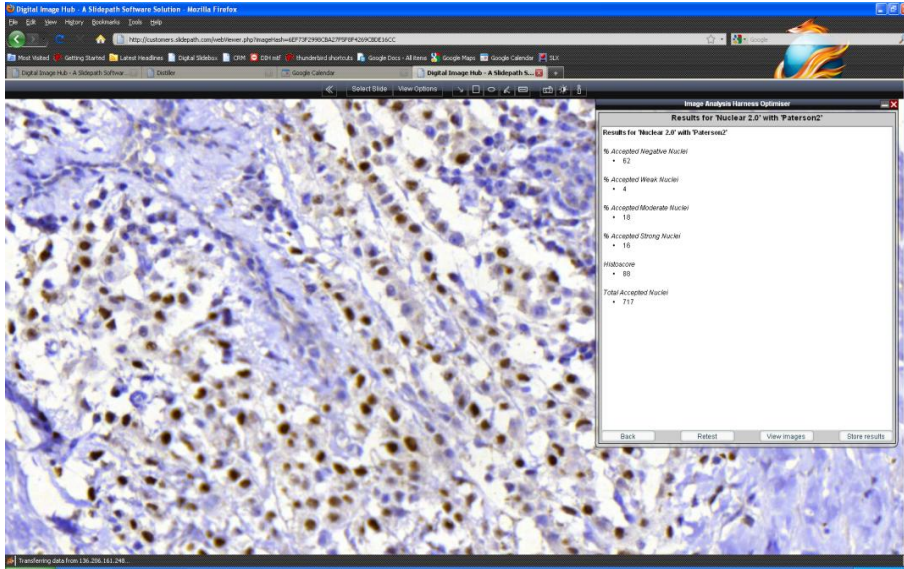


Cyclin D1 Intensity



Preliminary findings suggest that high cyclin D1 intensity is linked to bad prognosis regarding overall survival (opposite to ER-positive premenopausal breast cancer)

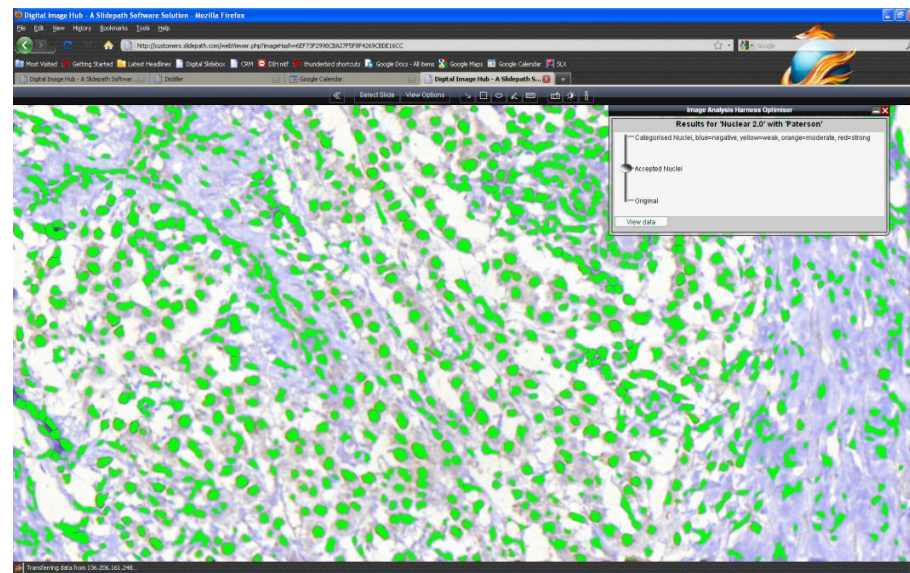
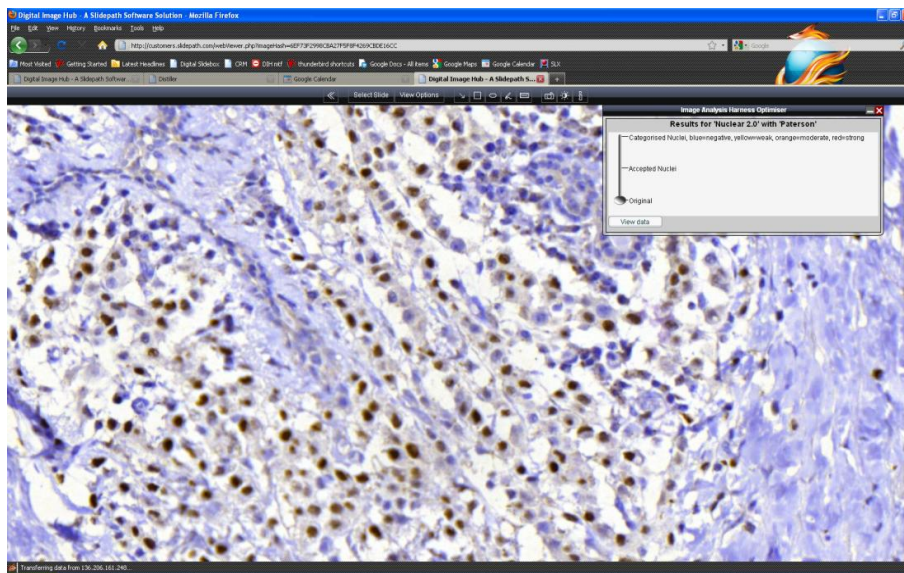




r-values between 0.42 – 0.5

How to Improve Significance Values?

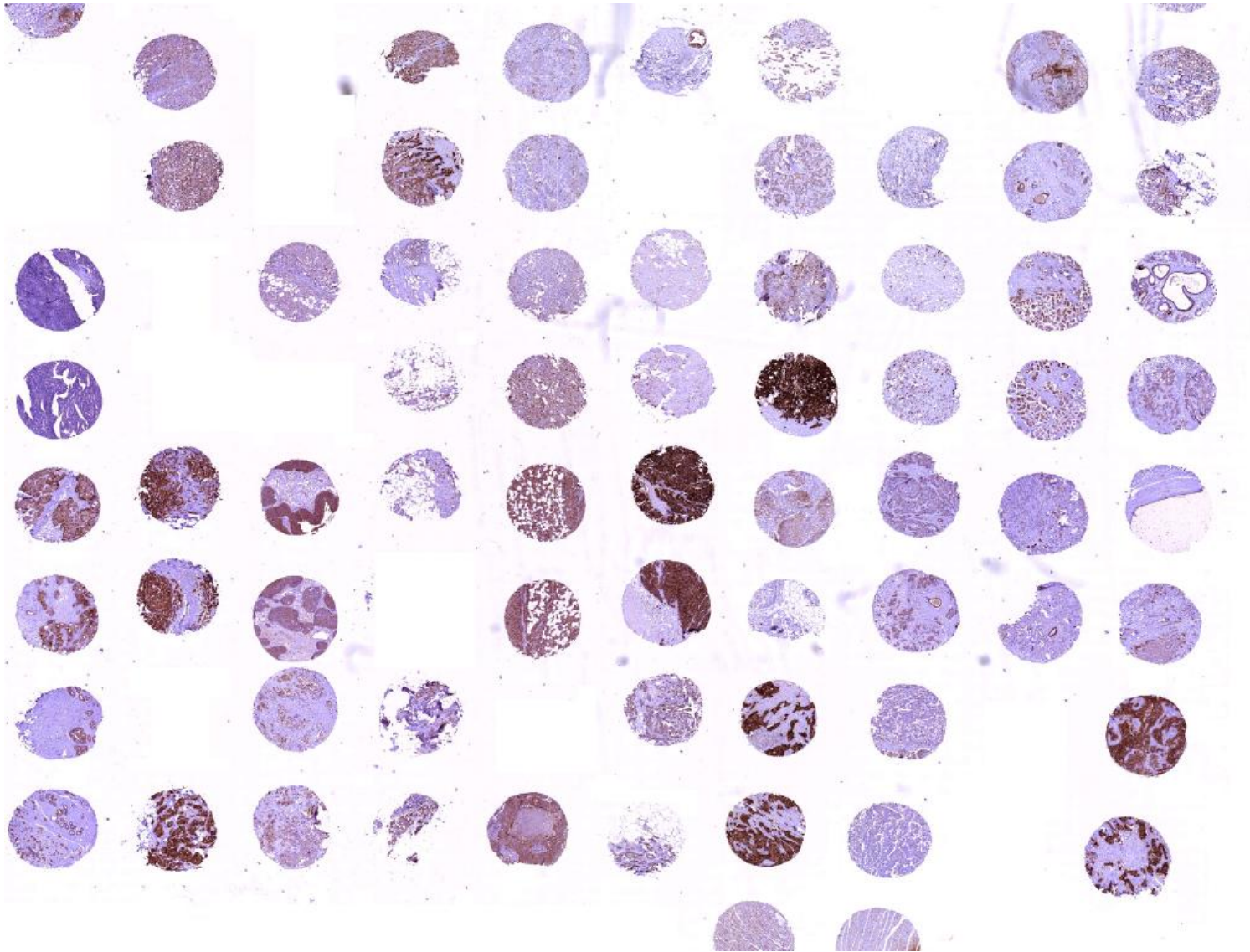
- Adjust algorithm
- Potentially a difficult subgroup



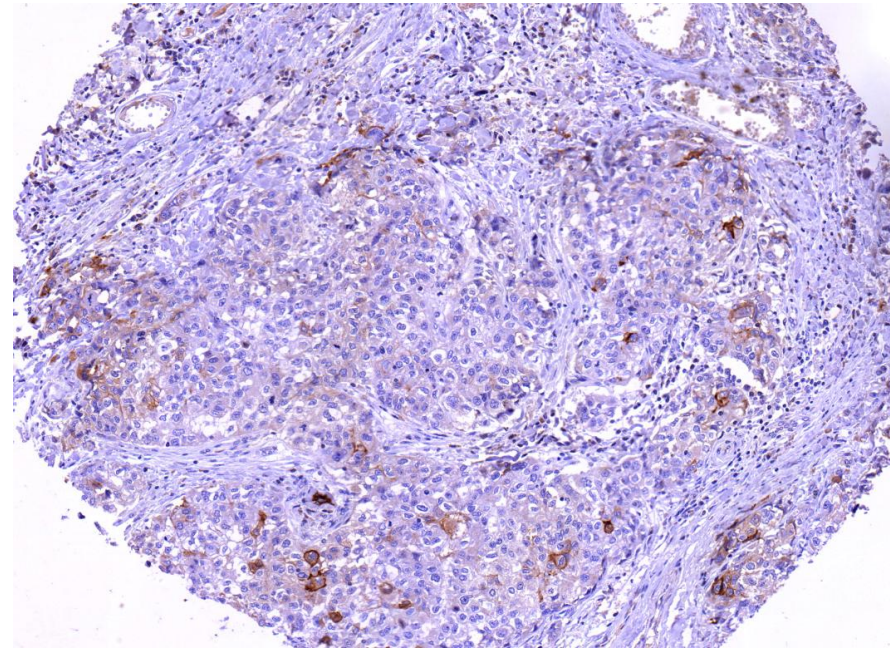
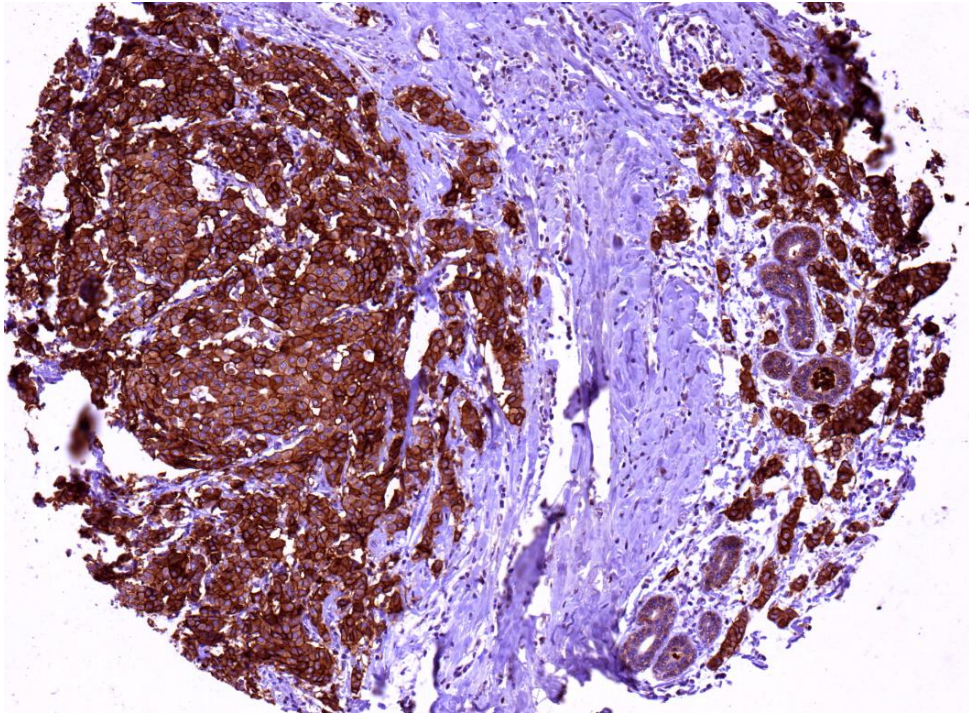
Automated analyses of $\alpha v \beta 6$ integrin (membrane antigen)

- Manual evaluation in a small training set of <100 tumours
 - Intensity
 - fraction
- Algorithm optimisation using the Distiller platform
- Analyses of concordance between automated histoscore and manual scoring

$\alpha\text{v}\beta\text{6}$



$\alpha v \beta 6$



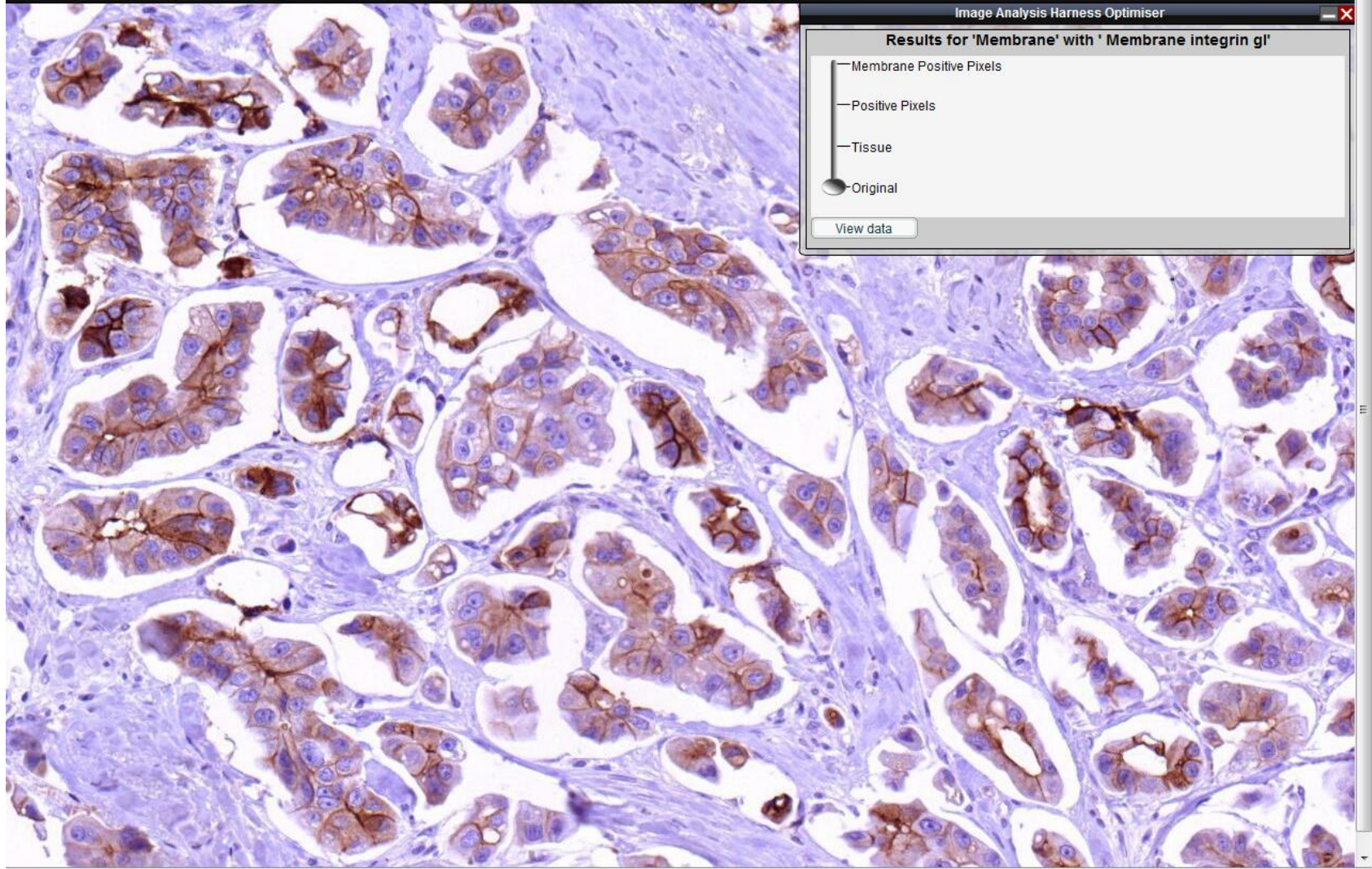


Image Analysis Harness Optimiser

Results for 'Membrane' with 'Membrane integrin g1'

- Membrane Positive Pixels
- Positive Pixels
- Tissue
- Original

View data

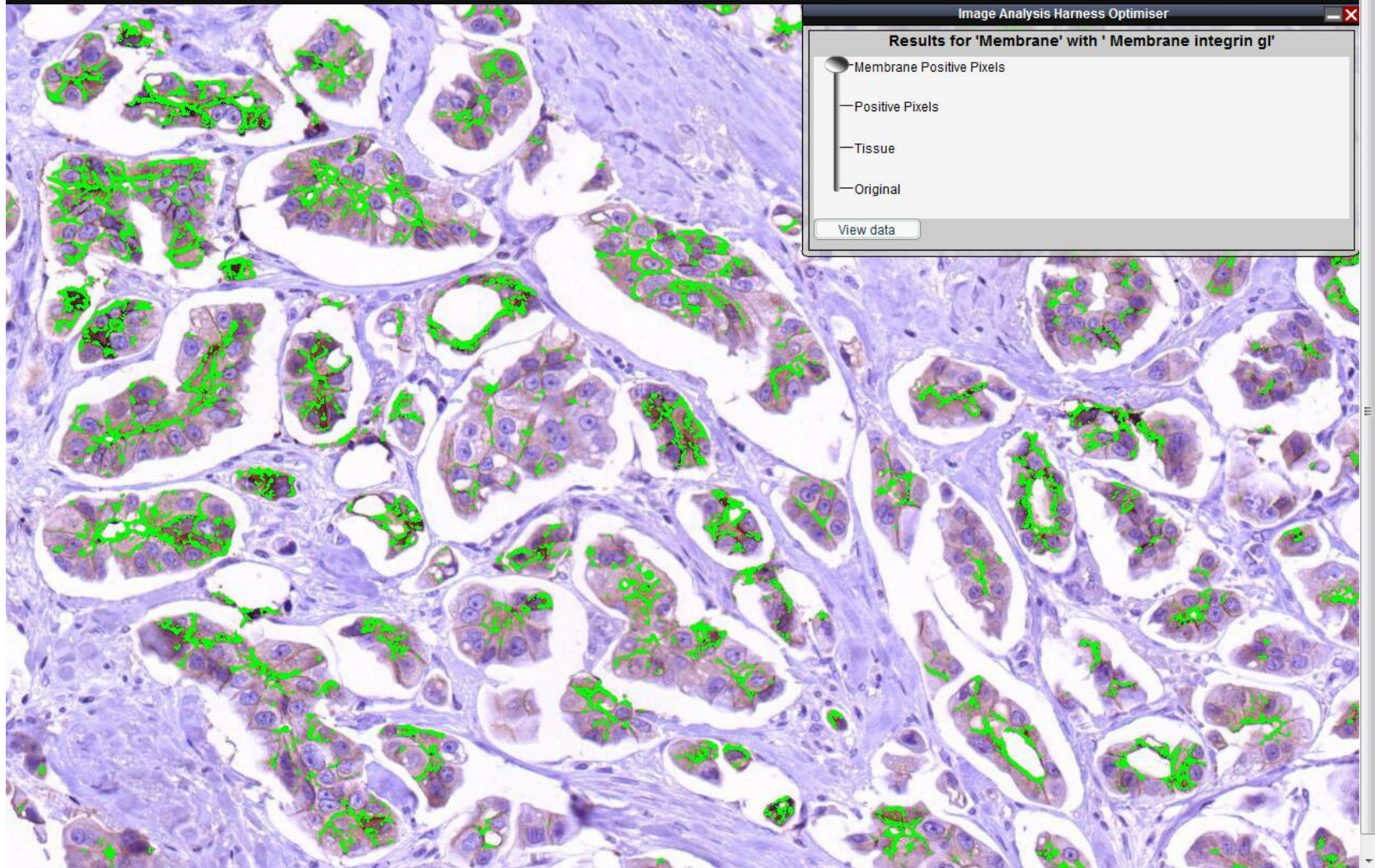


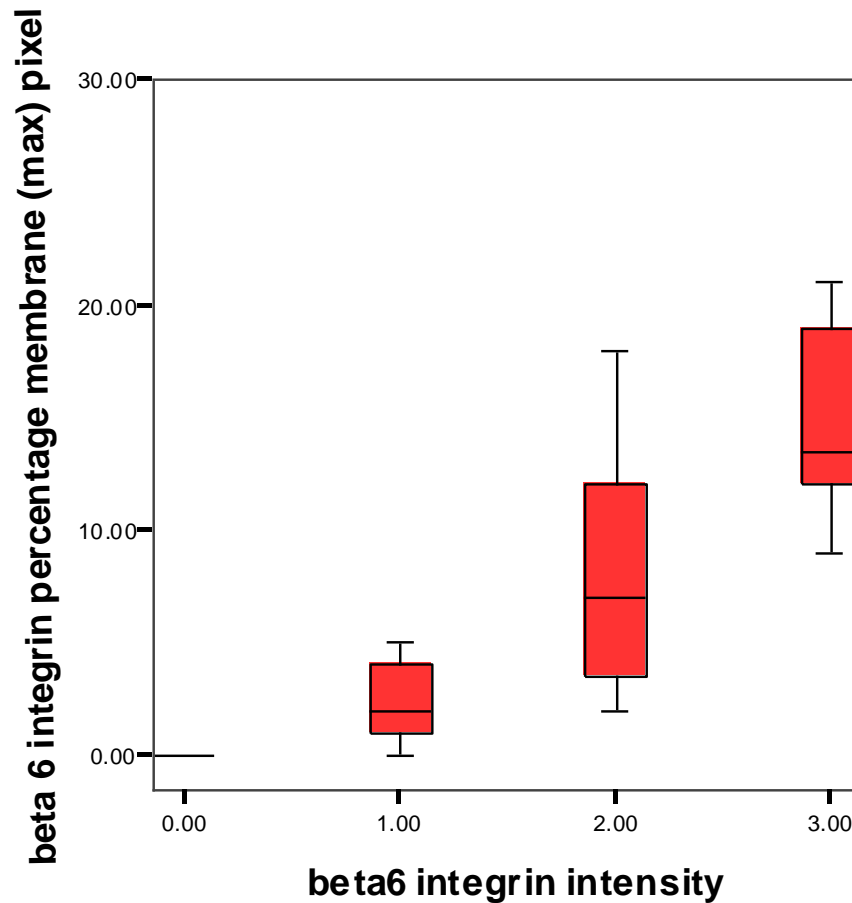
Image Analysis Harness Optimiser

Results for 'Membrane' with 'Membrane integrin gl'

- Membrane Positive Pixels
- Positive Pixels
- Tissue
- Original

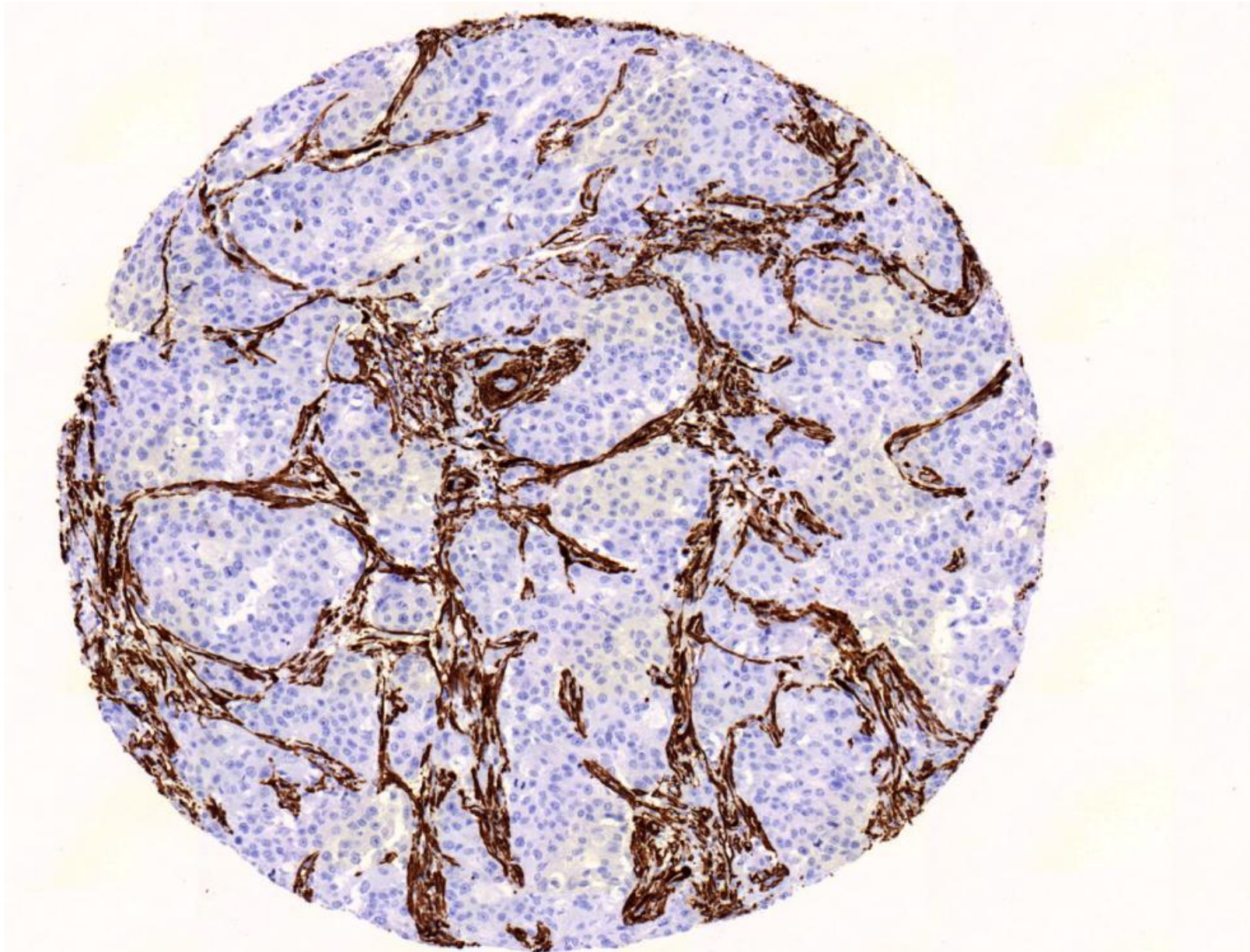
View data

$\alpha v\beta 6$ – automated and manual analyses



Spearman's correlation coefficient, $r = 0.767$

Stromal analyses - SMA



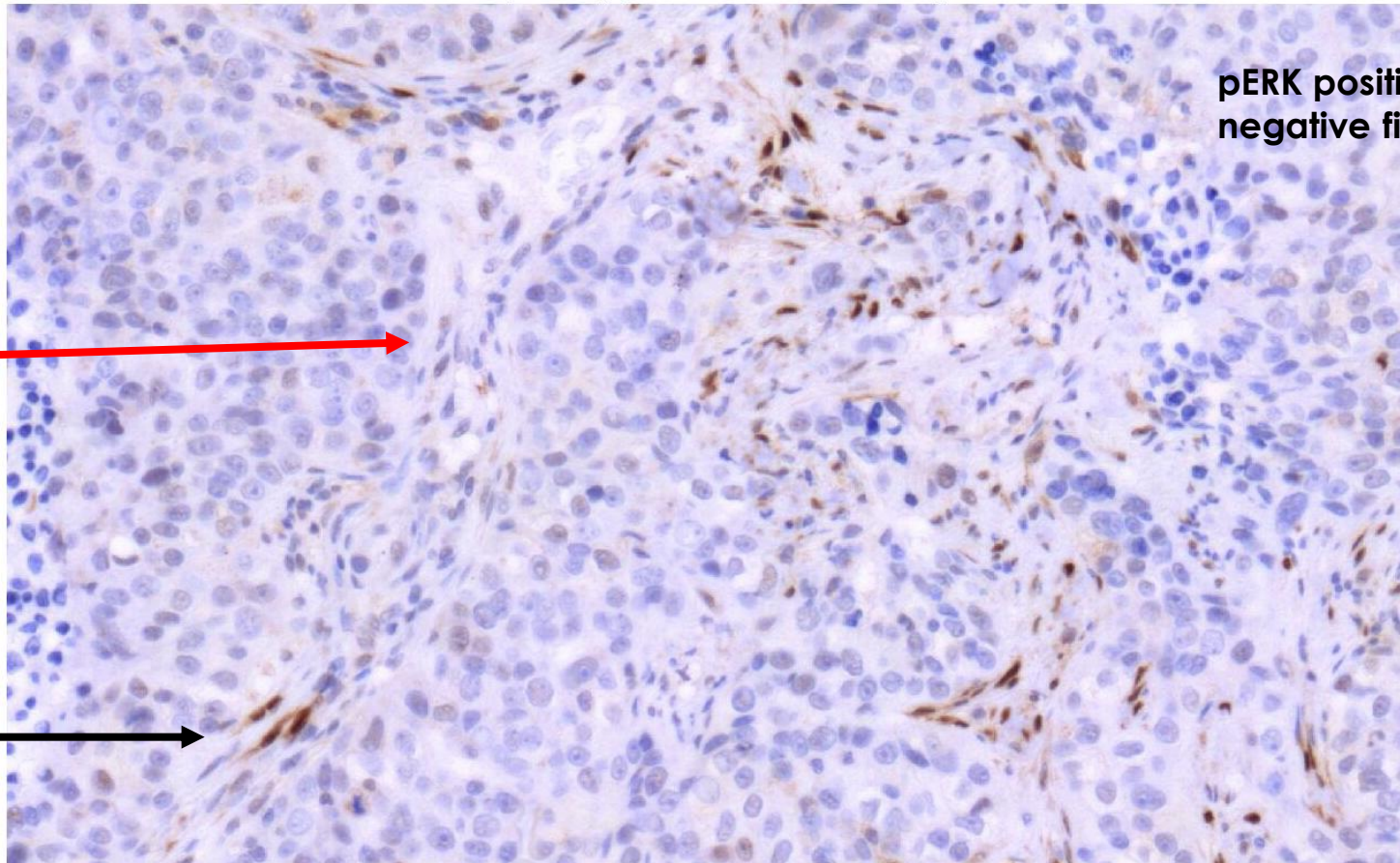
Heterogenous expression of pERK in fibroblasts within tumours



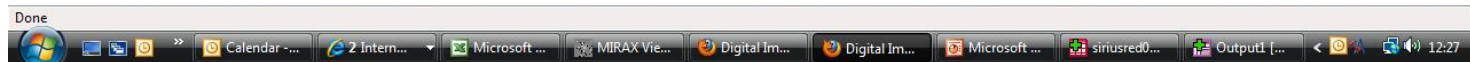
Static link for snapshot: <http://spath-dss.picr.man.ac.uk/dss/snapshot.php?snapshotID=73315160>

Dynamic link for opening snapshot in viewer: <http://spath-dss.picr.man.ac.uk/dss/webViewer.php?snapshotID=73315160>

To save: Right click image, choose 'Save Picture As...' or 'Save Image As...'



pERK positive and negative fibroblasts

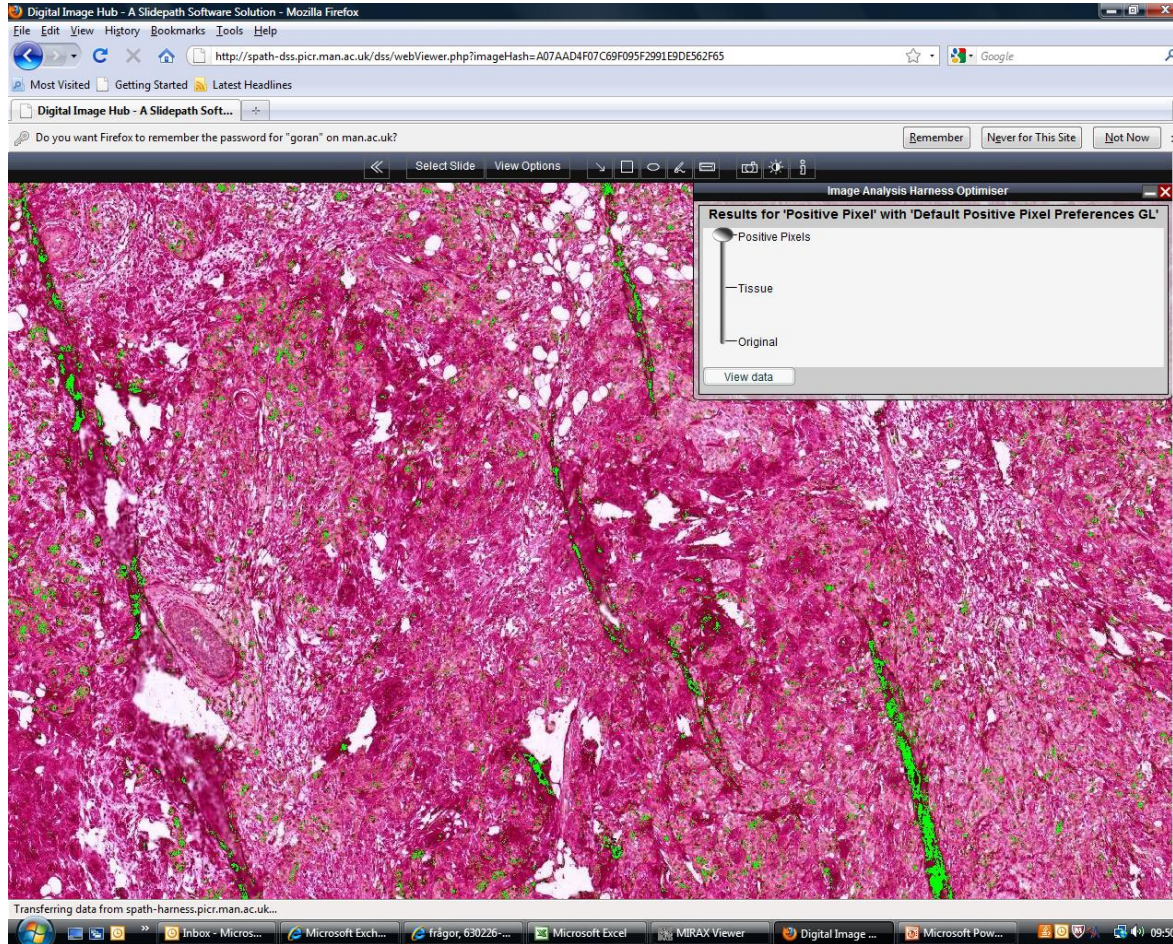


Why large variations of activated fibroblasts in the stromal compartment within the tumour?

Links to collagen-density?

Illustration of double staining procedures and automatic analyses regarding intra-tumour heterogeneity

Large variations in the presence of collagen in breast cancer samples determined by Picro-sirius red staining

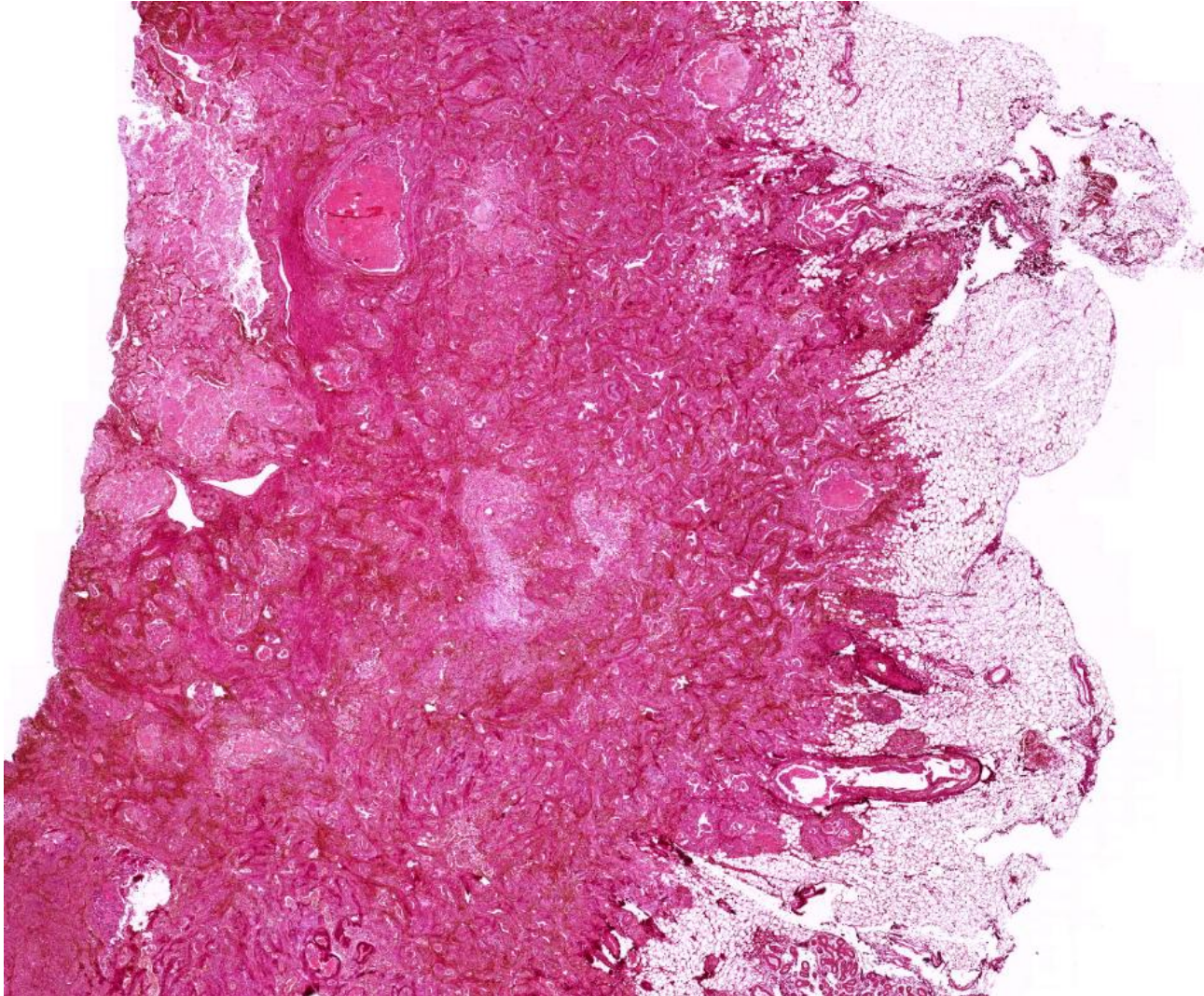


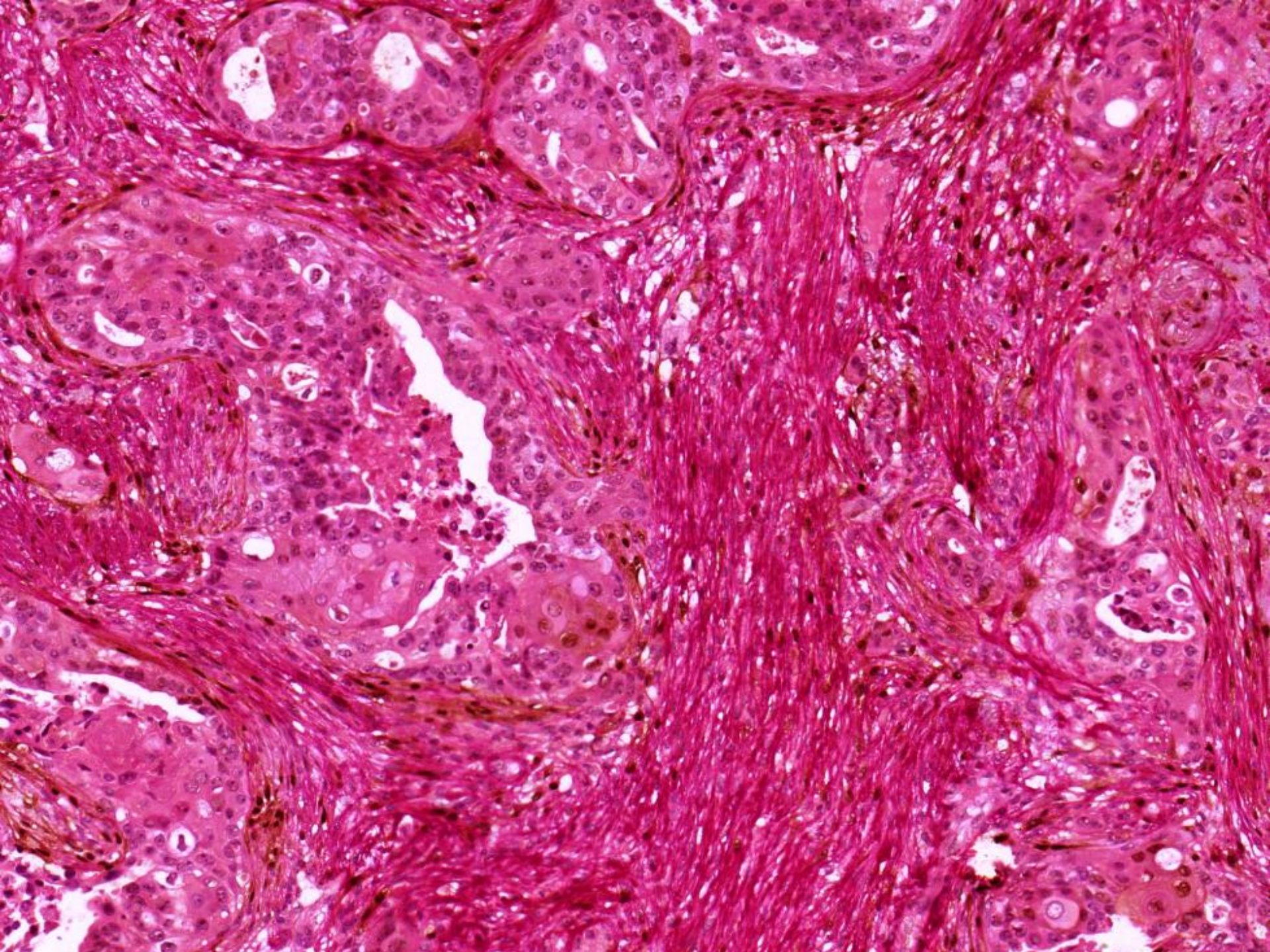
Fact

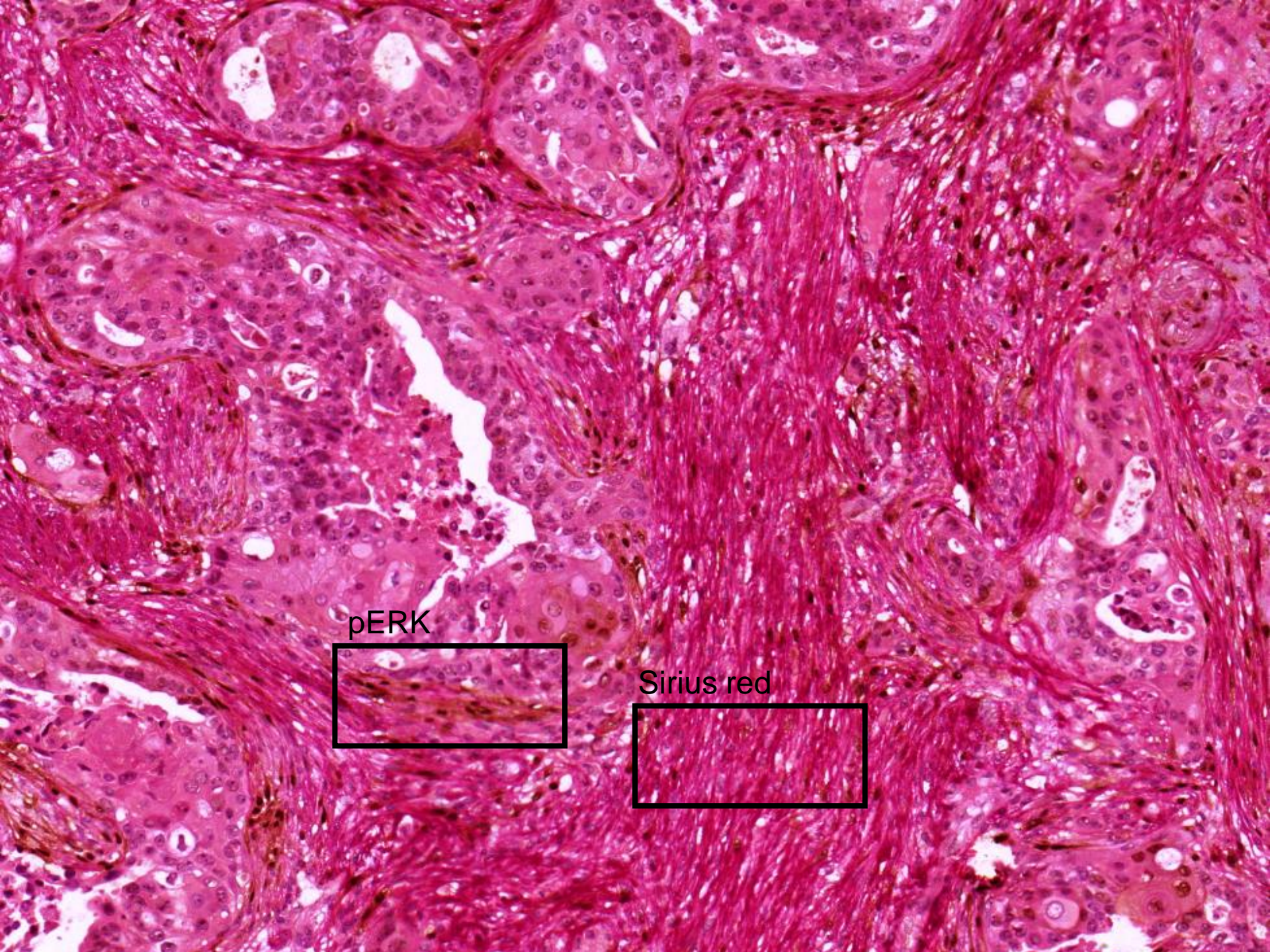
Sirius red in saturated picric acid binds selectively to fibrillar collagens (types I to V), specifically to the {Gly-X-Y}_n helical structure.

Double staining of sirius red (collagen) and pERK–brown

Invasive breast cancer with identified variations in stromal pERK







pERK



Sirius red



**An objective measurement of
presence of cells within different
areas**

Nuclear detections using Distiller

- **Based on colour definitions**
- **Nuclear algorithms**

Select Slide View Options

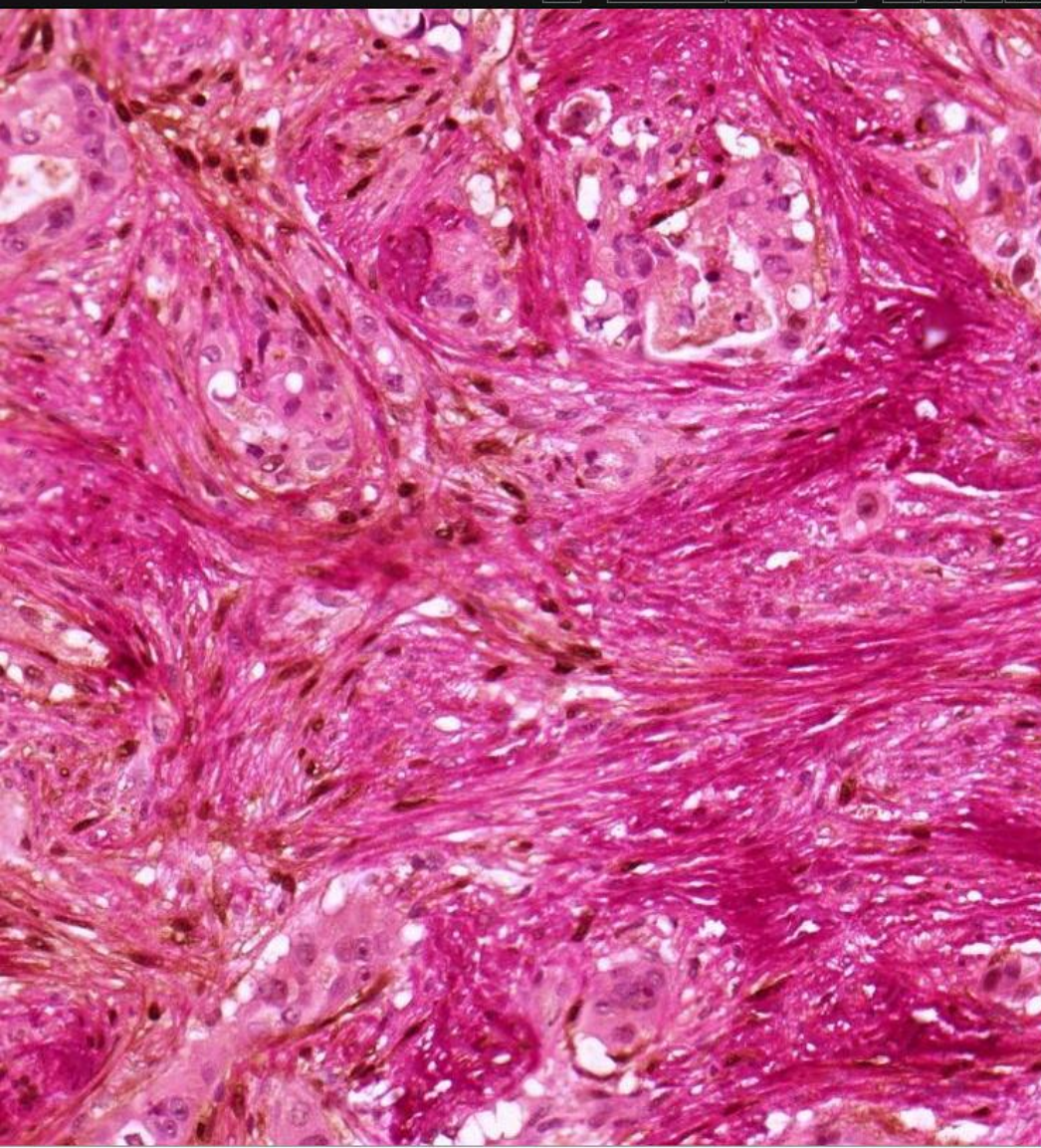


Image Analysis Harness Optimiser

Results for 'Nuclear 2.0' with 'Default Nuclear Density sofie'

— Categorized Nuclei, blue=negative, yellow=weak, orange=moderate, red=strong

— Accepted Nuclei

— Original

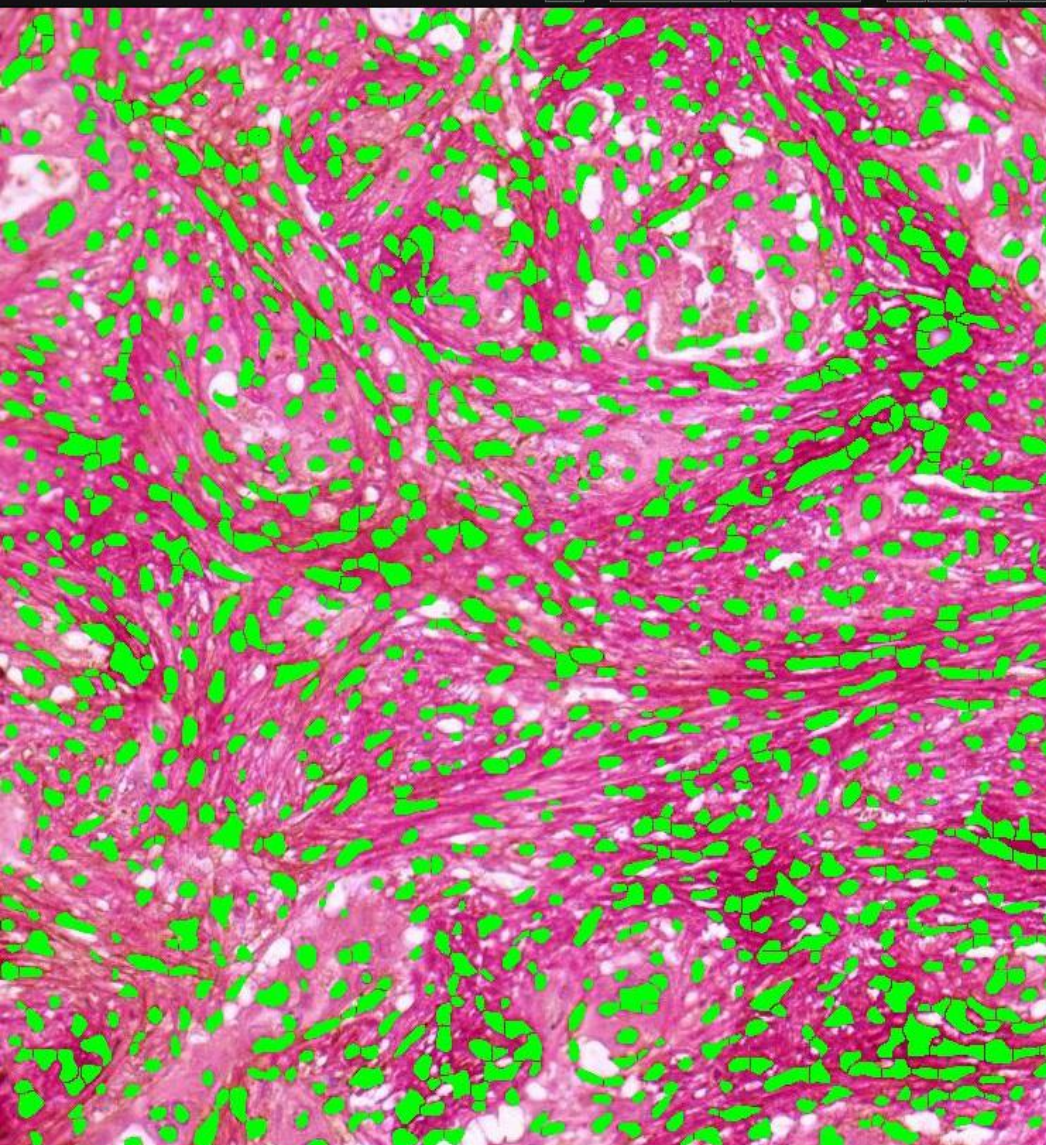


Image Analysis Harness Optimiser

Results for 'Nuclear 2.0' with 'Default Nuclear Density sofie'

Categorised Nuclei, blue=negative, yellow=weak, orange=moderate, red=strong

Accepted Nuclei

Original

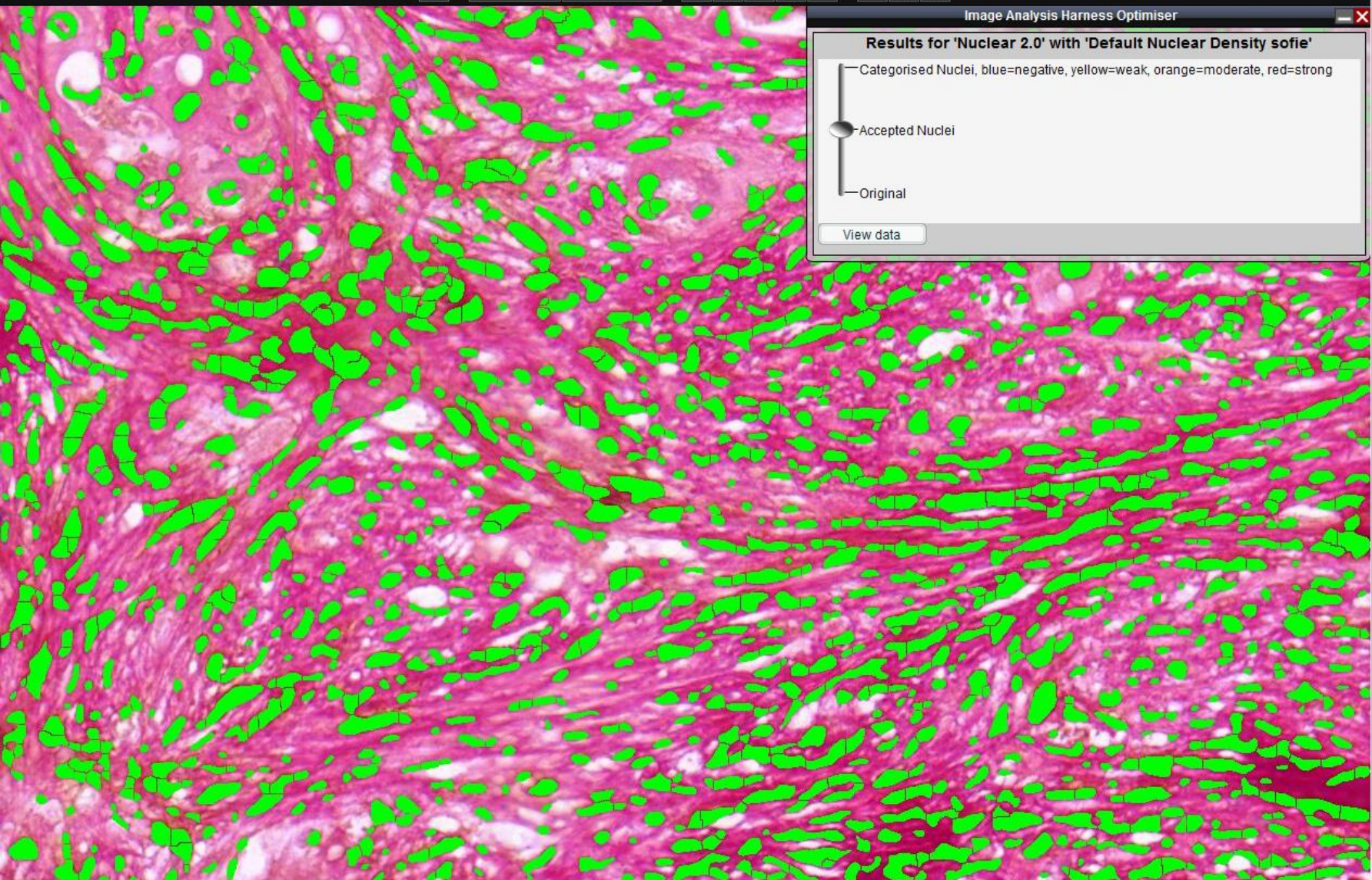
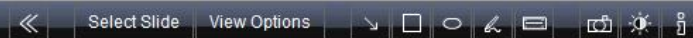


Image Analysis Harness Optimiser

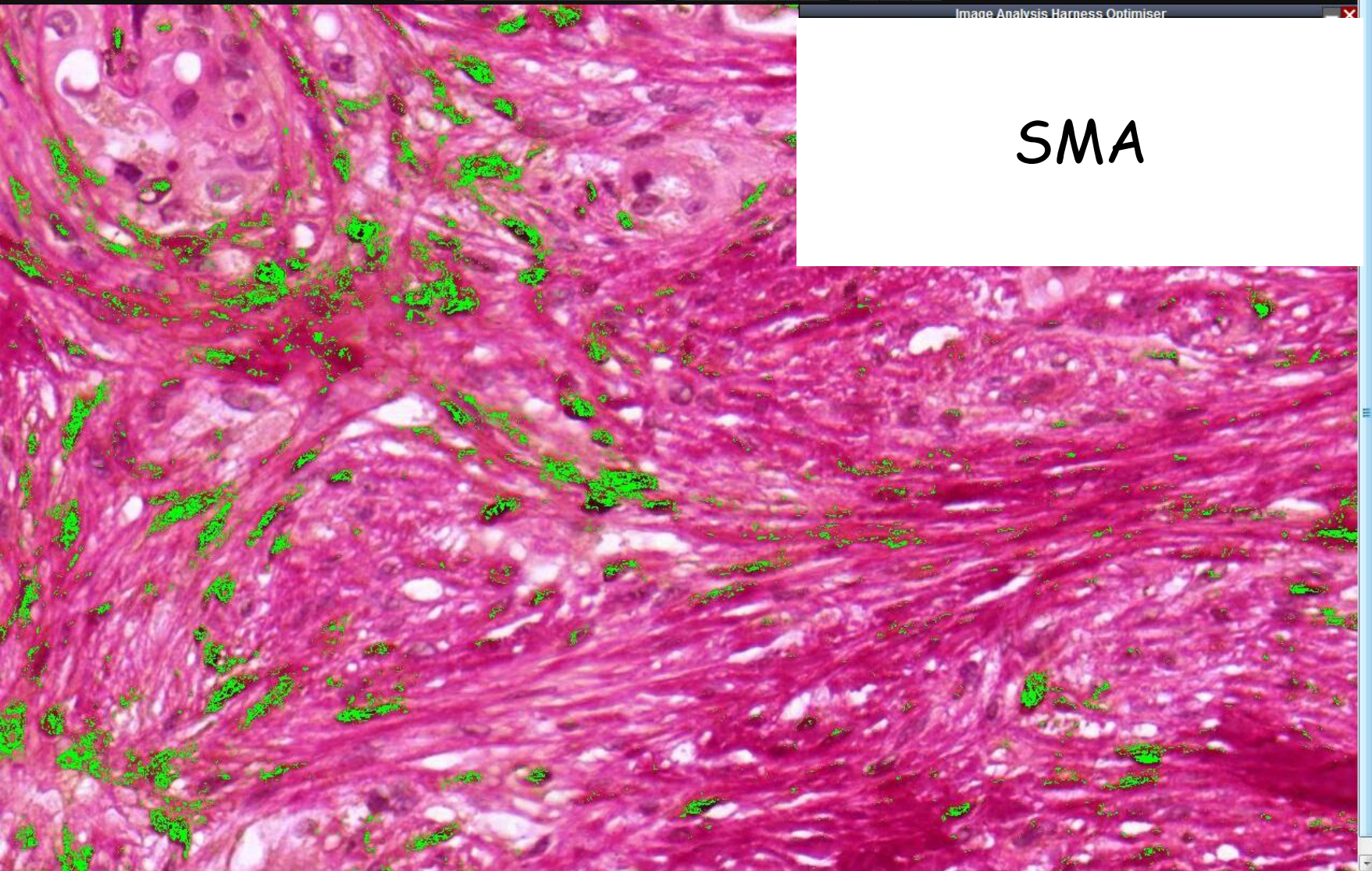
Results for 'Nuclear 2.0' with 'Default Nuclear Density sofie'

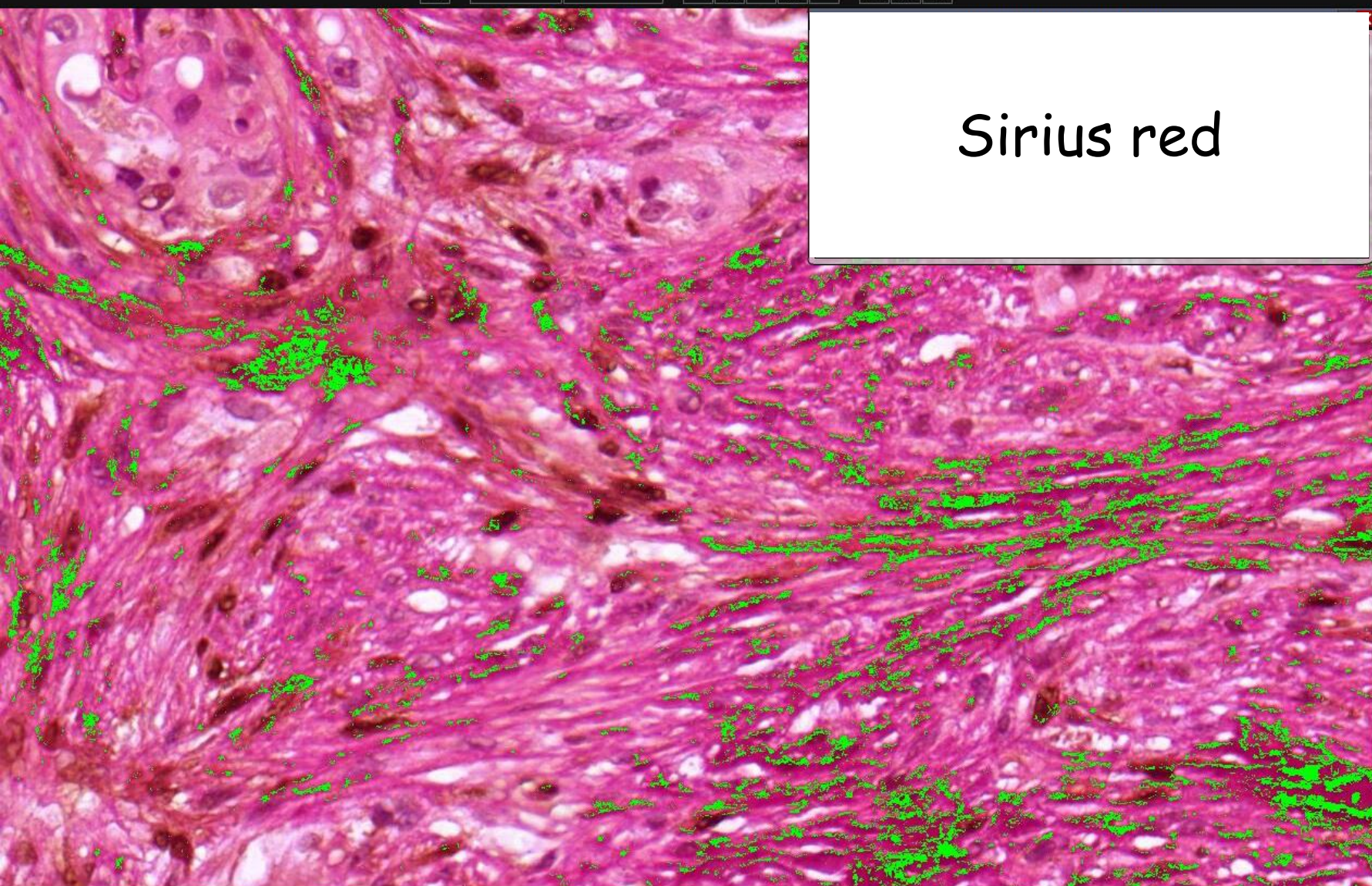
— Categorized Nuclei, blue=negative, yellow=weak, orange=moderate, red=strong

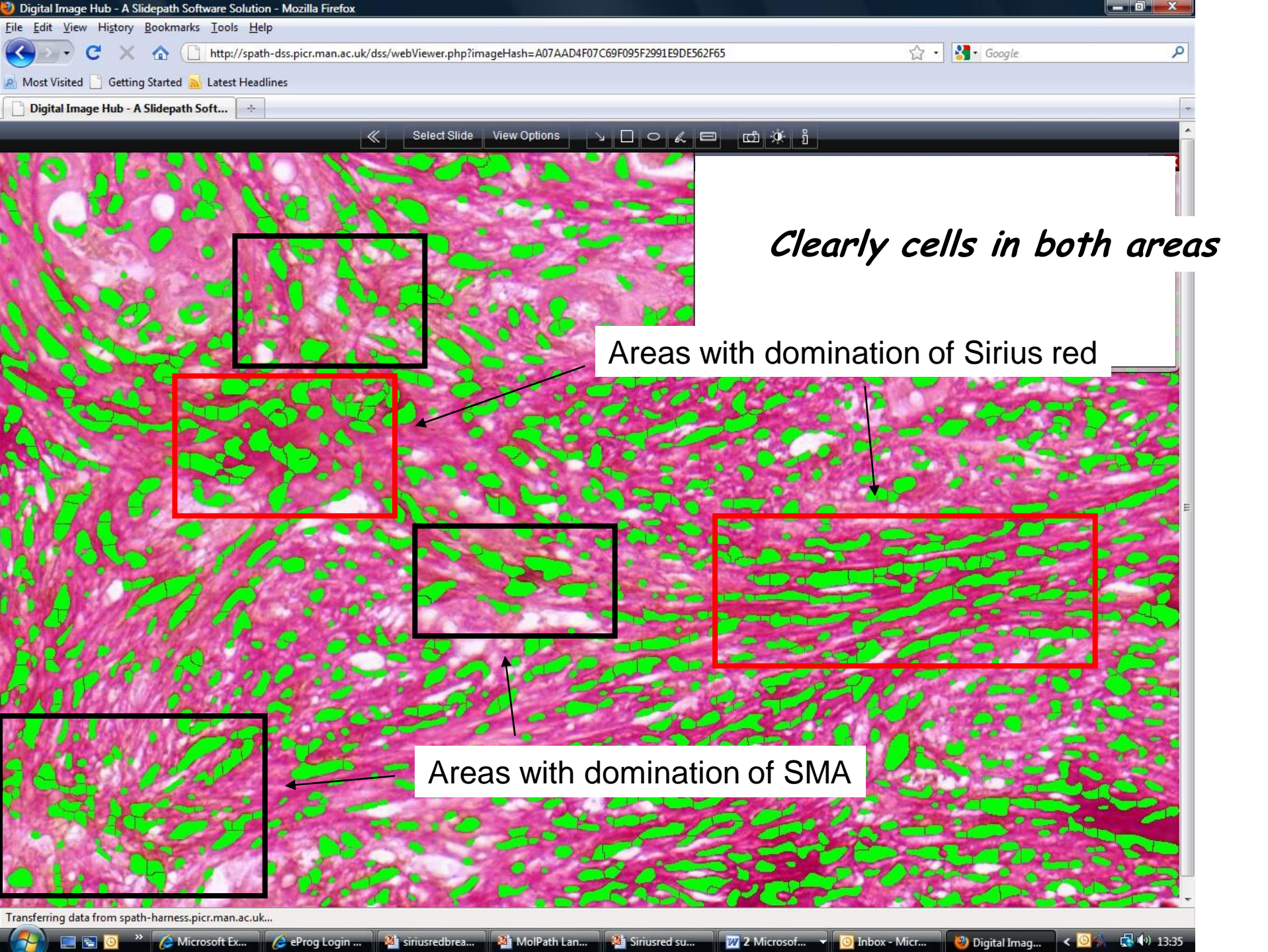
— Accepted Nuclei

— Original

SMA







Clearly cells in both areas

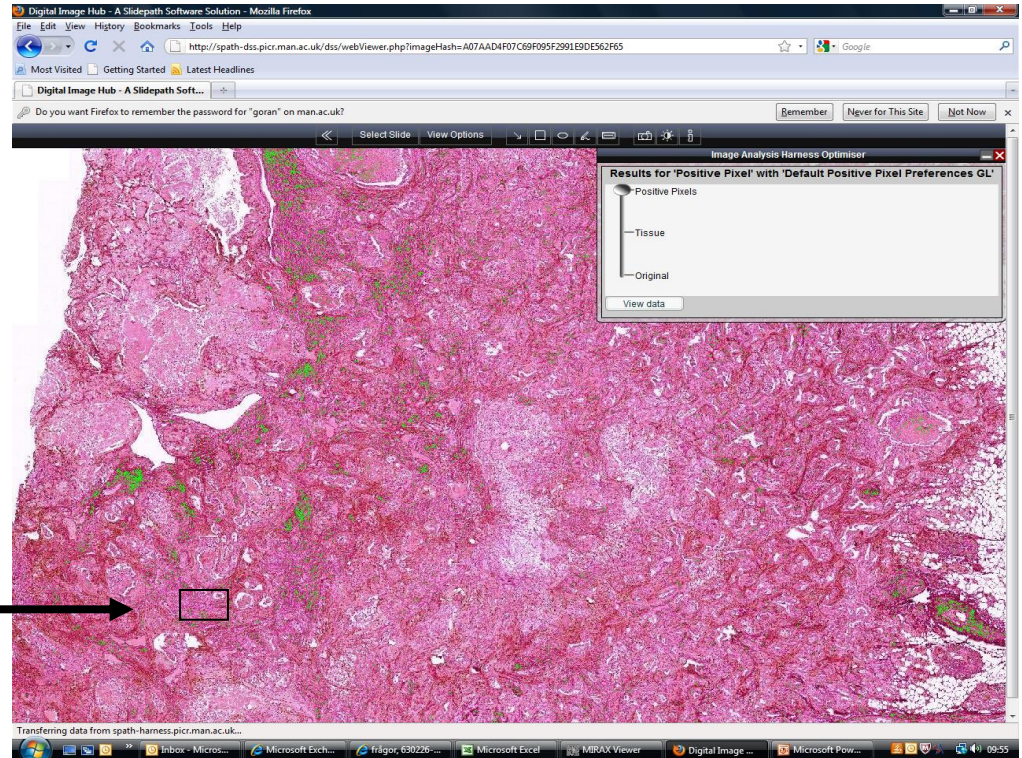
Areas with domination of Sirius red

Areas with domination of SMA

Automated detection of brown and red using image analyses

Quantification of the amount of red and brown in the area

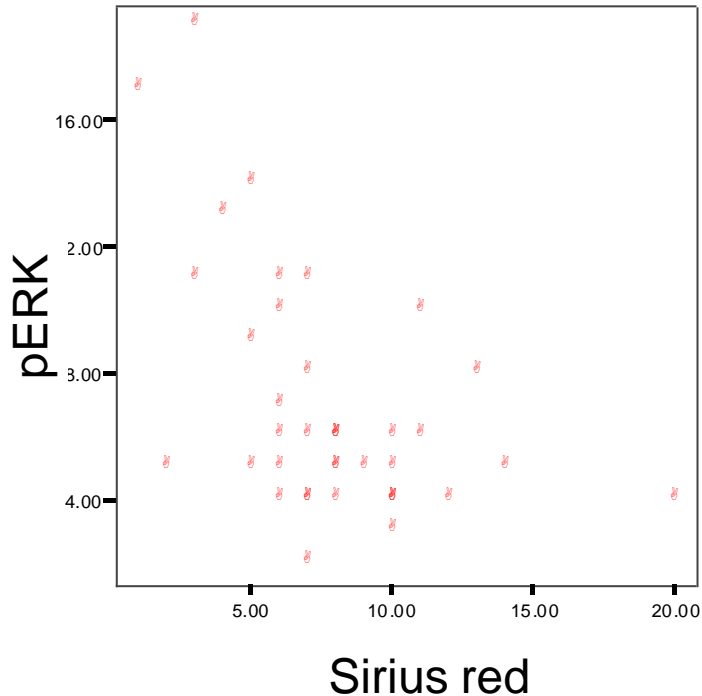
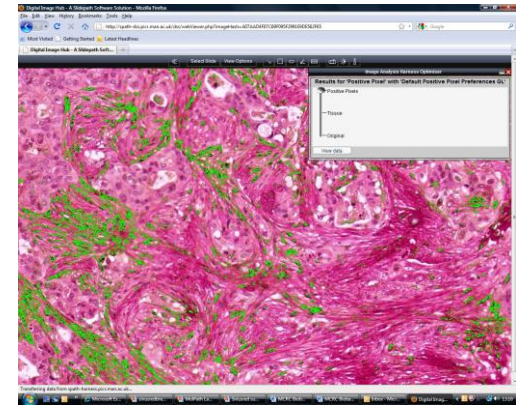
Multiple and random areas in the tumour analysed



Produce pixel intensity of the colour and percentage positivity in relation to tissue

Automated detection of pERK and sirius red

Each dot represents a characterised area within the tumour



Significant inverse relation between the presence of pERK in fibroblasts and Sirius red

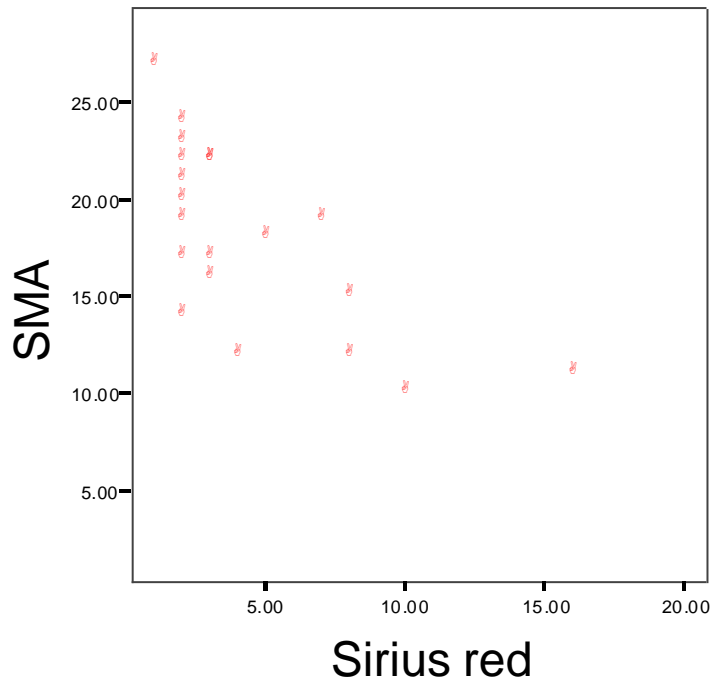
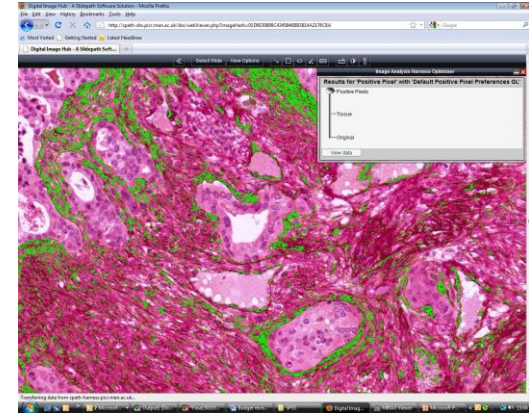
$$R = -0.508$$

$$P = 0.001$$

Strong link between collagen and pERK

Automated detection of SMA and sirius red

Each dot represents a characterised area within the tumour



Significant inverse relation between the presence of pERK in fibroblasts and Sirius red

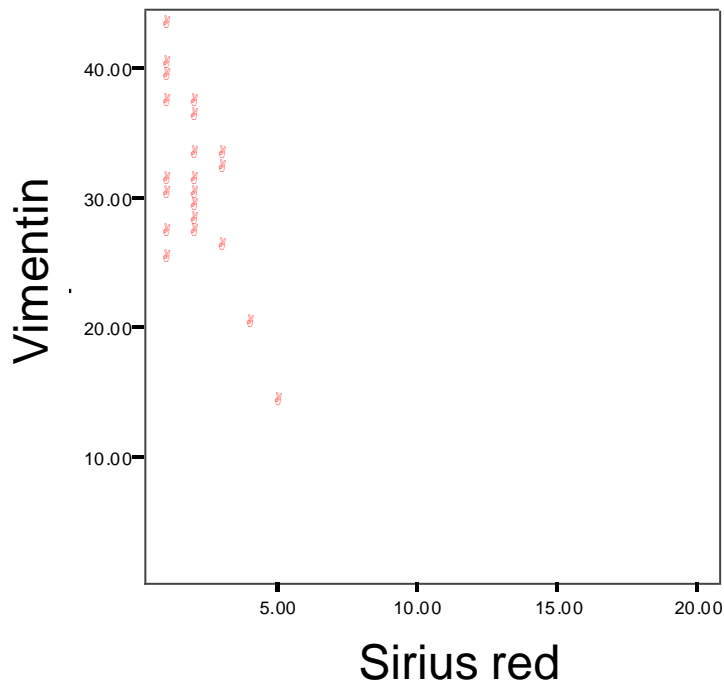
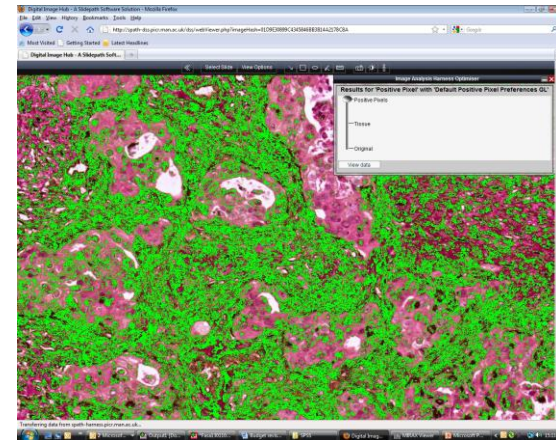
$$R=-0.709$$

$$P<0.001$$

Strong inverse link between collagen and SMA

Automated detection of Vimentin and sirius red

Each dot represents a characterised area within the tumour



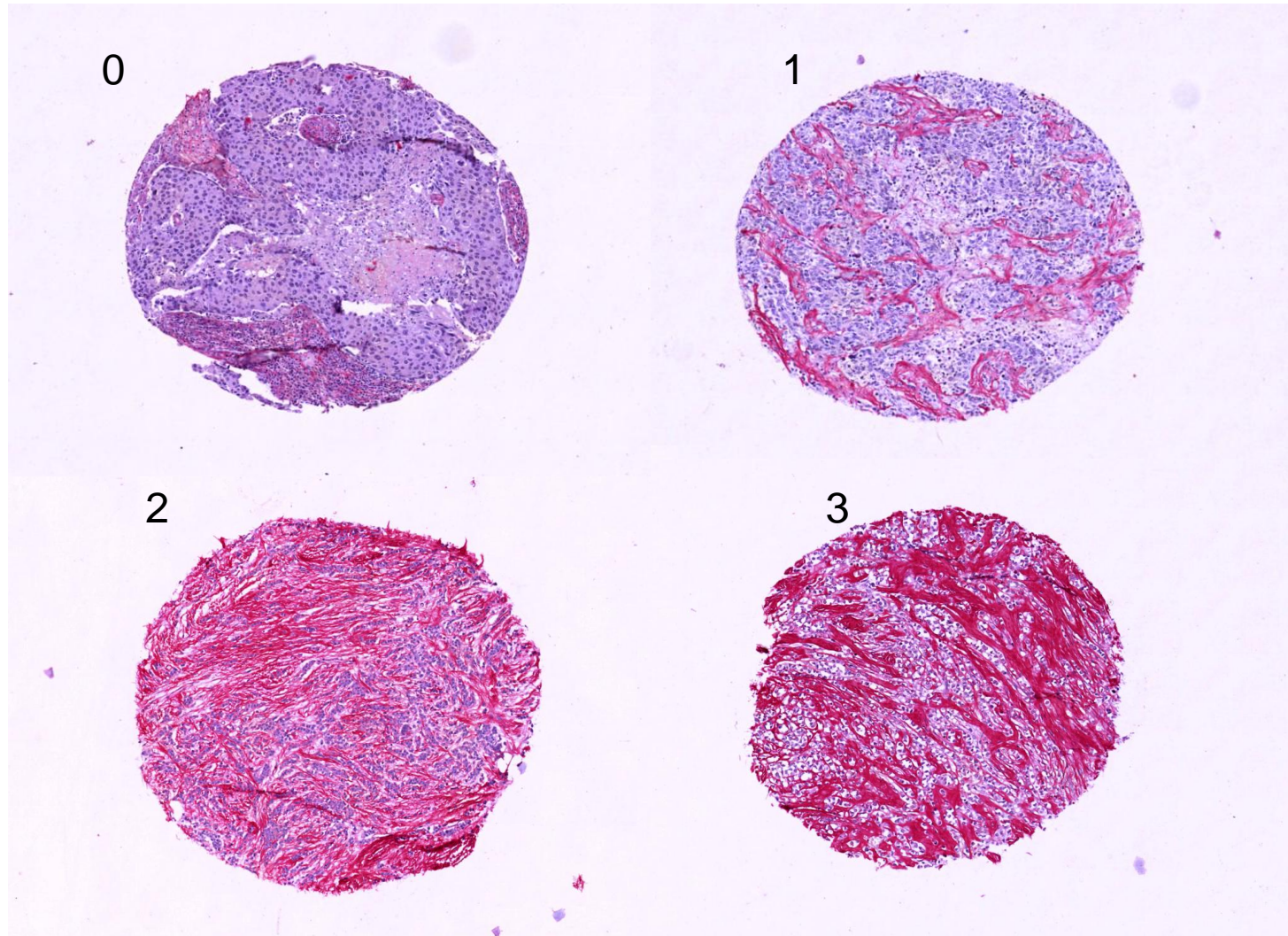
Borderline inverse relation between the presence of Vimentin and Sirius red
 $R=-0.432$
 $P=0.051$

- Only few areas with exclusive Sirius red staining
- In general co-localisation of Vimentin and Sirius red

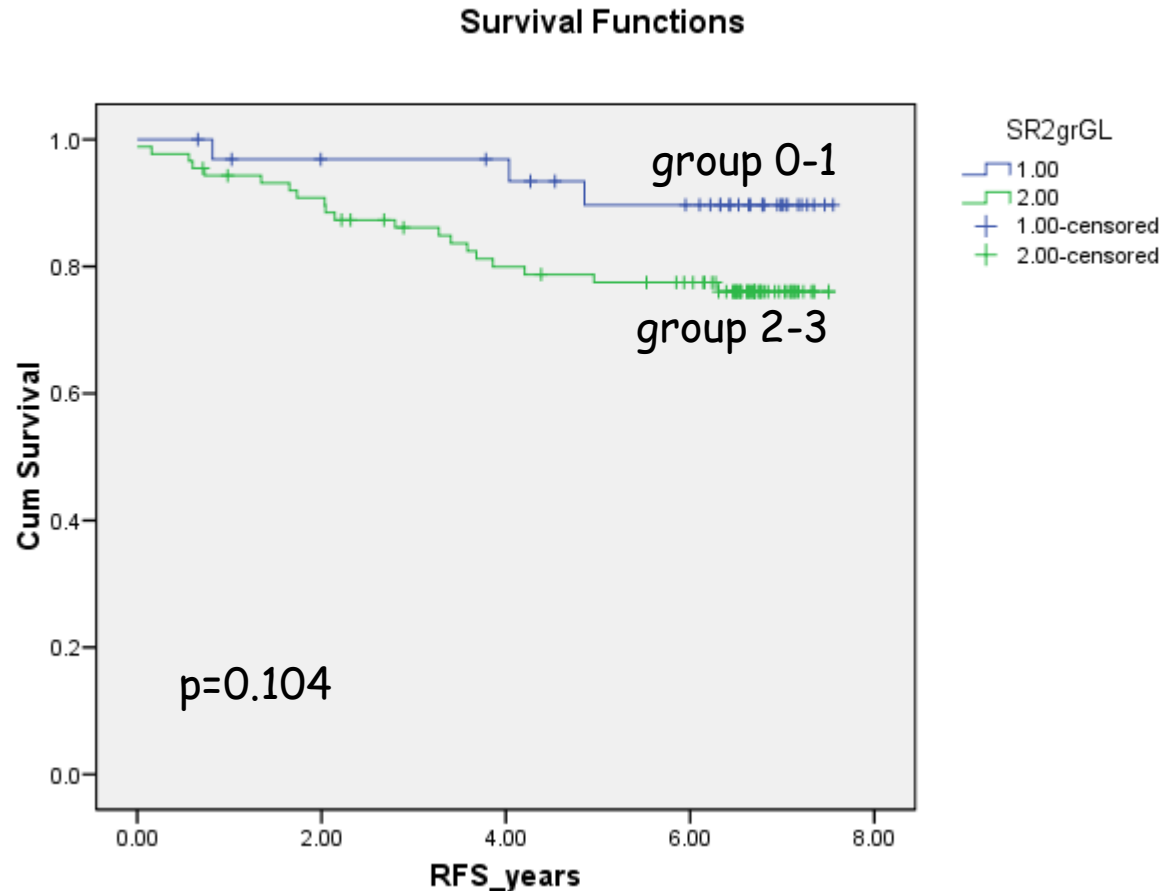
Complex association between collagen
and activated fibroblasts

Automated analyses can be useful for
monitoring intra-tumour heterogeneity and
stromal events in breast cancer

Analyses of Sirius red staining in breast cancer



Relation between Sirius red and disease recurrences



Trend towards a link to recurrences despite being inversely associated to many strong prognostic features as grade, proliferation...

Relation between Sirius red and disease recurrences in multivariate analyses

Sirius red

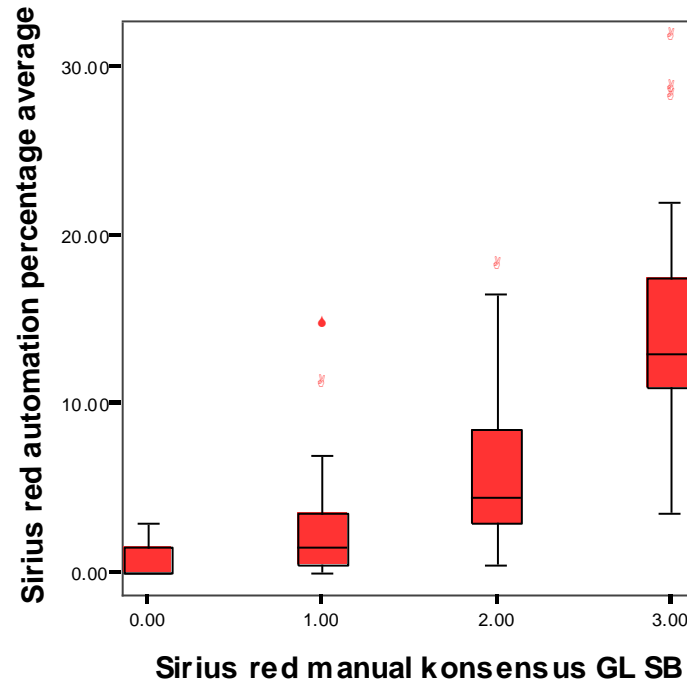
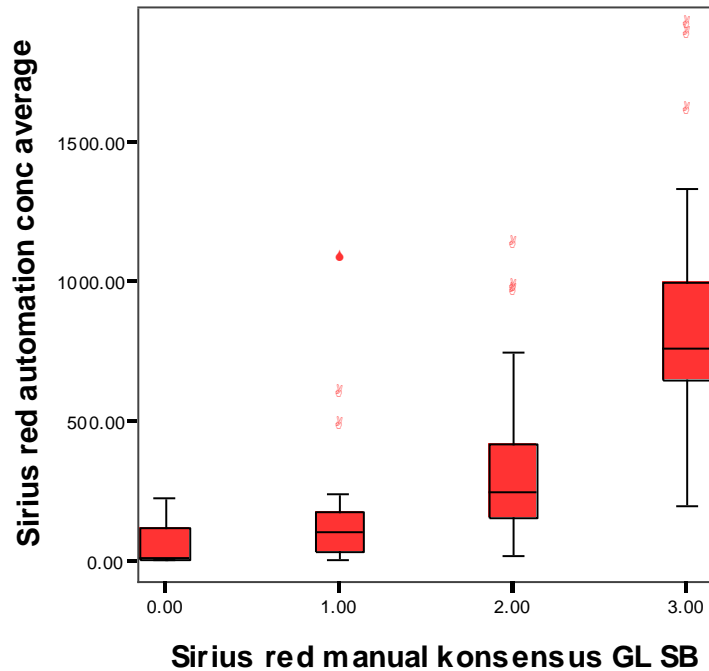
Variables in the Equation

	B	SE	Wald	df	Sig.	Exp(B)	95.0% CI for Exp(B)	
							Lower	Upper
SR2grGL	2.261	.686	10.848	1	.001	9.591	2.498	36.828
age	.042	.019	4.832	1	.028	1.043	1.005	1.083
nodstat2	1.650	.579	8.134	1	.004	5.208	1.676	16.190
nhg	.794	.463	2.945	1	.086	2.213	.893	5.481
size	.028	.010	8.099	1	.004	1.028	1.009	1.048
er	-1.873	.603	9.640	1	.002	.154	.047	.501

Strong independent prognostic factor together with size, ER and lymph node positivity

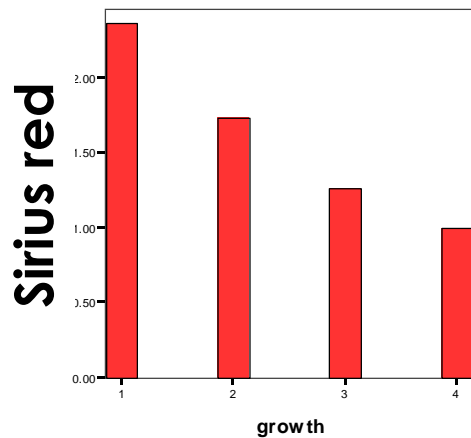
HR = 9.6 for Sirius red in two groups

Automated and manual analyses of Sirius red



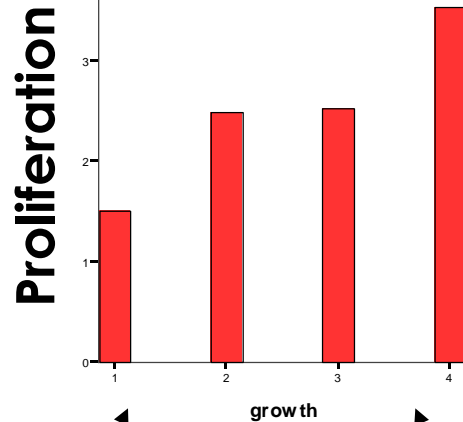
→ ***Strongly significant association $r = 0.740$***

Relation between tumour growth and Sirius red, proliferation and stromal pERK



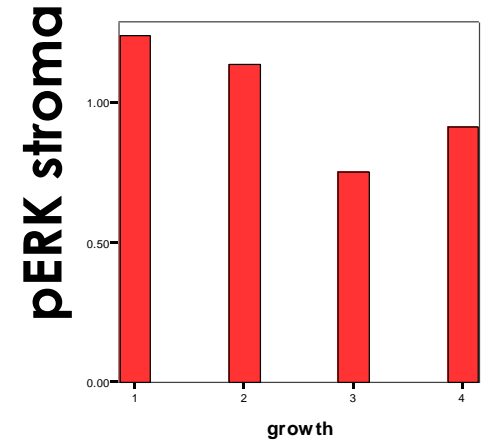
Growth

Bars show Mean



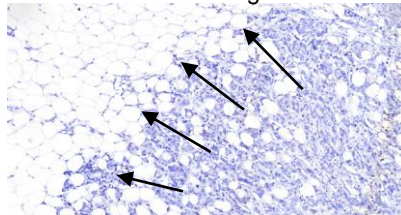
Growth

Bars show Mean

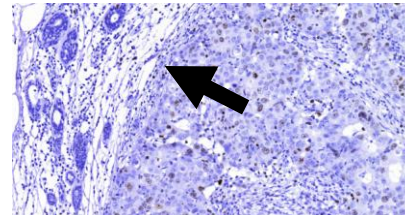


Growth

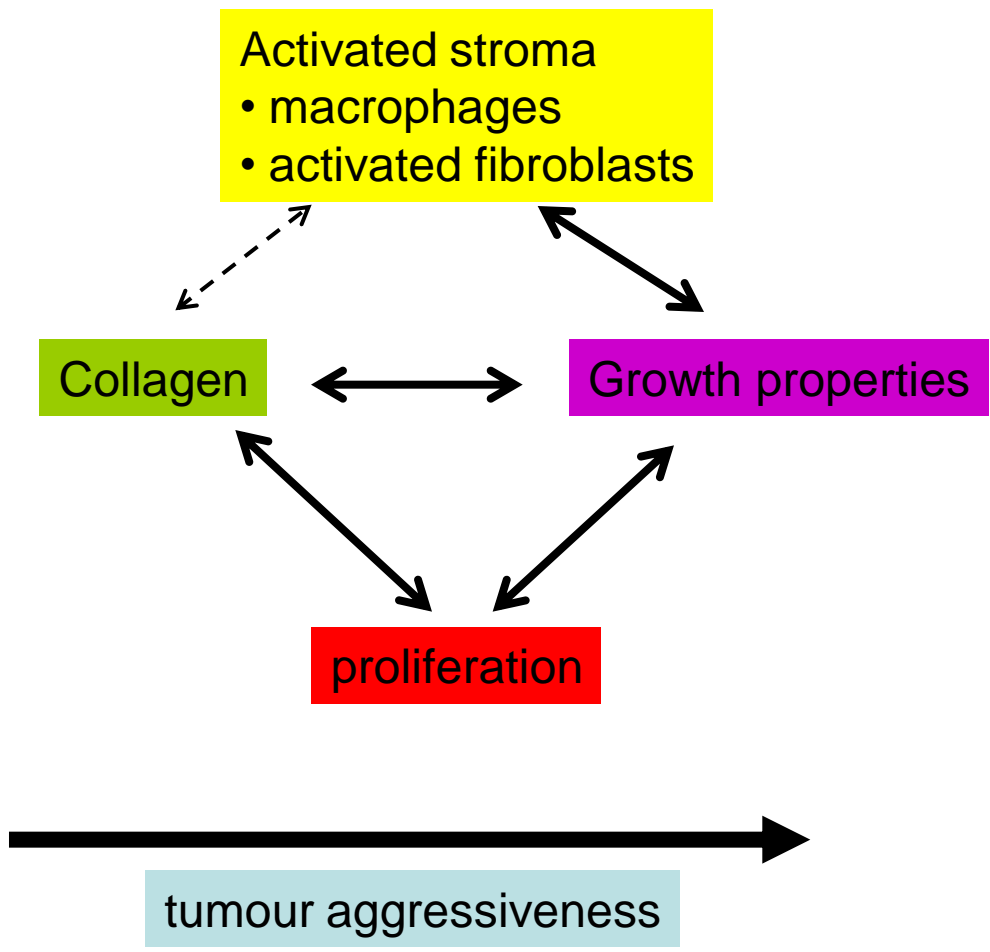
infiltrative growth



"pushing" growth



Infiltrative growth – high collagen content, low proliferation and “activated stroma”



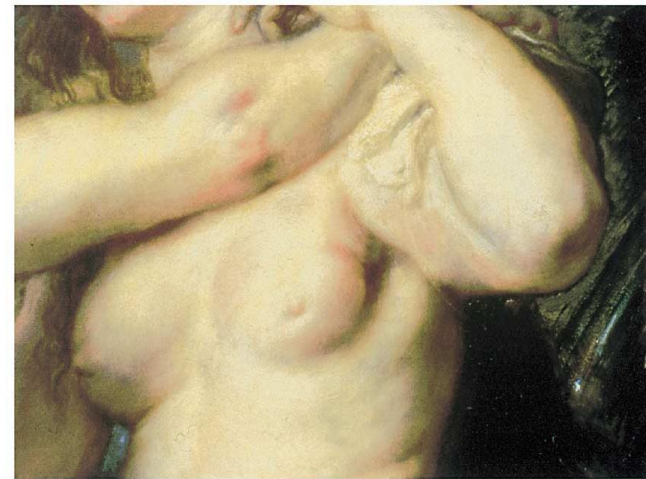
Future perspectives and summary

- Molecular Pathology – important in future research
- Automated analyses of TMAs - necessary
- Functional nuclear and membrane algorithms - Distiller
- Automated analyses not optimal for certain subgroups of breast cancer
- Stromal events can be monitored by automated analyses and are linked to key tumour biological properties

Breast cancer – a historical disease

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