NEW STANDARDS FOR DATA QUALITY AND DIAGNOSTIC WORKFLOWS IN THE PATHOLOGY LAB

AALBORG UNIVERSITY HOSPITAL

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NordiQC

New standards in pathology

The pathology labs faces major challenges

- Lack of experienced pathologists
- A growing volume of diagnostic tests
- Demands to reduce turnaround times
- Demands to reduce overall costs of pathology
- Demands for assessing new prognostic and therapeutic markers, subclassifications, gradings …
- Demands for optimization and standardization of the diagnostic work

New standards in pathology

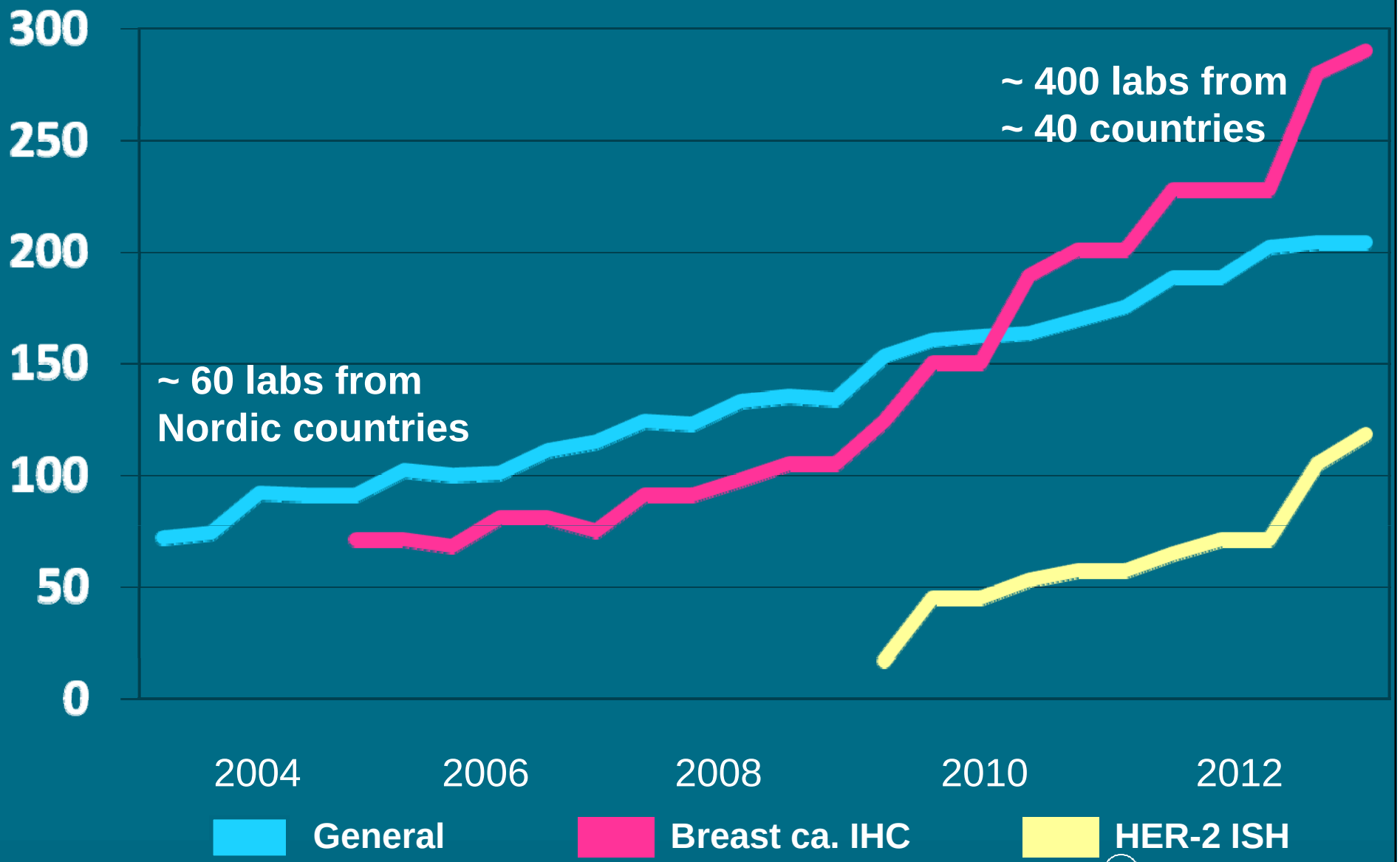
To obtain optimization and standardization of diagnostic we need

- Lab tools and procedures that can aid pathologists in
  - obtaining data of a better quality
  - achieve such data faster and cheaper
- External quality assurance schemes to identify the best and the less successful reagents and laboratory procedures.

Nordic immunohistochemical Quality Control

- Founded 2003 by Nordic pathologists
- Independent, scientific, not-for-profit organisation
- Institute of Pathology, Aalborg University Hospital
- General module: 3 runs/year
  - 15-18 different markers
- Breast cancer IHC module: 2 runs/y
  - HER-2, ER/PR, Ki67/E-Cad ...
- HER-2 ISH module: 2 runs/year
  - BRISH, FISH
- www.nordiqc.org

NordiQC participants



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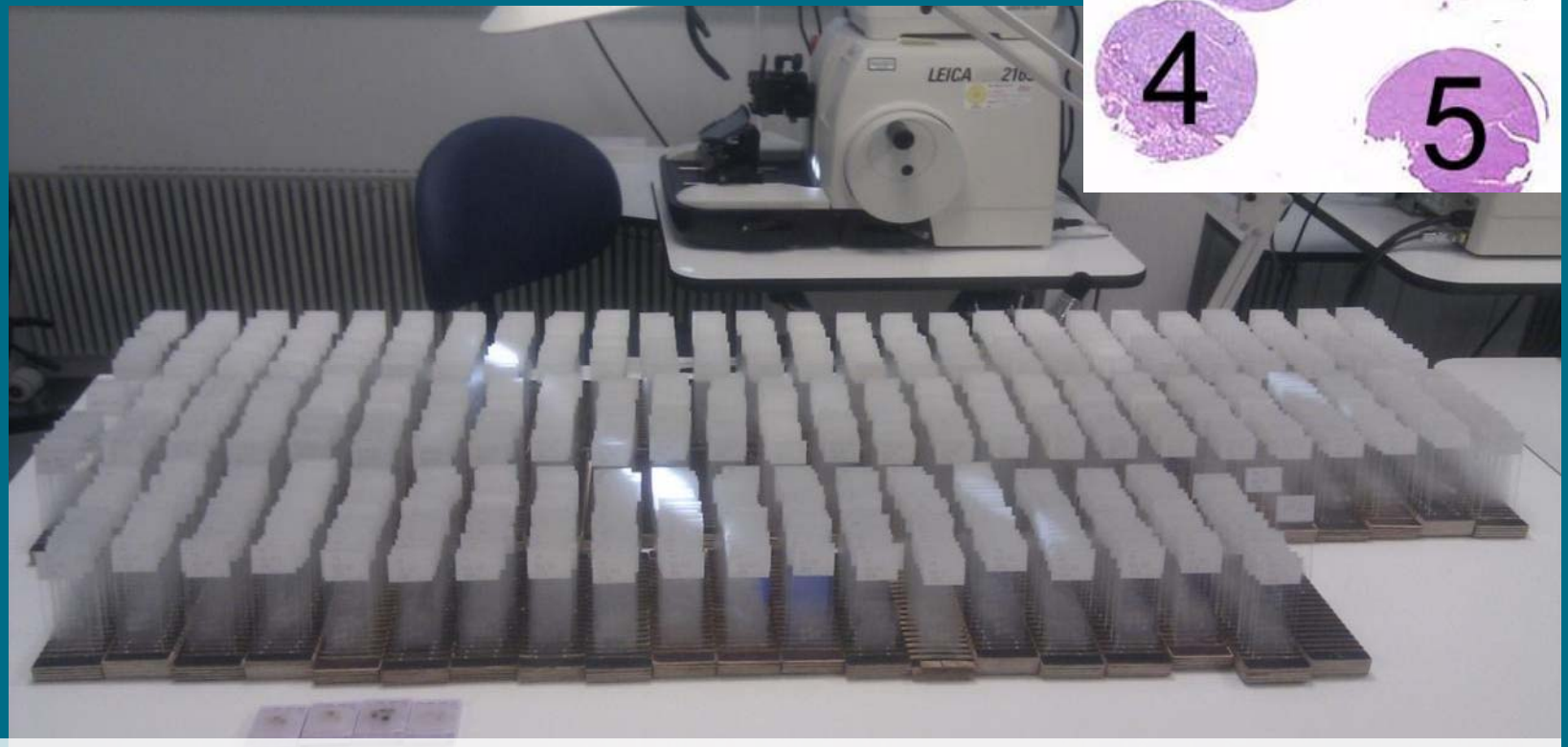
NORTH DENMARK REGION

Test material

Multi-tissue FFPE blocks

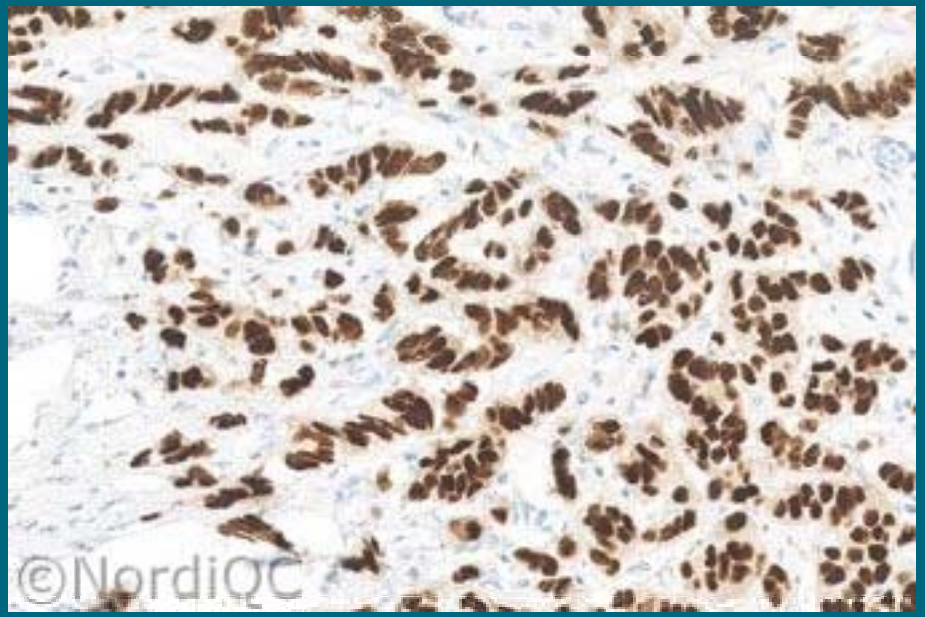
Normal and clinically relevant tumour tissues

- Different levels of antigen expression
  - high, moderate, low, none

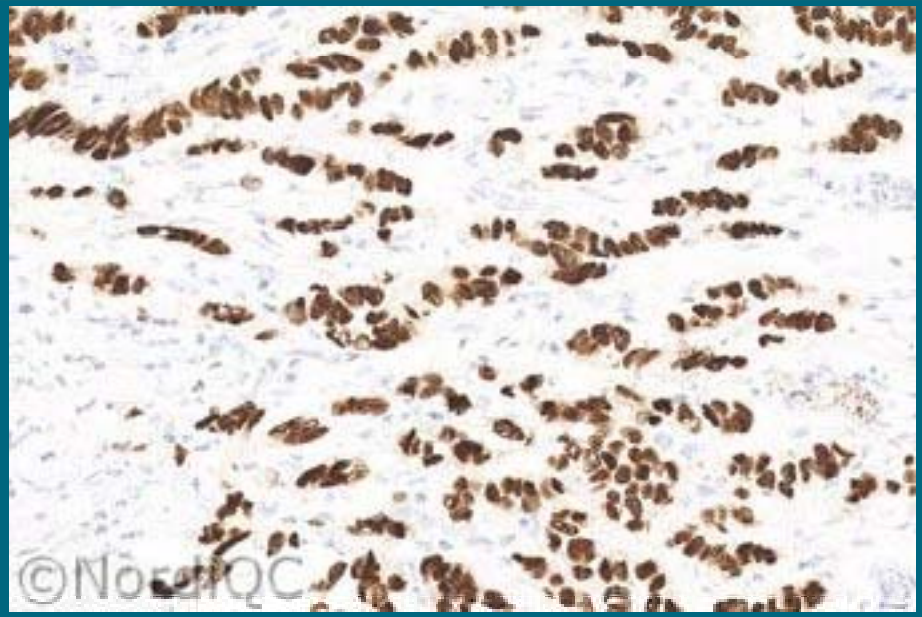


2 unstained slides for each marker send to the participants
1 stained slide returned for central assessment

Serial sections stained for Estrogen receptor



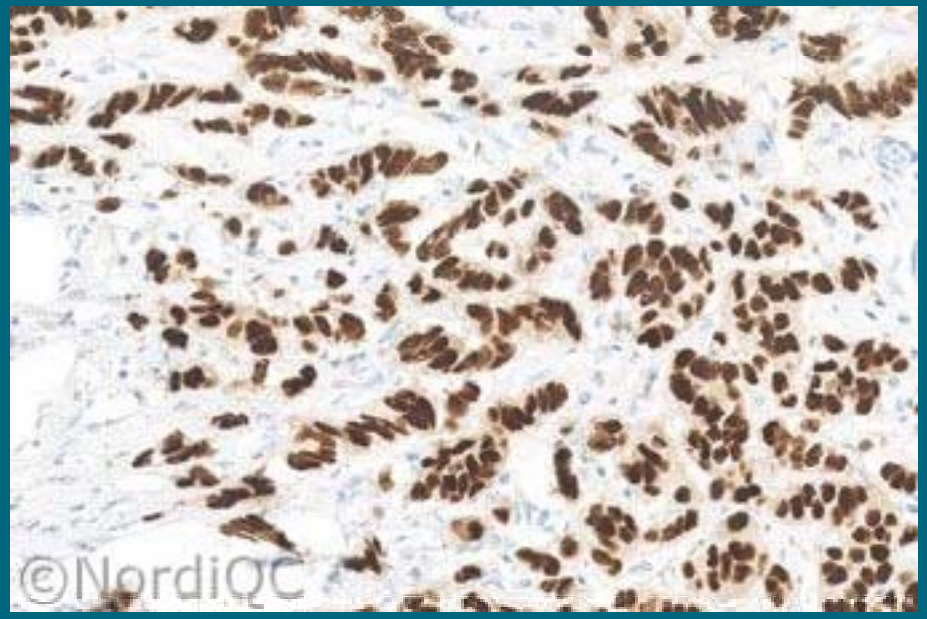
Lab. A

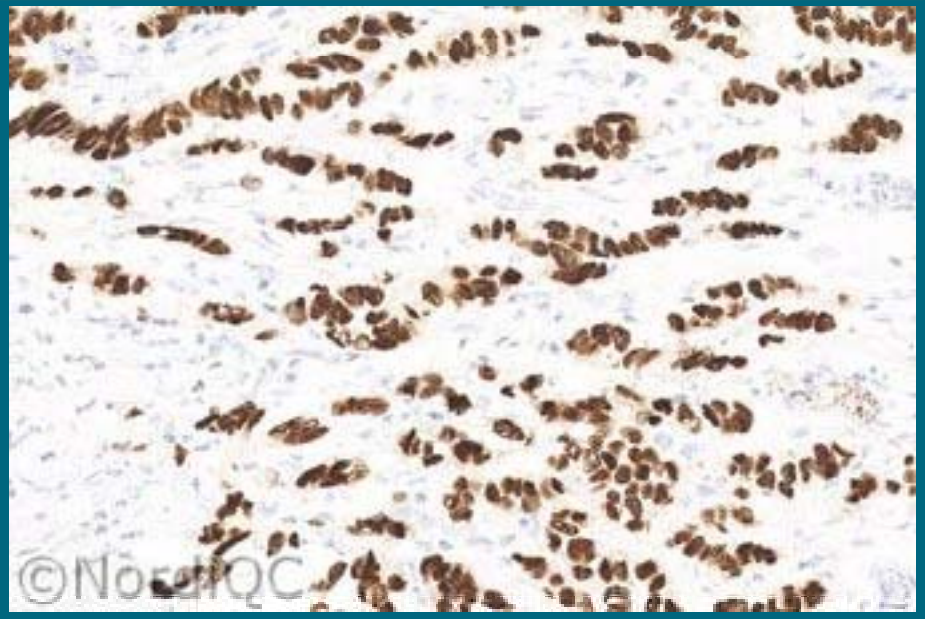


Lab. B

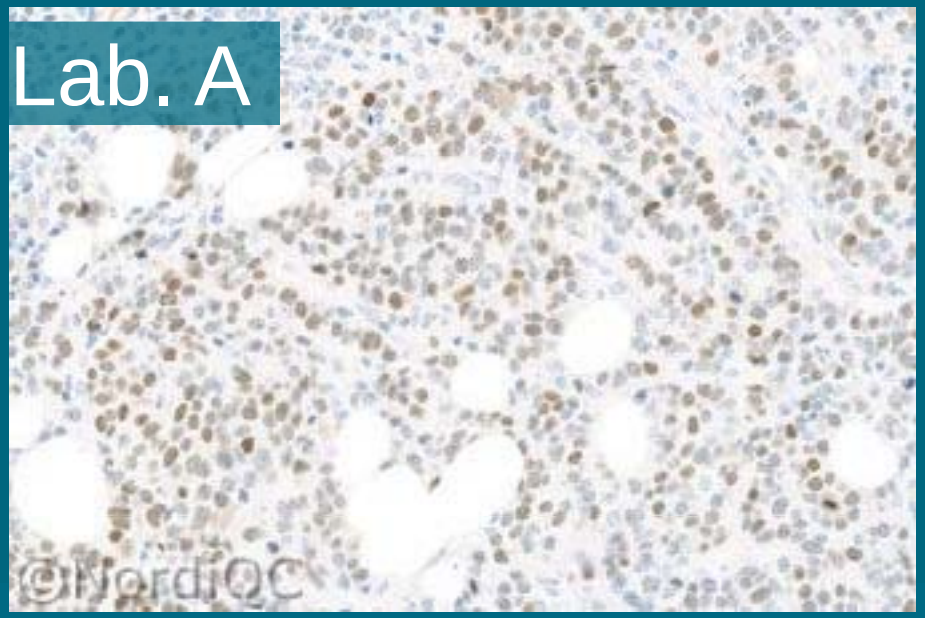
Ductal breast carcinoma

Serial sections stained for Estrogen receptor

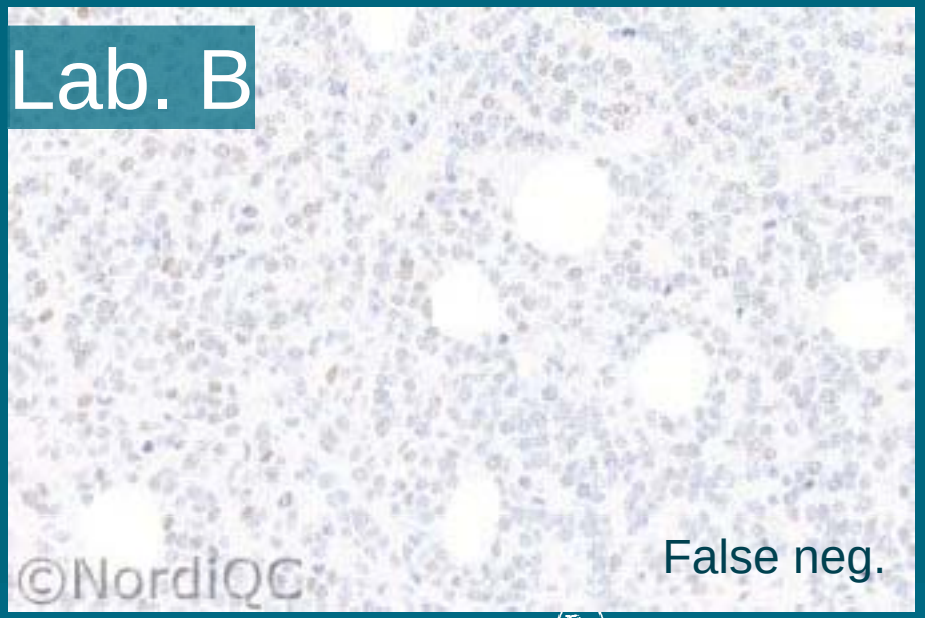




Lab. A

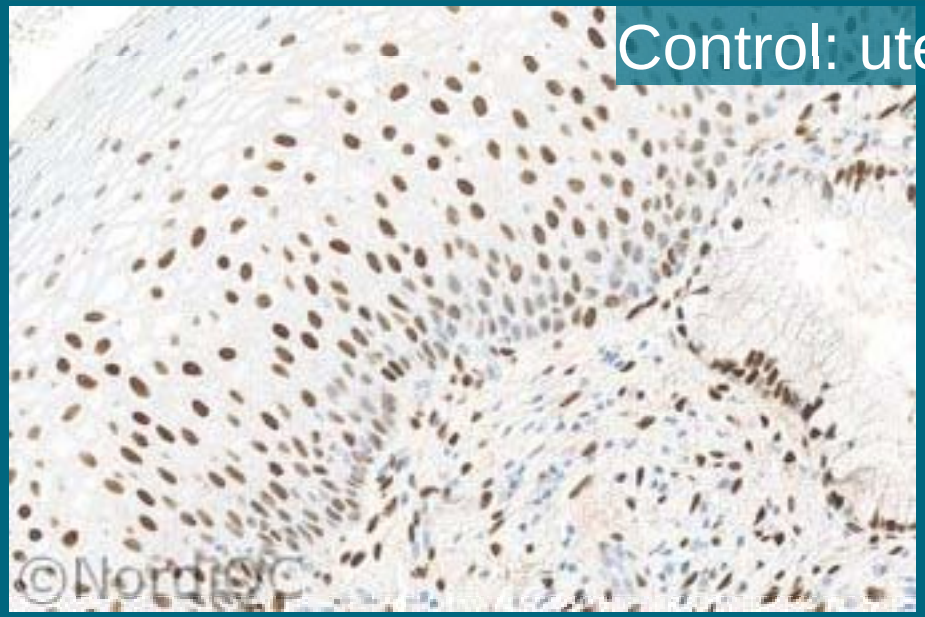


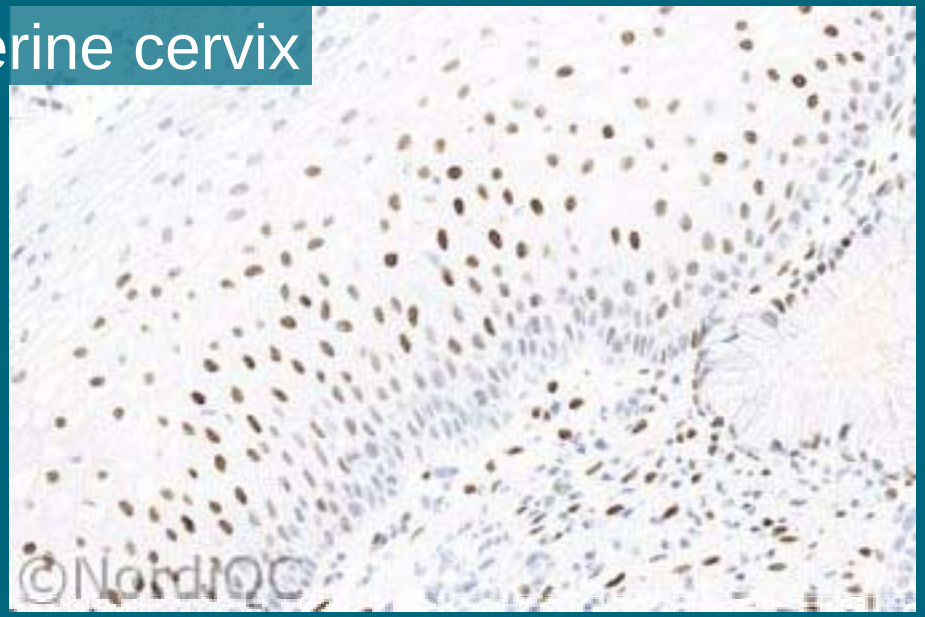
Lab. B

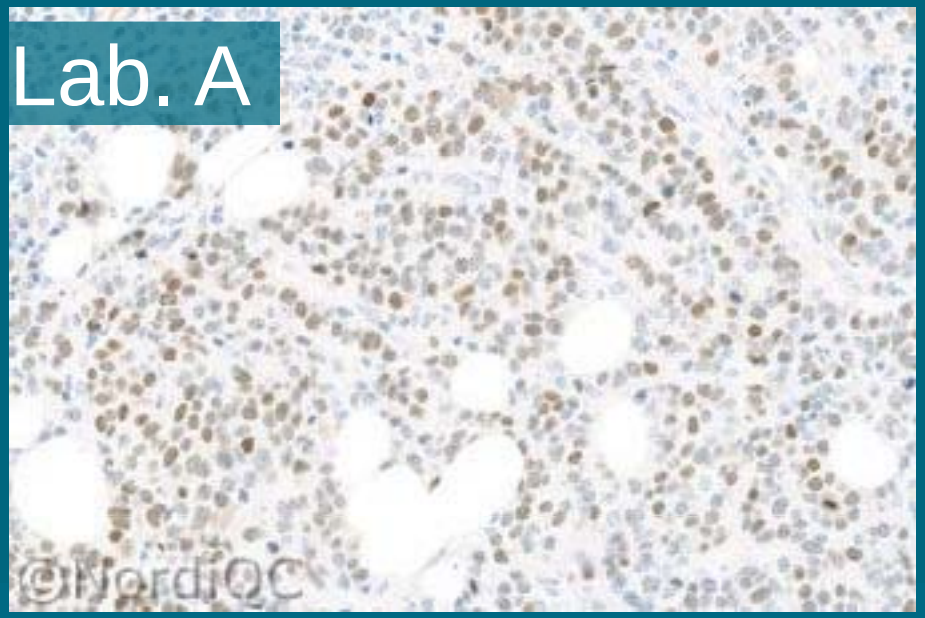


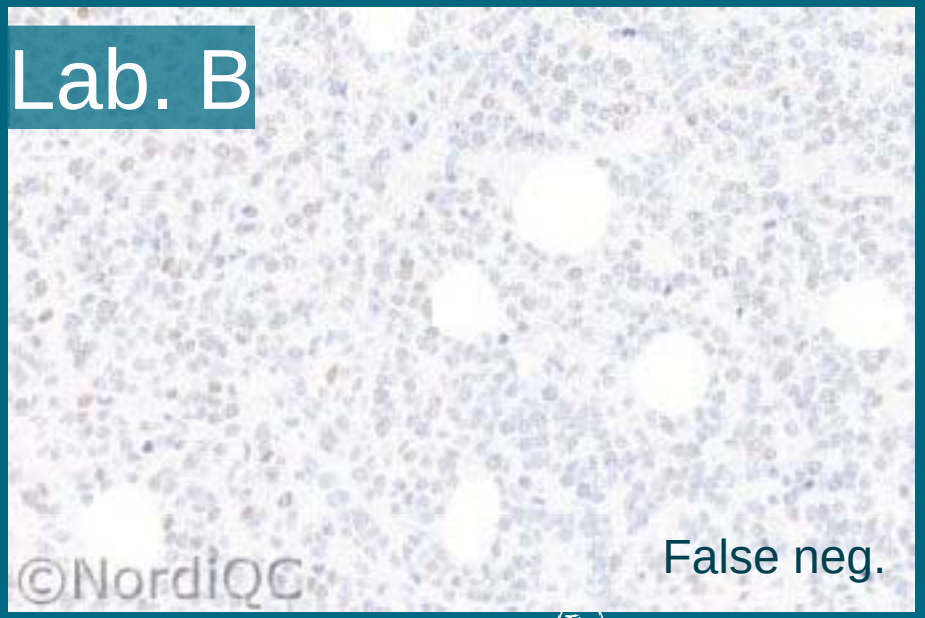
False neg.

Serial sections stained for Estrogen receptor

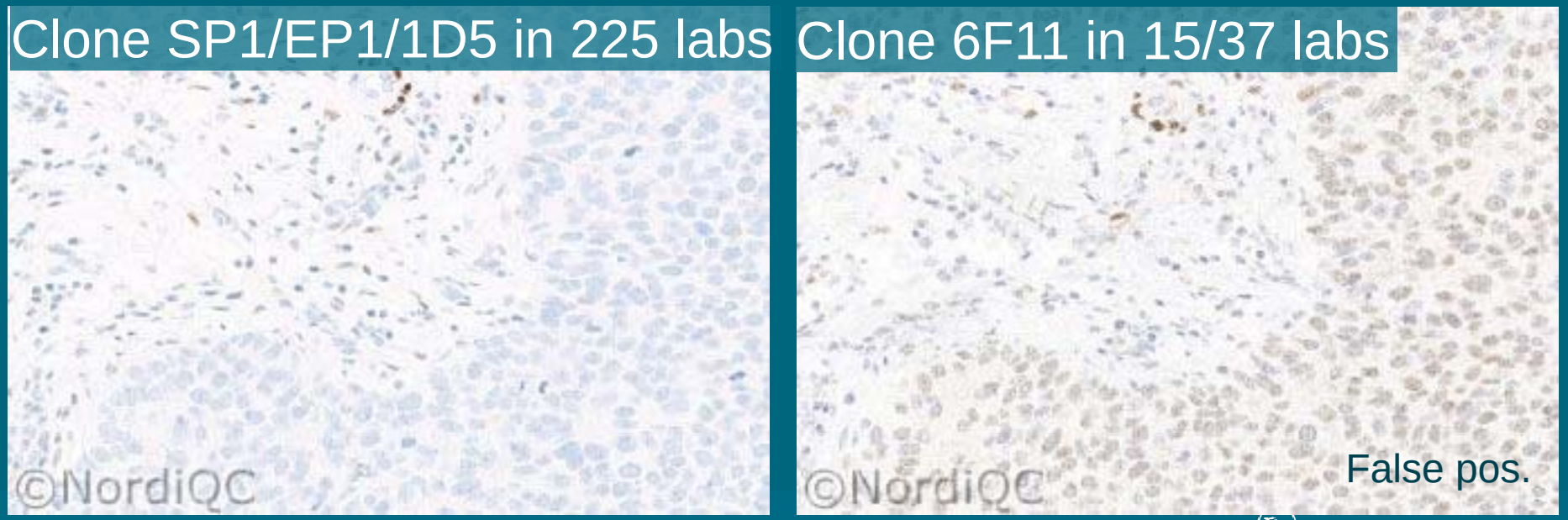




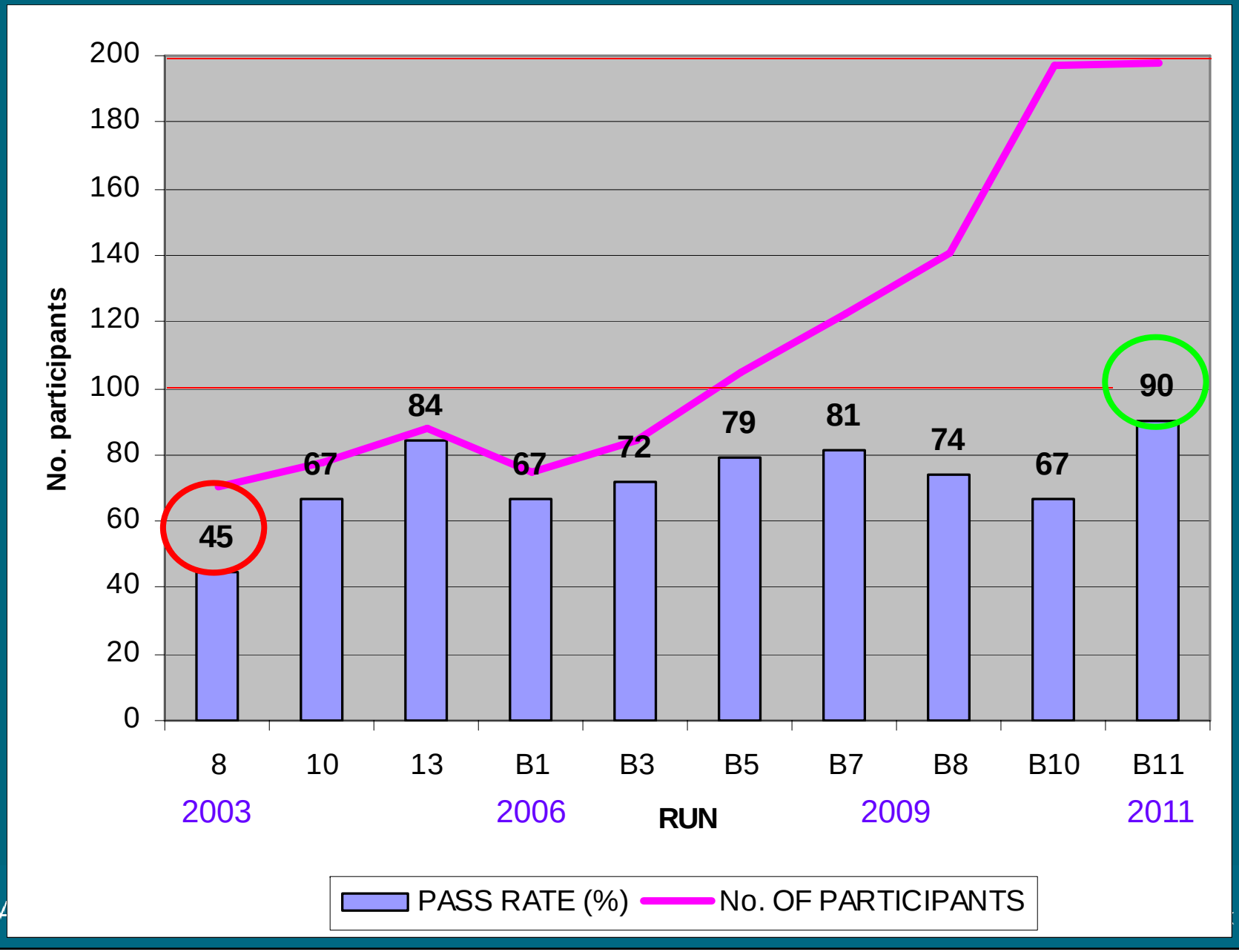




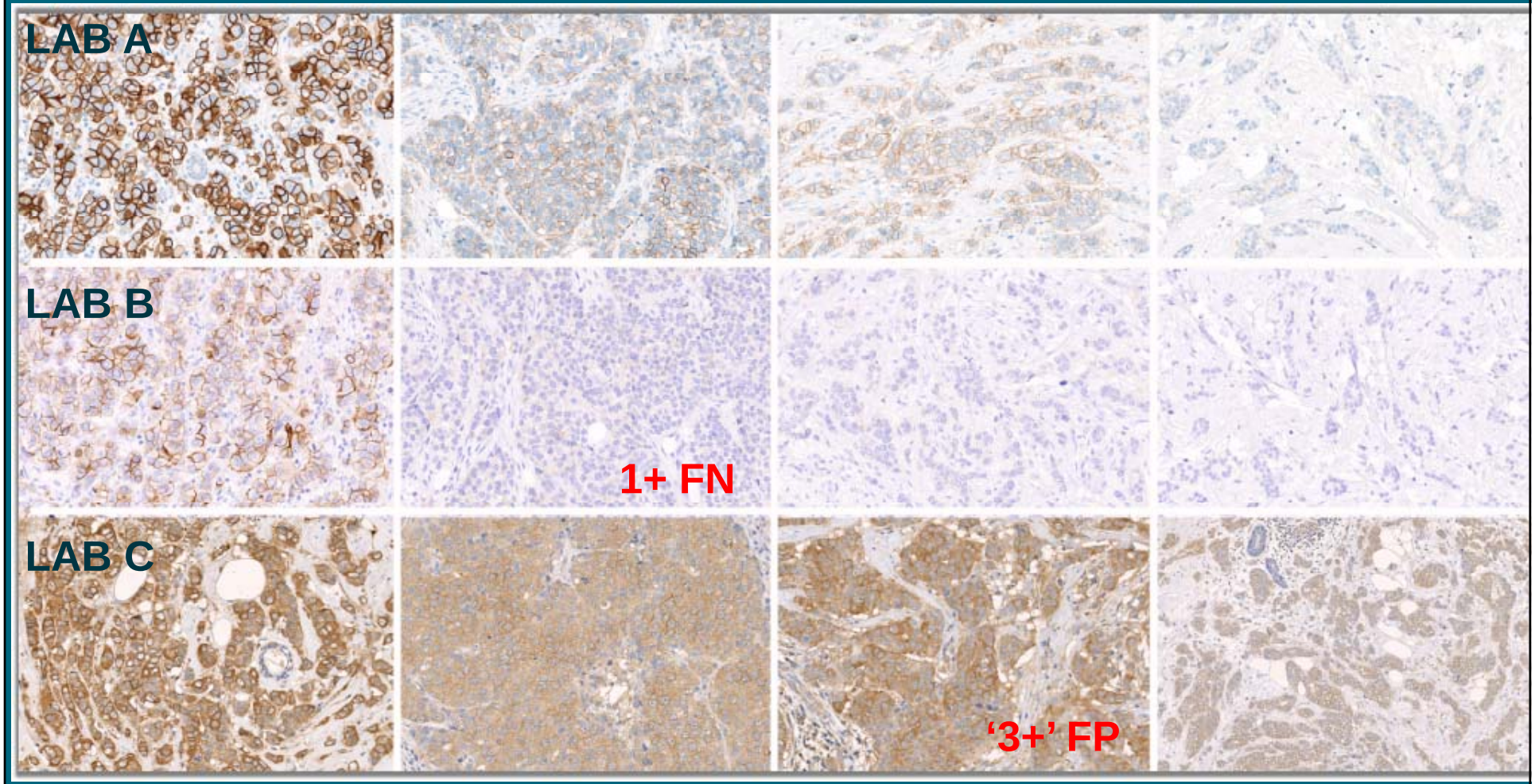
Serial sections stained for Estrogen receptor



NordiQC EQA: Estrogen Receptor **2003-11**

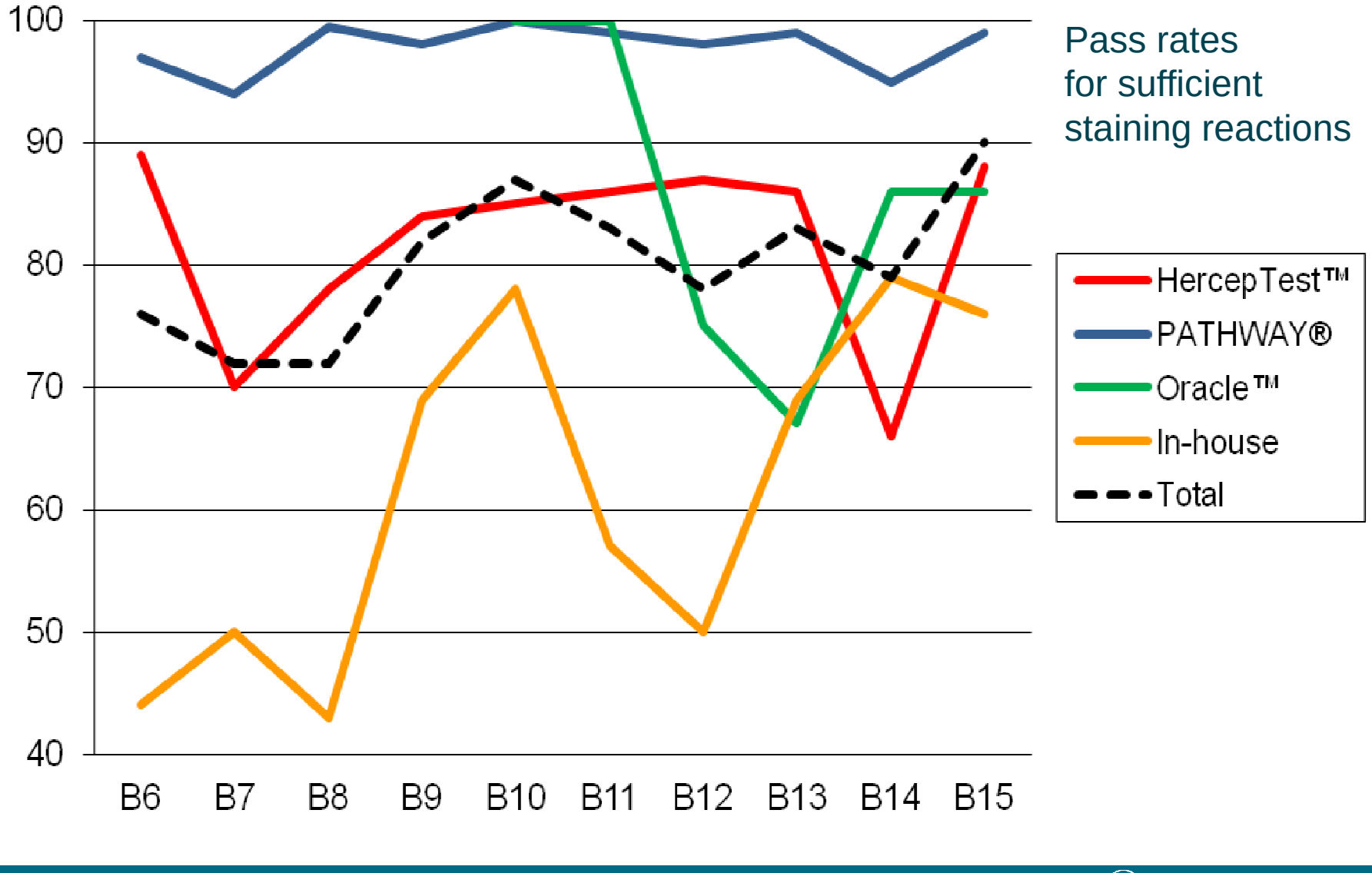


HER-2 staining

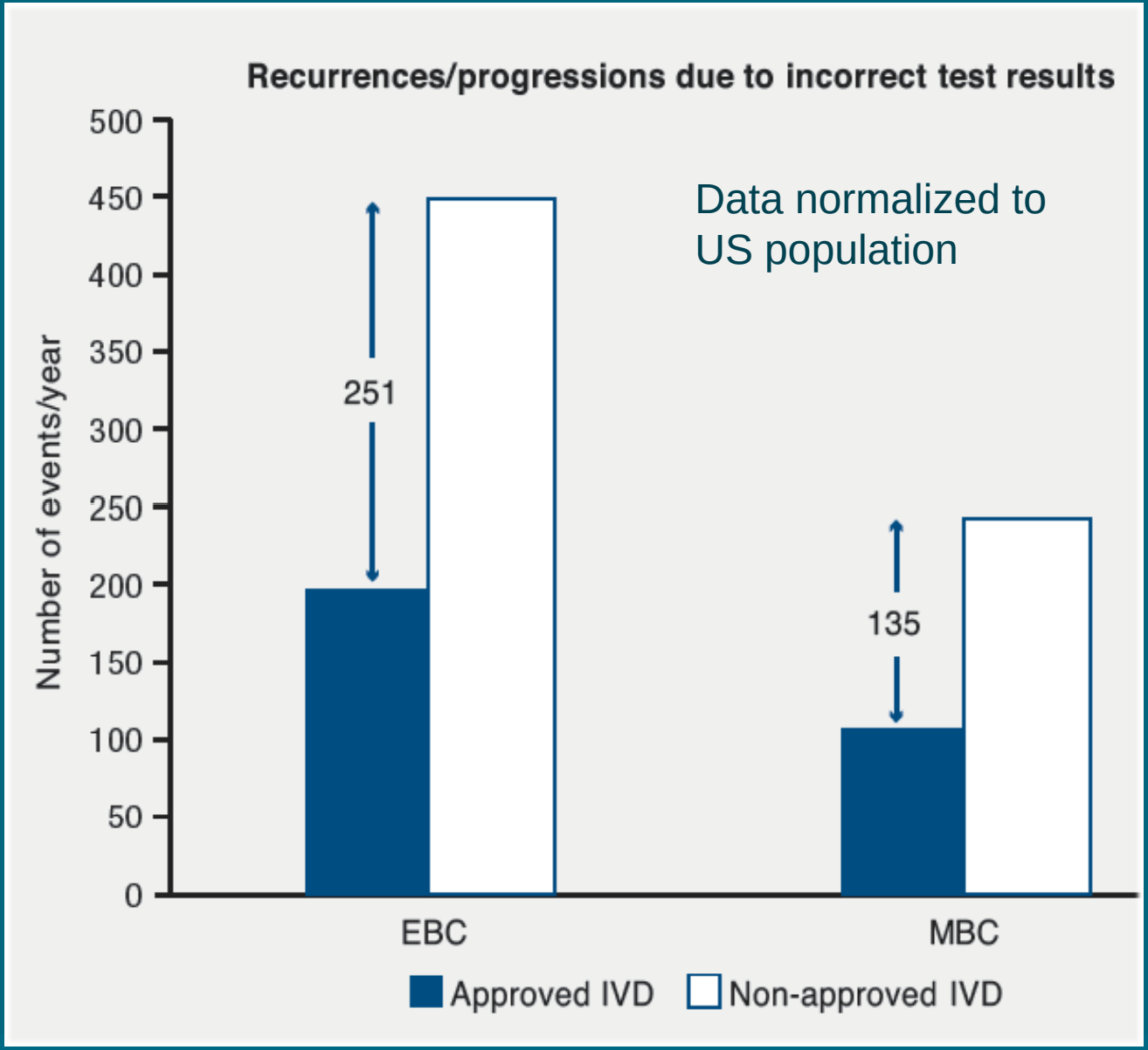


* A: Amplified, i.e., HER2/chromosome 17 ratio >2. NA: Non-amplified

HER-2 staining results in 10 runs



Roche – NordiQC joint venture



Roche – NordiQC joint venture

Annual, US	Approved	Non-approved
Primary testing costs	$M 11	$M 2
Additional direct costs	$M 18	$M 72

For each **1$** saved by the pathology lab by usage of cheaper reagents, the healthcare system is ultimately burdened with **7$**.

Submitted for publication

NordiQC

Nordic immunohistochemical Quality Control

Home ■ Participation ■ Modules/tests ■ Assessments ■ Epitopes ■ Protocols ■ Techniques ■ Links

NordiQC is an independent scientific organization, promoting the quality of immunohistochemistry by arranging schemes for pathology laboratories, assessing tissue stains, giving recommendations for improvement and providing good protocols.

Last update: 30-03-2012

The results of **Run 34** are uploaded by 1st April. See **Newsletter**.
Individual results are e-mailed.

Run 35 (General module), **Run B13** (Breast cancer module) and **Run H1** (HER-2 ISH module) in scheme 2012 is **open** for **protocol submission**, deadline is **11th April**.

Slides are circulated about **18th April**, and deadline for protocol corrections and return of slides is **7th May.**

Note that **HER2-ISH** now belongs to a new module (and is no longer included in the Breast cancer module, in run B13 replaced by Ki67).
The new HER-2 ISH module comprises two annual runs with in situ hybridization tests for HER-2. This module includes both BRISH and FISH.

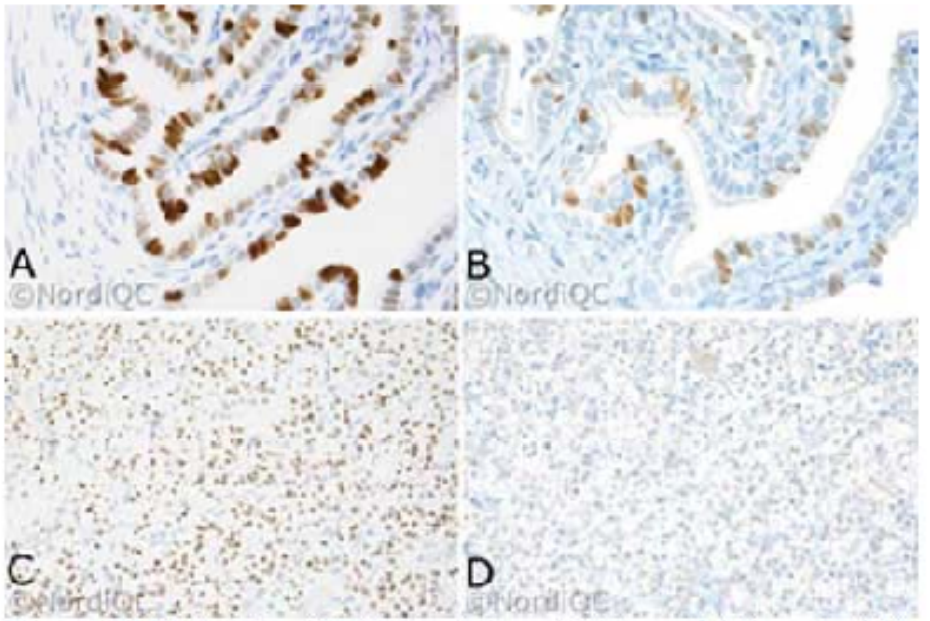


Figure: PAX8 staining of Fallopian tube, which is useful for control (A-B), and renal cell carcinoma (C-D). An optimal protocol stains all the epithelial cells in the control tissue (A) and tumour (C), while the insufficient protocol gives a weak staining of the control (B) and false negative staining of the tumour (D).

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Enrol in NordiQC

EPITOMICS · Leica MICROSYSTEMS · mtm

NordiQC Workshops and seminars

Roche · Thermo SCIENTIFIC · visiopharm

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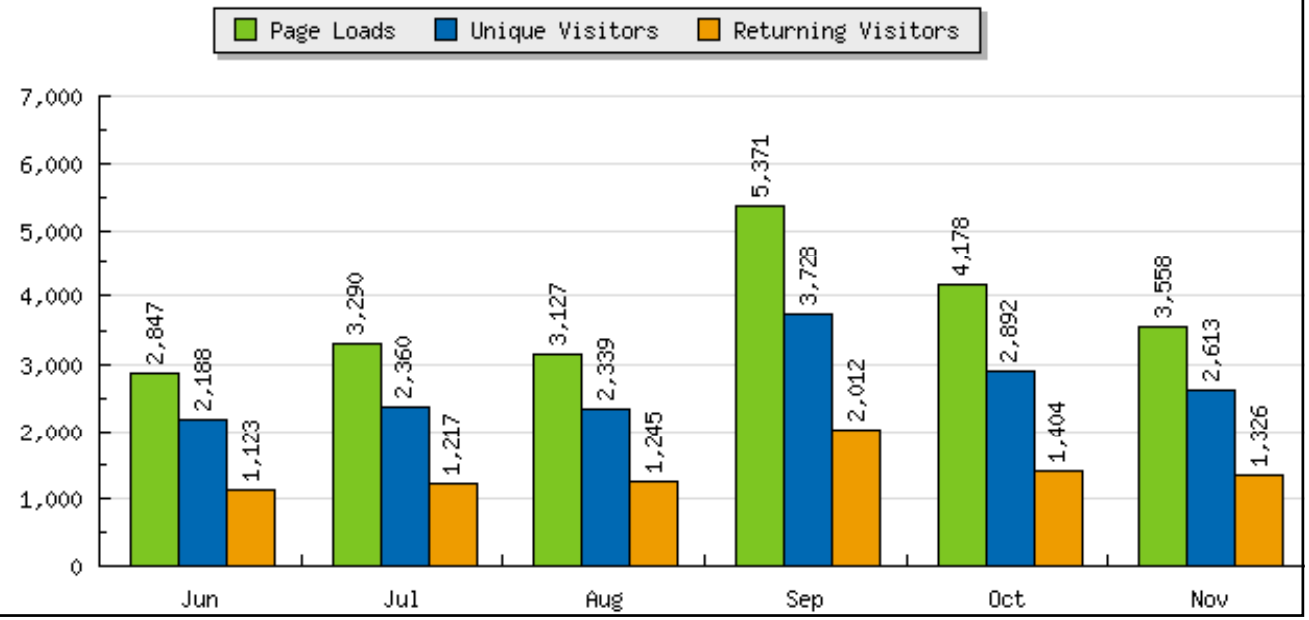
Organization ■ Subscription ■ Newsletter ■ Accompany letters ■ Seminars ERFA (Danish)

Visits to www.nordiqc.org

Monthly visits to www.nordiqc.org in the last 6 months 2010.



Feed-back

PDF file e-mailed to participants with assessment marks, explanations and recommendations

Nordic immunohistochemical Quality Control

Institute of Pathology, Aalborg Hospital, Ladegaardsgade 3, P.O.Box 561, DK-9100 Aalborg, Denmark

Assessment of Run 28 2010: Individual results

Aalborg, April 2010

The core group has assessed your submitted stains as shown in the table below.

The assessment is generally based on the staining intensity and distribution in cells expected to stain, background staining, cross-reactivity, counter-staining and preservation of tissue structures. More specific criteria for each marker are described on www.nordiqc.org → Assessments.

Each stained slide is marked as *optimal*, *good*, *borderline* or *poor*.

Optimal staining: The stain is considered perfect or close to perfect in all of the included tissues.

Good staining: The stain is considered fully acceptable in all of the included tissues. However, the protocol may be optimized to ensure the best sensitivity or signal-to-noise ratio.

Borderline staining: The stain is considered insufficient because of, e.g., a generally too weak staining or a false negative staining of one of the included tissues, or a false positive staining reaction. The protocol should be optimized.

Poor staining: The stain is considered very insufficient because of, e.g., false negative staining of several of the included tissues, or a marked false positive staining reaction. An optimization of the protocol is urgently needed.

Moderate or strong cross reaction (due to, e.g., the character of the primary antibody) or other false positive staining reaction (due to, e.g., endogenous biotin) is not compatible with an optimal result and will usually cause down marking.

For stains assessed as borderline or poor, comments and recommendations are given to the protocols. Also a good stain may be given a comment if a specific problem is identified.

Please compare the optimal stains and recommended protocols published on www.nordiqc.org with your own stains and protocols. A protocol recommended by NordiQC as well as changes suggested in this letter must be tested carefully in your own laboratory before implementation into the diagnostic work. NordiQC cannot take any responsibility for the consequences of changes of protocols or methods in a laboratory.

In case of a borderline or poor staining result, the laboratory may - not later than at the deadline for the

Marker	CD23	CR	CyD1	Ki67	Podop	TTF1
Assessment:	Poor	Optimal	Optimal	Good	Good	Borderline
Comments to the protocol:	False negative	-	-	Excessive counterstain	Weak	Weak*
Suggestions for improvement:	Consider change of primary Ab and recalibrate	-	-	-	-	Increase primary Ab conc. and/or prolong HIER

* Please read the epitope description and assessment summary carefully, as the choice of the Ab clone will influence the sensitivity and specificity.

Publications

AJCP 2005,124:782

Antibody Selection in Immunohistochemical Detection of Cyclin D1 in Mantle Cell Lymphoma

Emina Torlakovic, MD, PhD,[1,2] Søren Nielsen, HT,[3] and Mogens Vyberg, MD[3]

RESEARCH ARTICLE

AIMM 2011, 19:437

Thyroid Transcription Factor-1 in Primary CNS Tumors

Marianne Højsgaard Kristensen, MS, Søren Nielsen, HT, and Mogens Vyberg, MD

RESEARCH ARTICLE

AIMM 2013, 21:64

Demonstration of CDX2 is Highly Antibody Dependant

Martine Borrisholt, MS, Søren Nielsen, HT, and Mogens Vyberg, MD

RESEARCH ARTICLE

AIMM 2013, in print

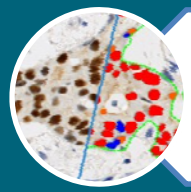
Carb-3 Is the Superior Anti-CD15 Monoclonal Antibody for Immunohistochemistry

Rasmus Røge, MD, Søren Nielsen, HT, and Mogens Vyberg, MD

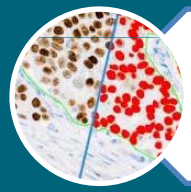
New standards in pathology

- Use of image analysis for diagnostic reading and interpretation
  - Optimization and standardization of data quality
  - Optimization of workflows

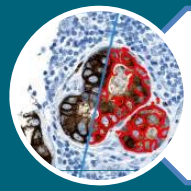
Multicenter validation of VDS Breast Panel



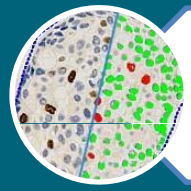
ER



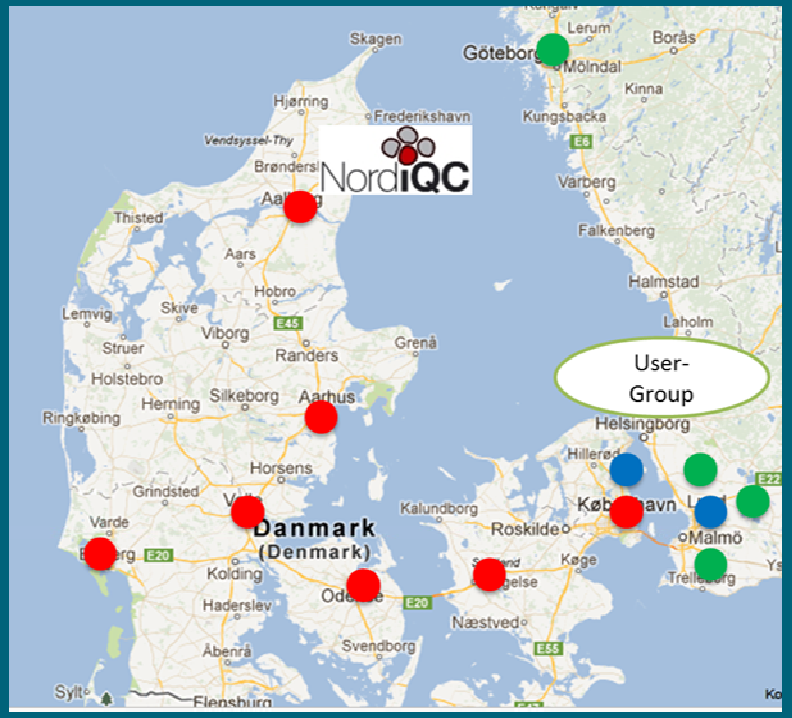
PR



Her2 CE

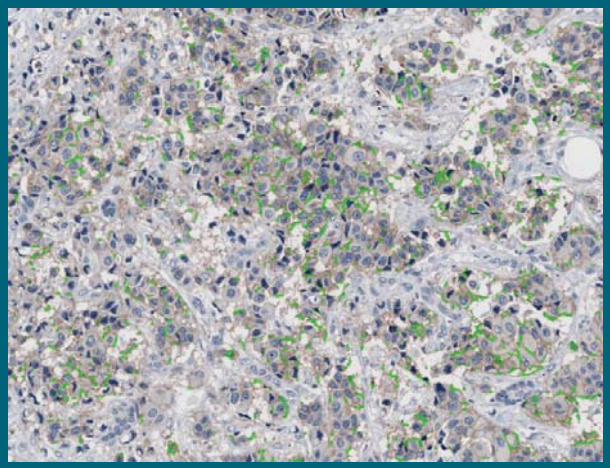
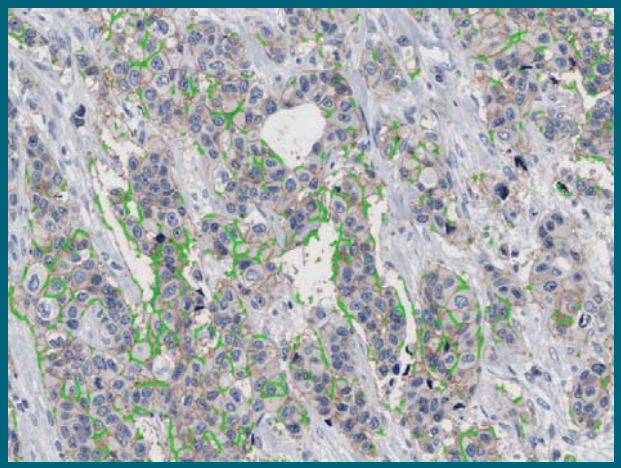
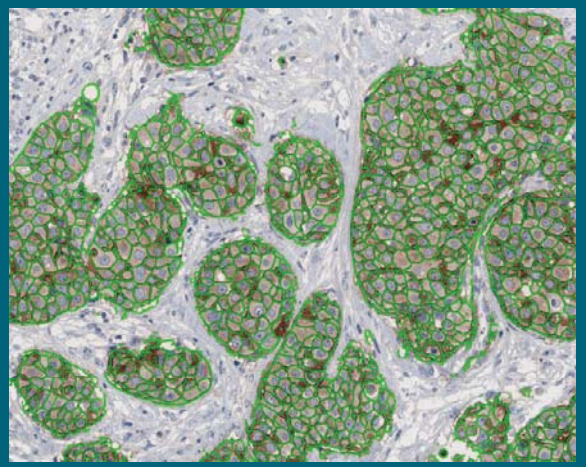


Ki67 & PHH3



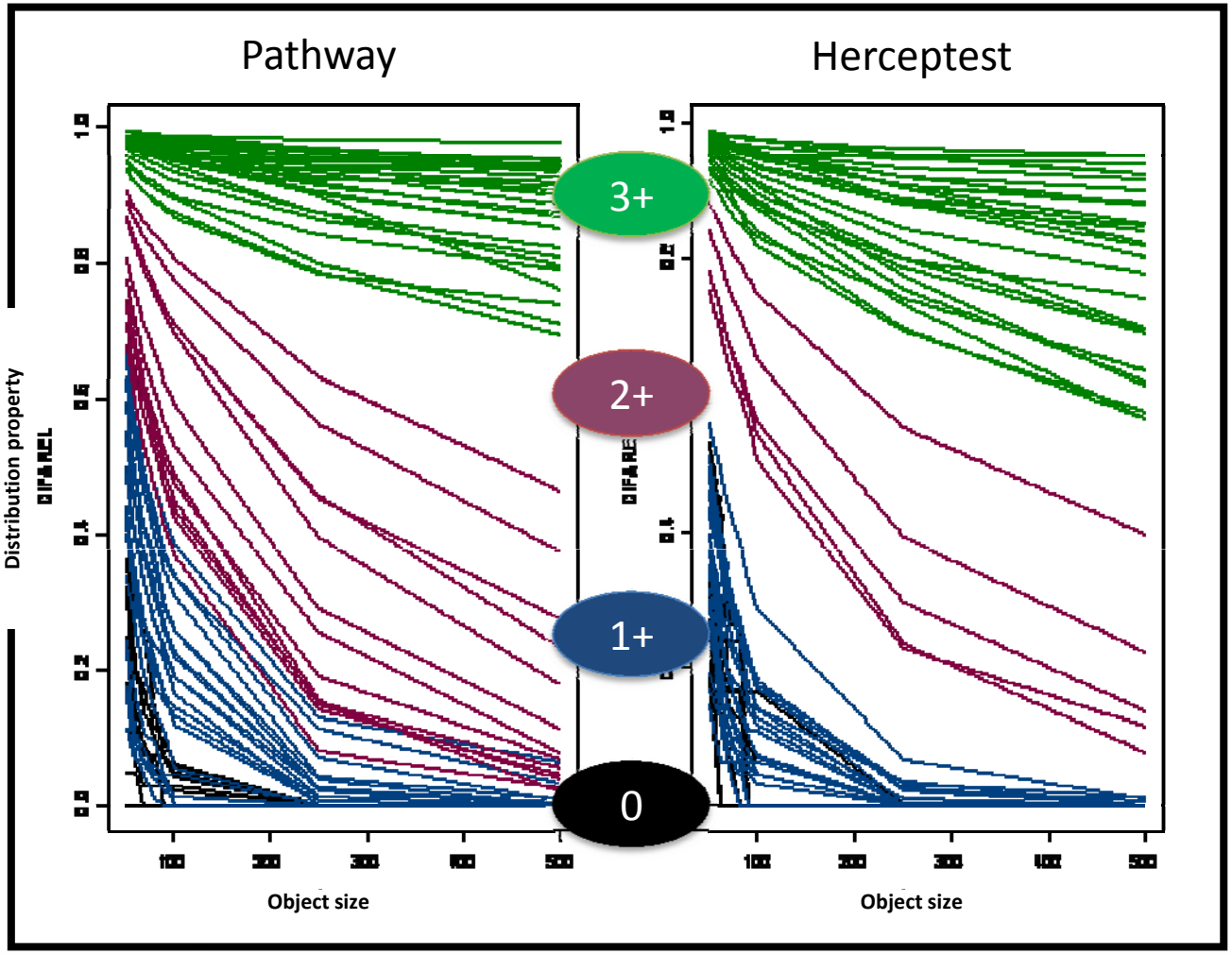
Work in progress as part of national and inter-Scandinavian programs (NordiQC organization and participants, and Visiopharm)

Visiopharm HER-2 CONNECT™
Connectivity captures the size distribution of membrane obejcts

0/1+	2+	3+
Very small and disconnected membrane objects	Small mixed with larger connected membrane objects	Dominated by large connected membrane objects

Connectivity captures the size distribution of membrane obejcts



HER2-CONNECT ™ - a reagent agnostic principle

Herceptest

IA \ H	0/1+	2+	3+	
0/1+	110	17	0	120
2+	0	7	4	16
3+	0	5	72	79
	110	29	76	215

Percent agreement: 87.9%
Cohen's Kappa: 0.79

DR by 5 pathologists

Pathway

IA \ P	0/1+	2+	3+	
0/1+	125	9	0	121
2+	0	6	4	22
3+	0	0	71	72
	125	15	75	215

Percent agreement: 94.0%
Cohen's Kappa: 0.88

Sensitivity/Specificity when compared to HER2 FISH: 100%/100%

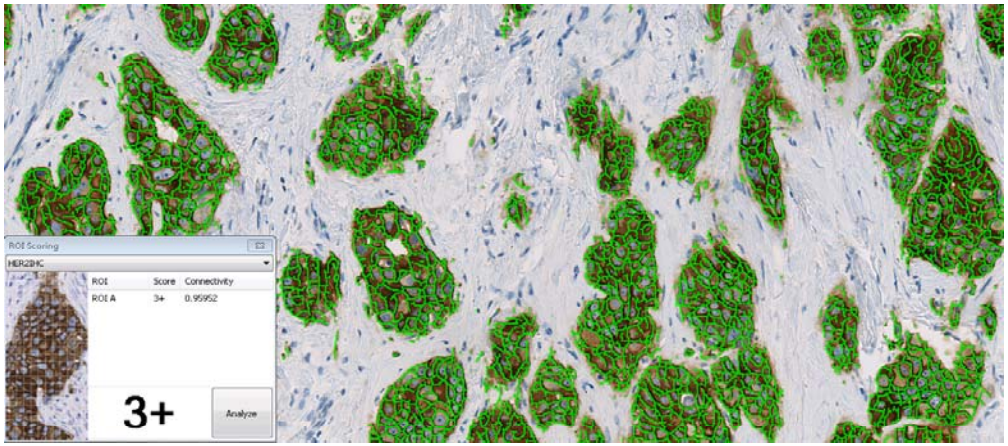
REF.: Digital image analysis of membrane connectivity is a robust measure of HER2 immunostains Brügmann et al. **Breast Cancer Res Treat.** 2012 Feb;132(1):41-9.

Data from >176 laboratories

	Sensitivity	Specificity	Inconclusive (2+)	HER2 IHC Test	Scanner	Site
HER2-CONNECT	100% (77/77)	100% (127/127)	5% (11/215)	HercepTest	NanoZoomer	Aalborg Hospital (cores)
Manual	100% (73/73)	97.3% (100/113)	13% (29/215)			
HER2-CONNECT	100% (71/71)	100% (134/134)	5% (10/215)	Pathway HER2	NanoZoomer	Aalborg Hospital (cores)
Manual	100% (75/75)	100% (125/125)	7% (15/215)			
HER2-CONNECT	63.9% (63/83)	98.1% 406/414)	2% (8/504)	Pathway HER2	ScanScope	Vilnius University Hospital (cores)
Manual	65.4% (51/78)	98.3% (393/400)	5% (27/505)			
HER2-CONNECT+ Pathology Review	100% (64/64)	81% (17/21)	41% (59/144)	HercepTest	ScanScope	Intermountain Central Laboratory (tissue)
Manual	100% (61/61)	0%[1] (0/15)	47% (68/144)			
HER2-CONNECT	98.2% (333/339)	99.4% (505/508)	4% (33/880)	Multiple	NanoZoomer	176 labs (cores)

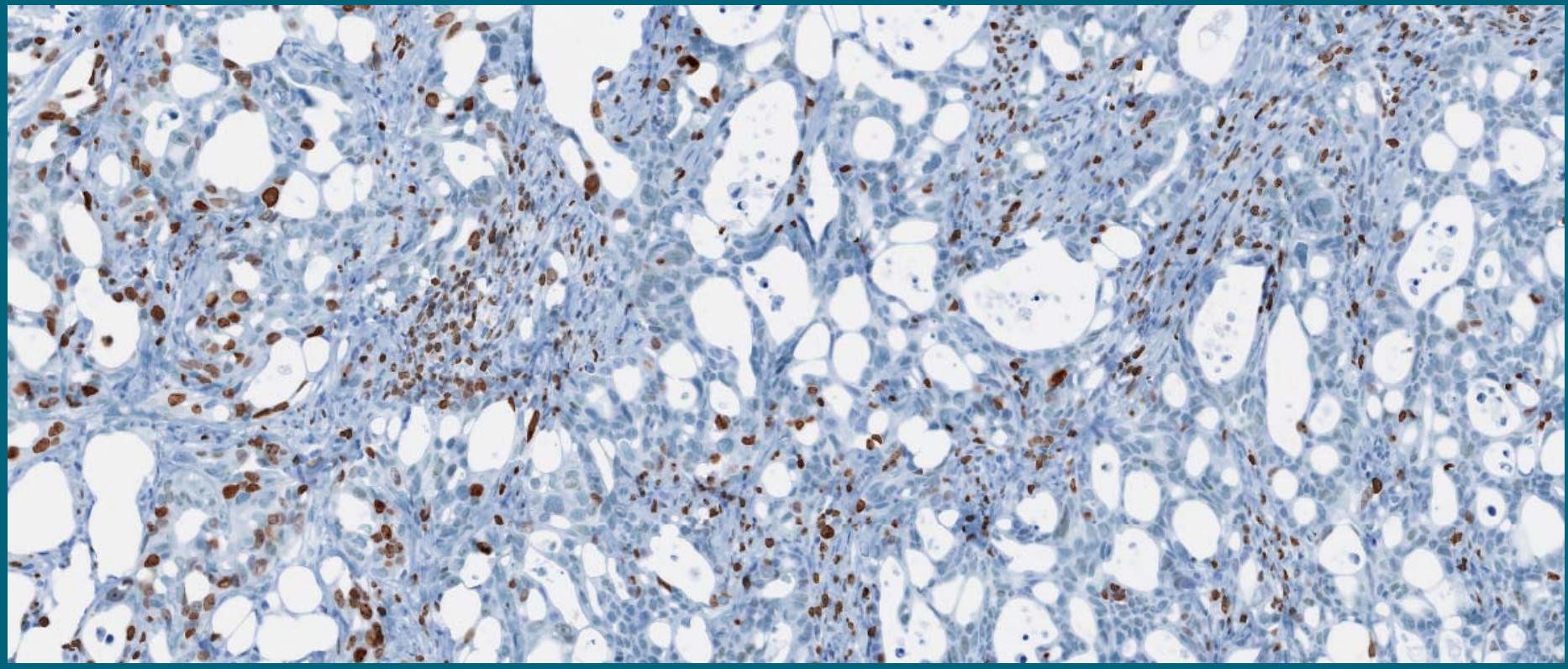
Cleared for diagnostic use in Europe

CE
IVD



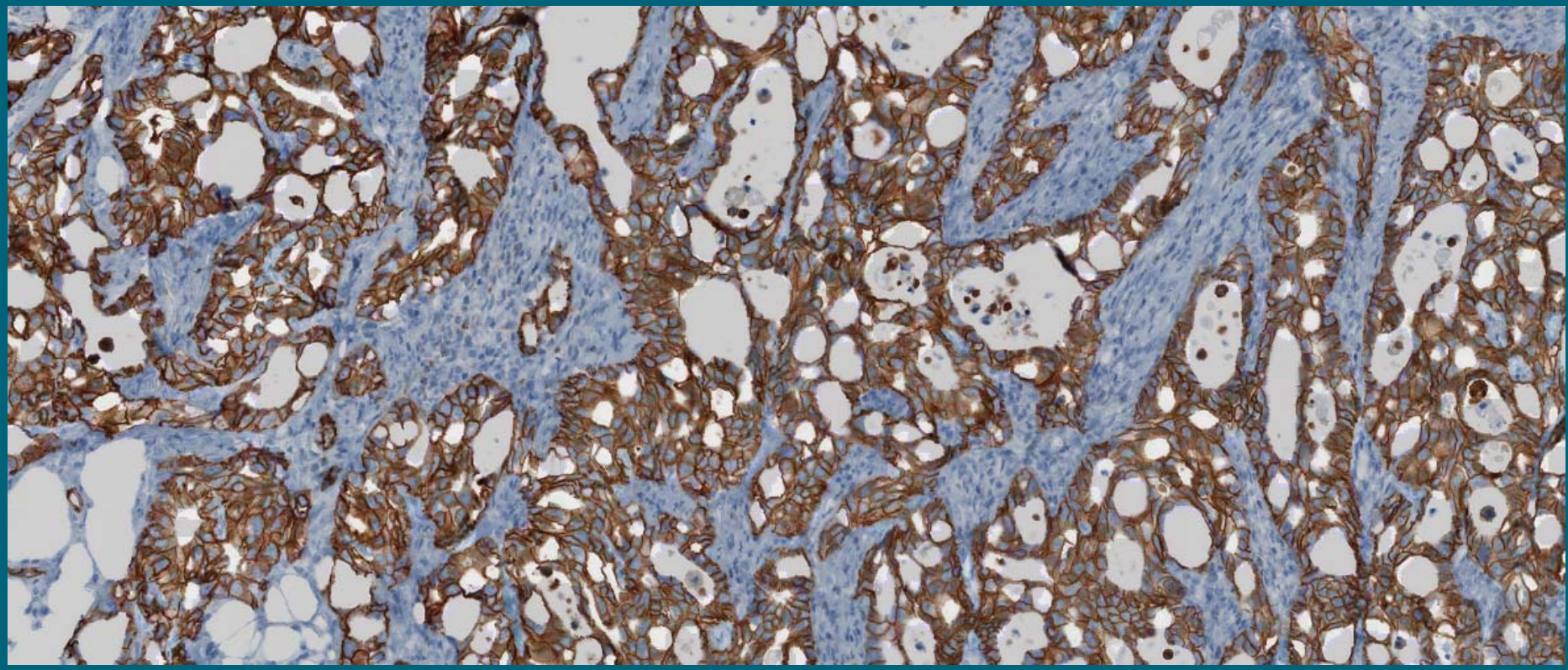
Used in a routine diagnostic setting at several Danish hospitals.

Nuclear markers: Tumour cells vs stromal cells



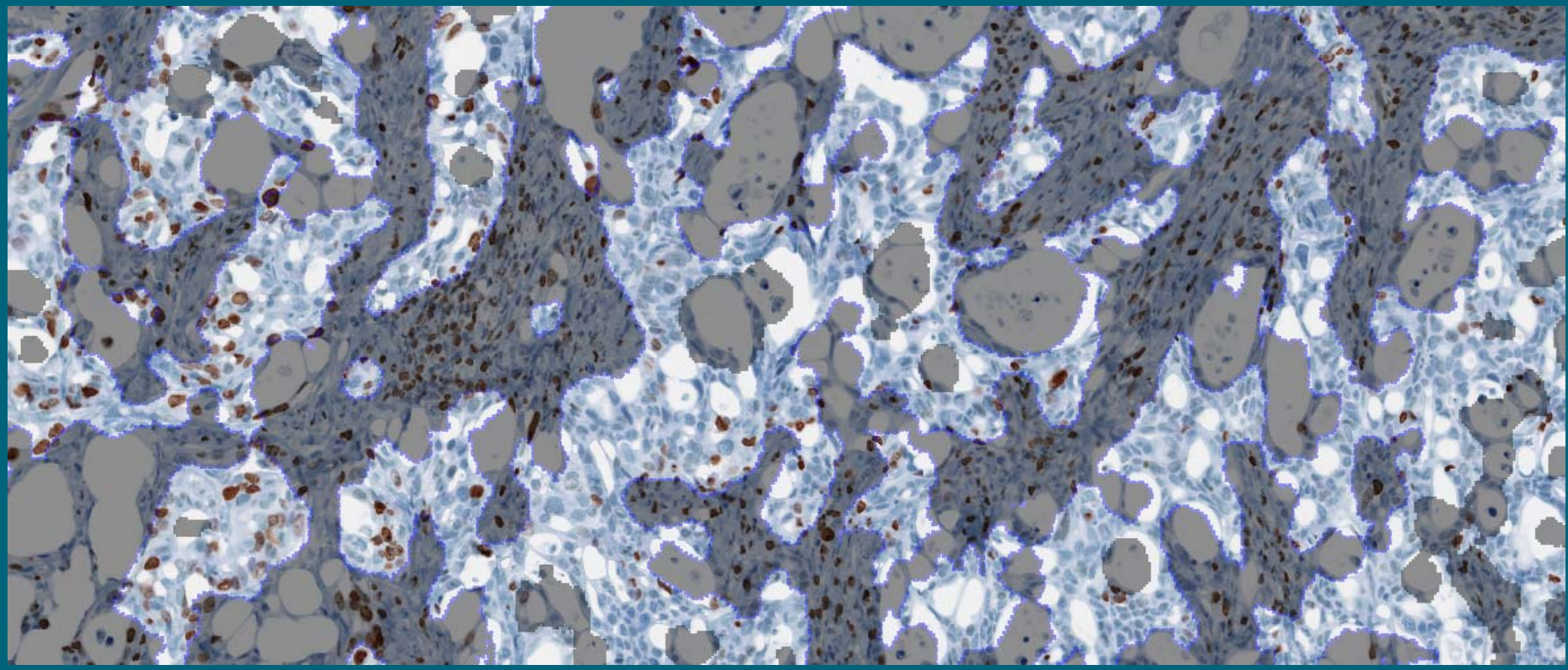
Discriminating between tumor cells and stromal cells is important for obtaining reliable data when reading nuclear marker indices – but cumbersome and prone to variation

Nuclear markers: Tumour cells vs. stromal cells



A solution: Using a ‘tumor marker’ (e.g. Pan-cytokeratin) combined with a high-precision alignment of two serial sections allow to determine tumor cells / regions . . .

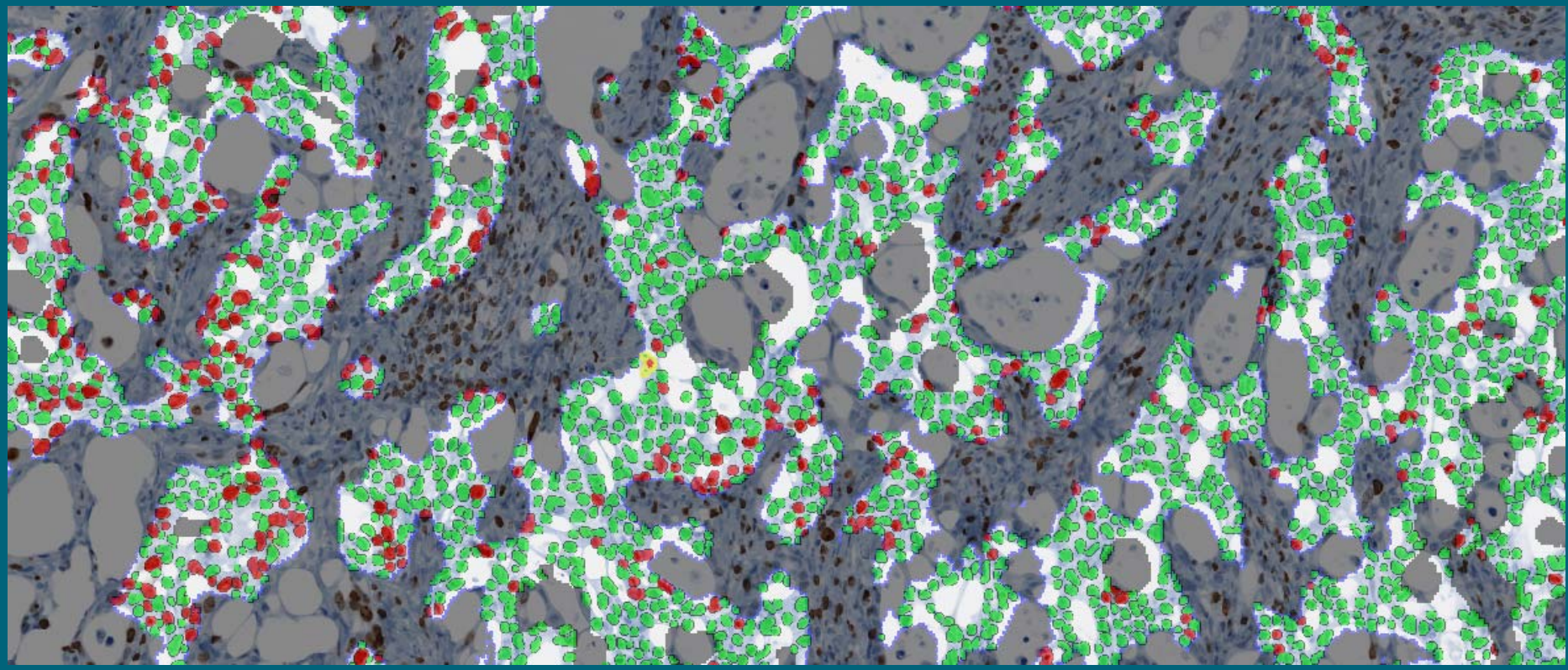
Nuclear markers: Tumour cells vs. stromal cells



… creating a mask which selects the region of interest . . .

NordiQC

Nuclear markers: Tumour cells vs. stromal cells



… allowing for a precise quantification of relevant nuclei

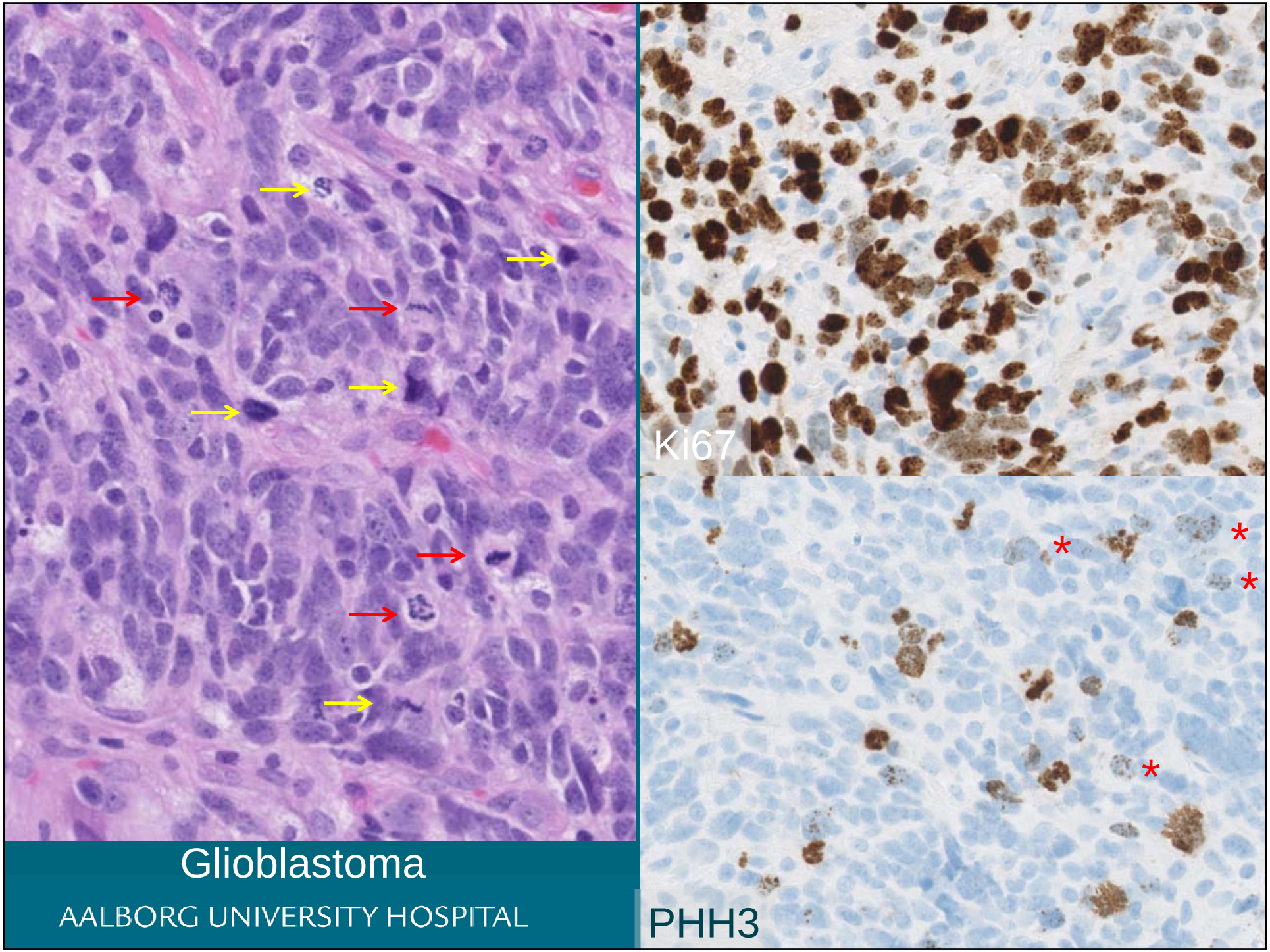
Mitotic activity and PHH3 labelling

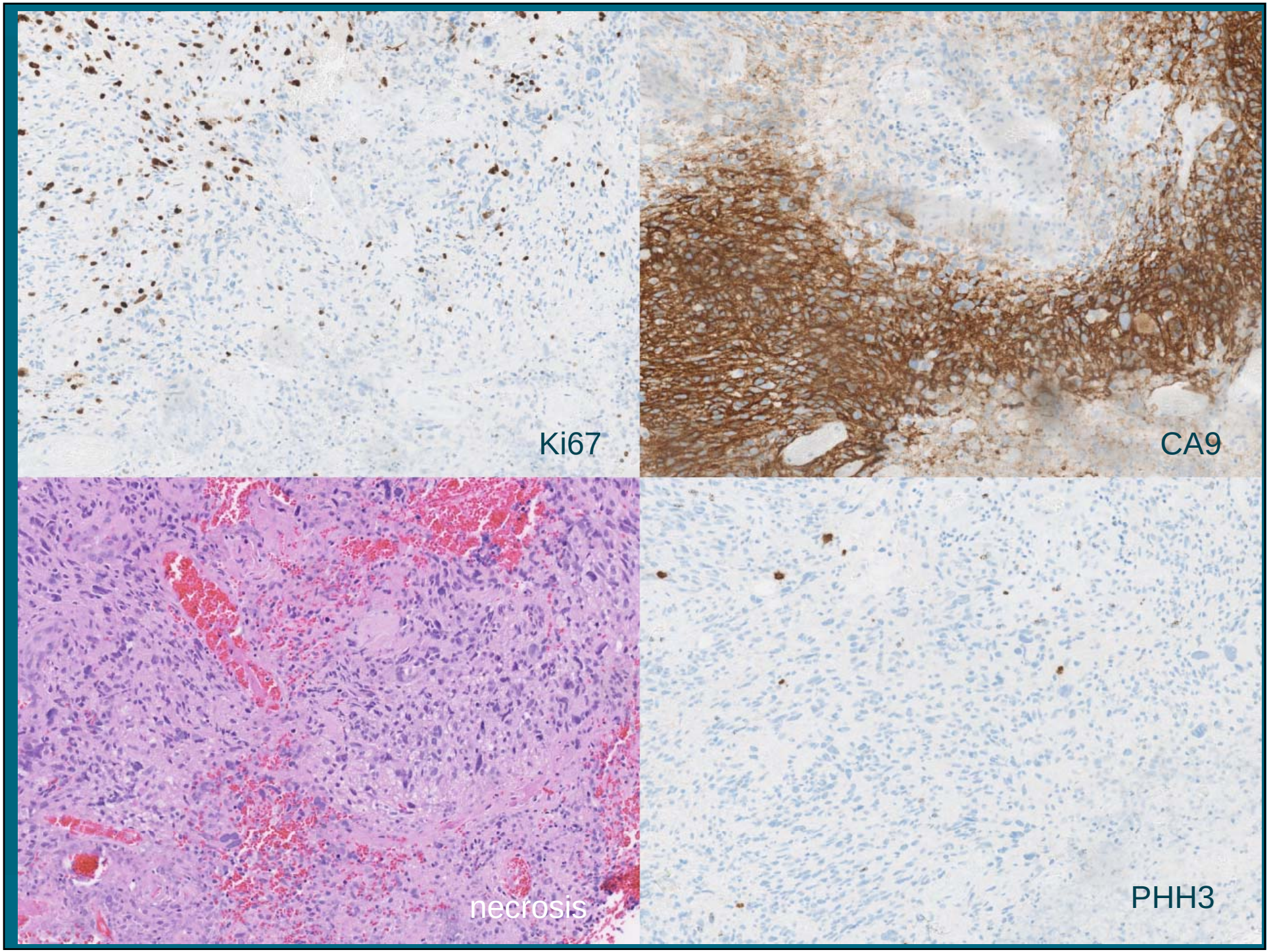
- Mitotic activity is important to assess malignancy grade
- Quantification of mitotic figures in H&E-stained sections is time-consuming and prone to inter-observer variability
- Mitotic index based on high power fields irrespective of cell number and size of HPF

<u>Phosphohistone H3 staining</u>

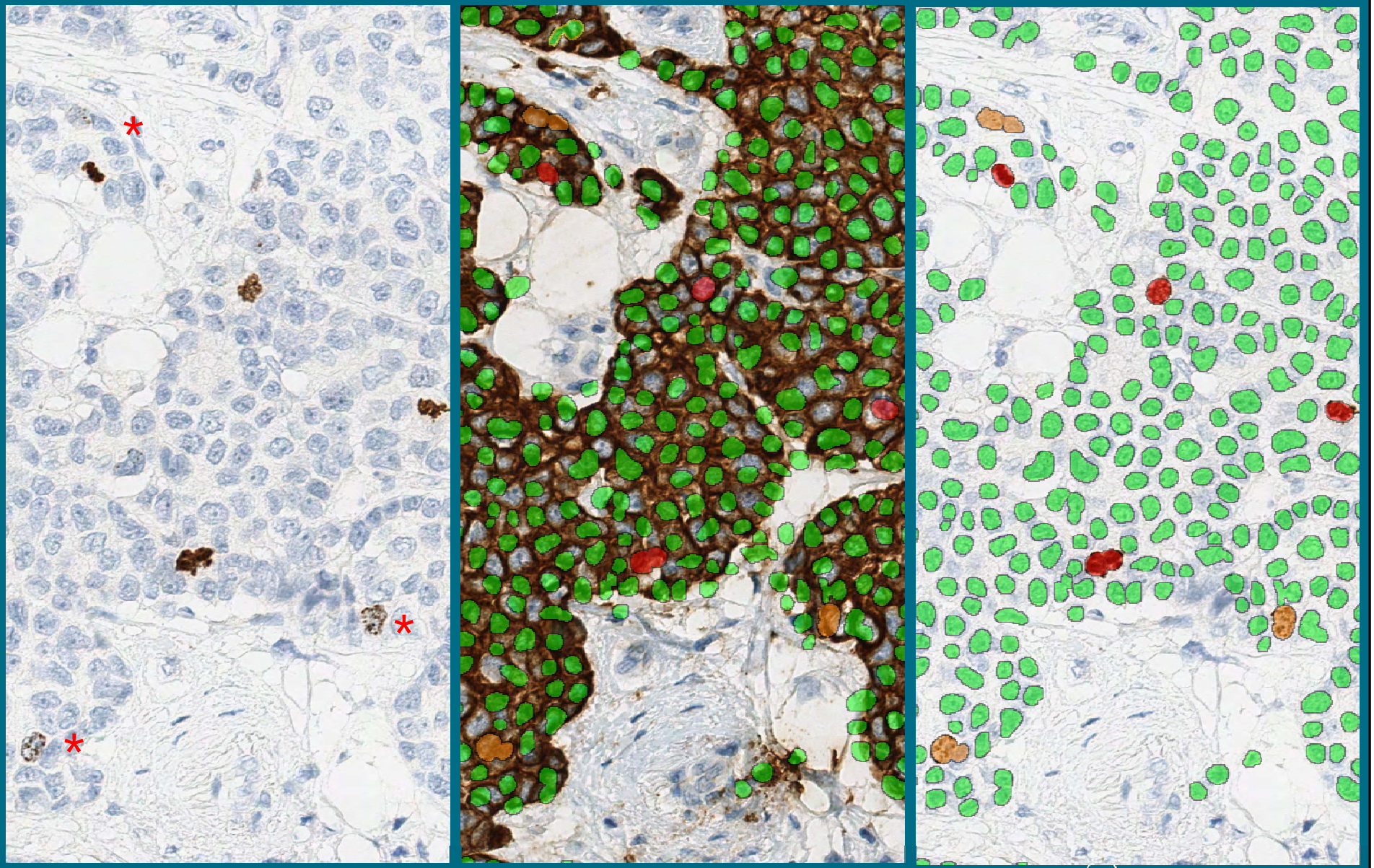
- PHH3 is highly correlated to mitotic phase and index
- PHH3 more easily identified visually
- PHH3 index can be determined by image analysis based on cell number

Bossard et al., J Clin Pathol. 2006, 59:706
Williams et al., AIMM 2011,19:431
Zbytek et al., AIMM 2012 PAP

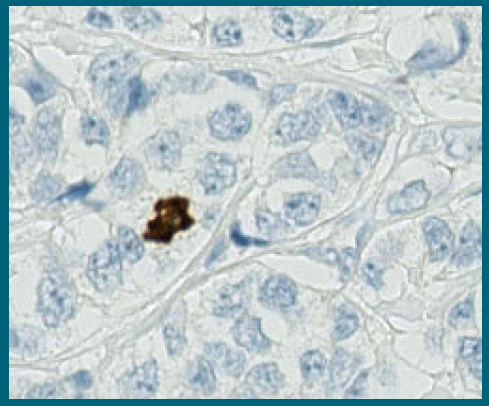

Ki67
PHH3
Glioblastoma
AALBORG UNIVERSITY HOSPITAL

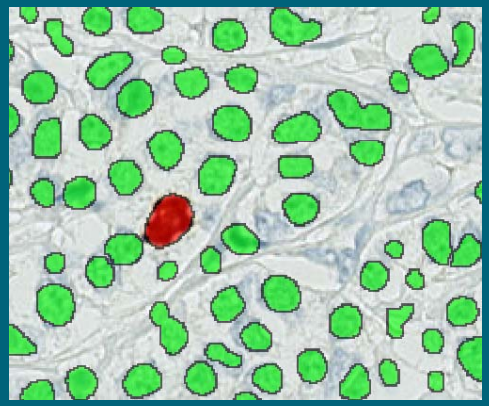

Ki67
CA9
necrosis
PHH3

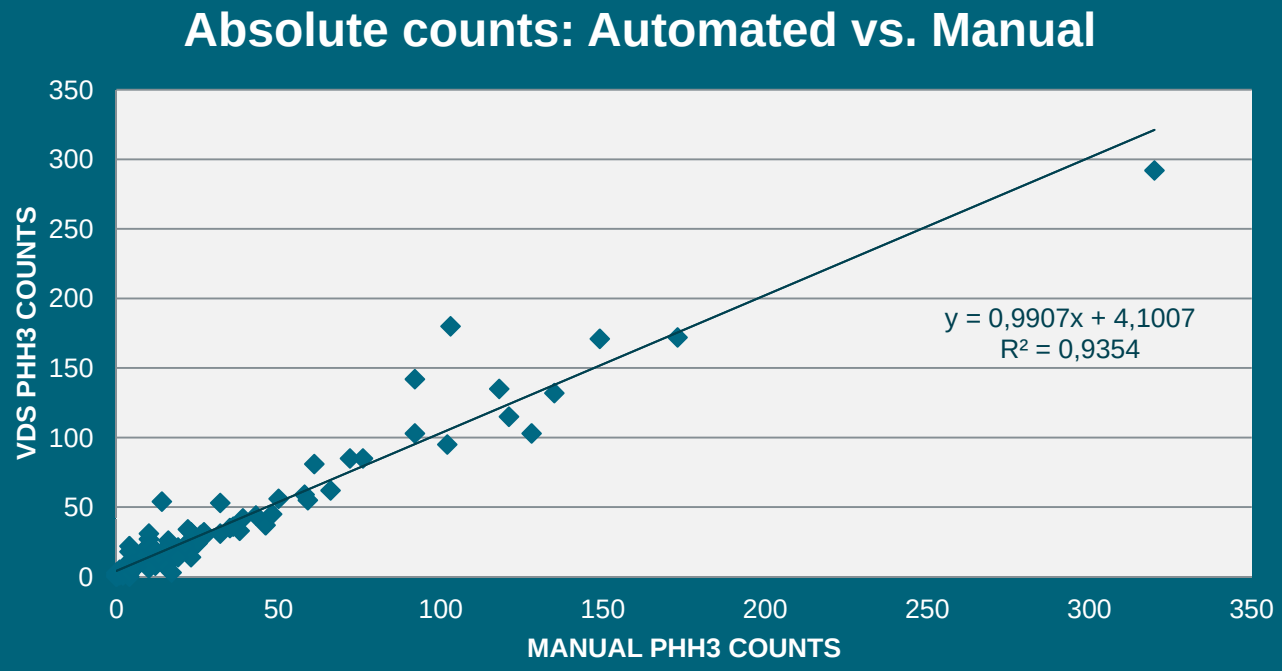
Phosphohistone H3 virtual double staining



PHH3: IA Concordance with manual counting



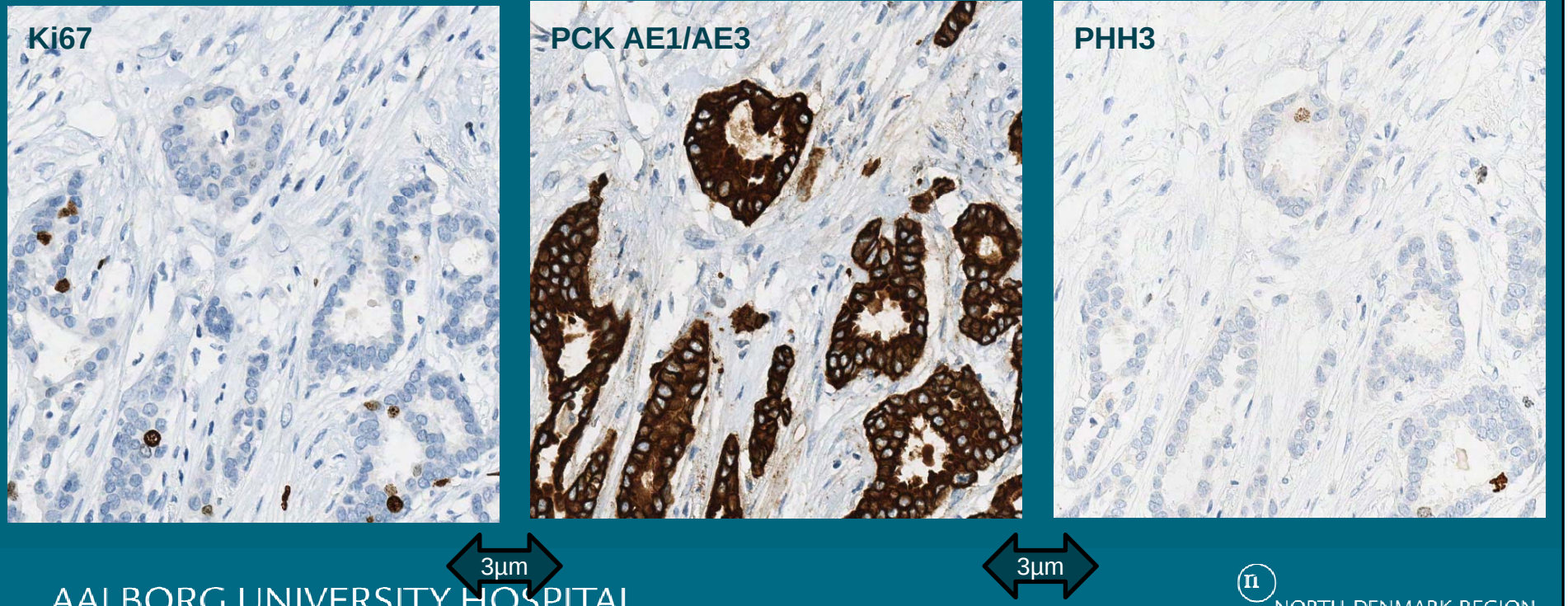




Average number tumour cells per TMA core = **6,368**
Average number of PHH3 positive cells per TMA core = **20**

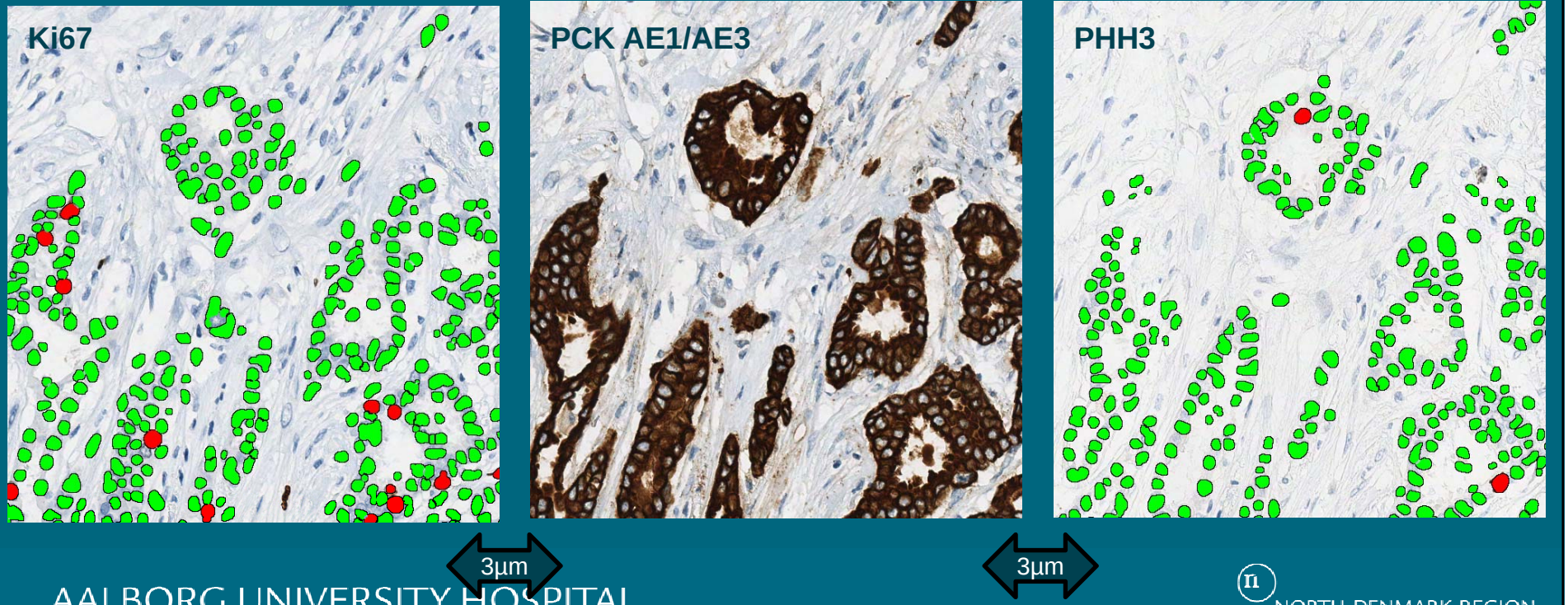
Ki67 vs. Phosphohistone H3

- Serial 3µm sections
- Stained with Ki67, PCK AE1/AE3, and PHH3 respectively
- Scanned using Aperio and Hamamatsu scanners
- Pairwise high-precision aligment between Ki67/PCK and PHH3/PCK

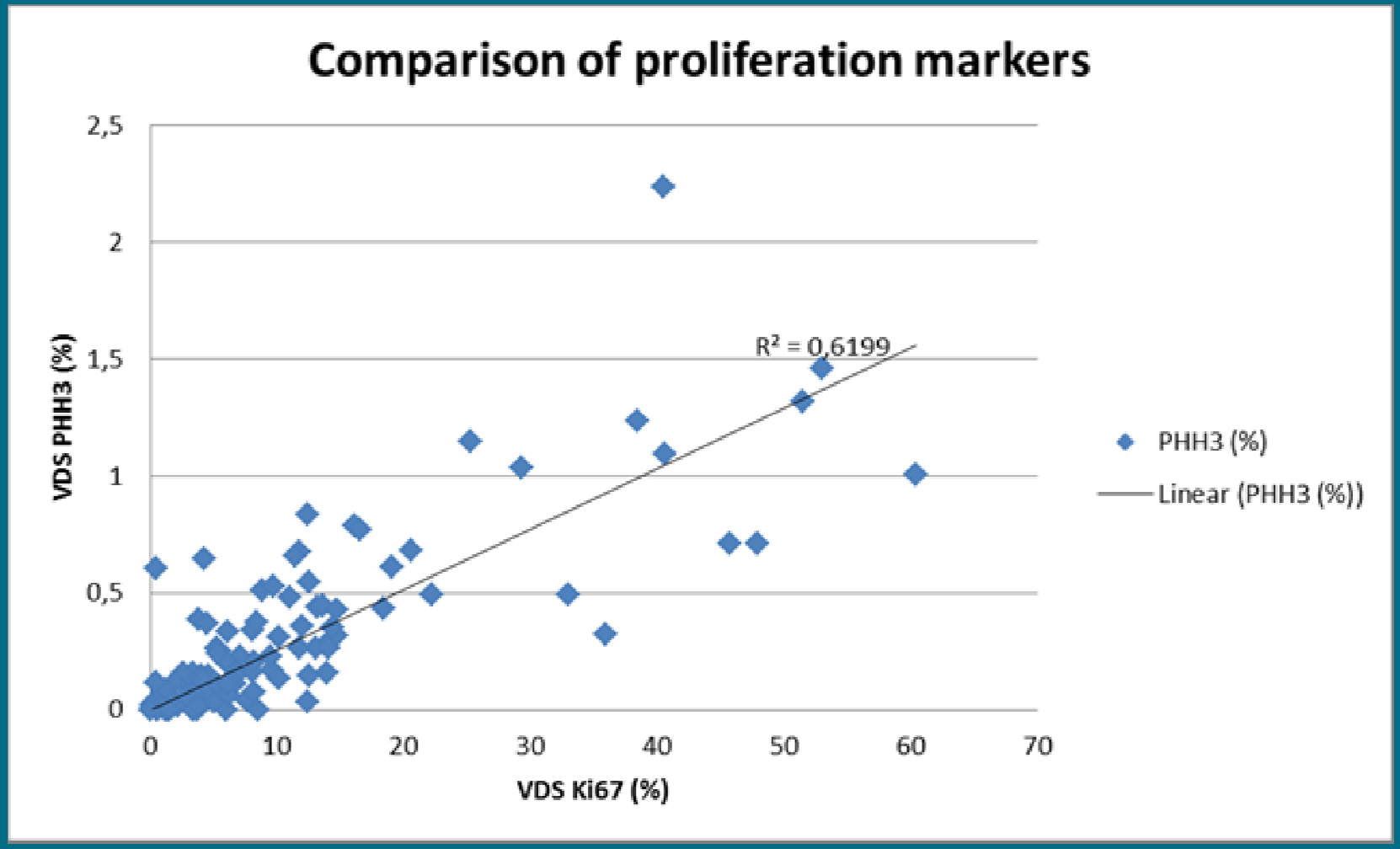


Ki67 vs. Phosphohistone H3

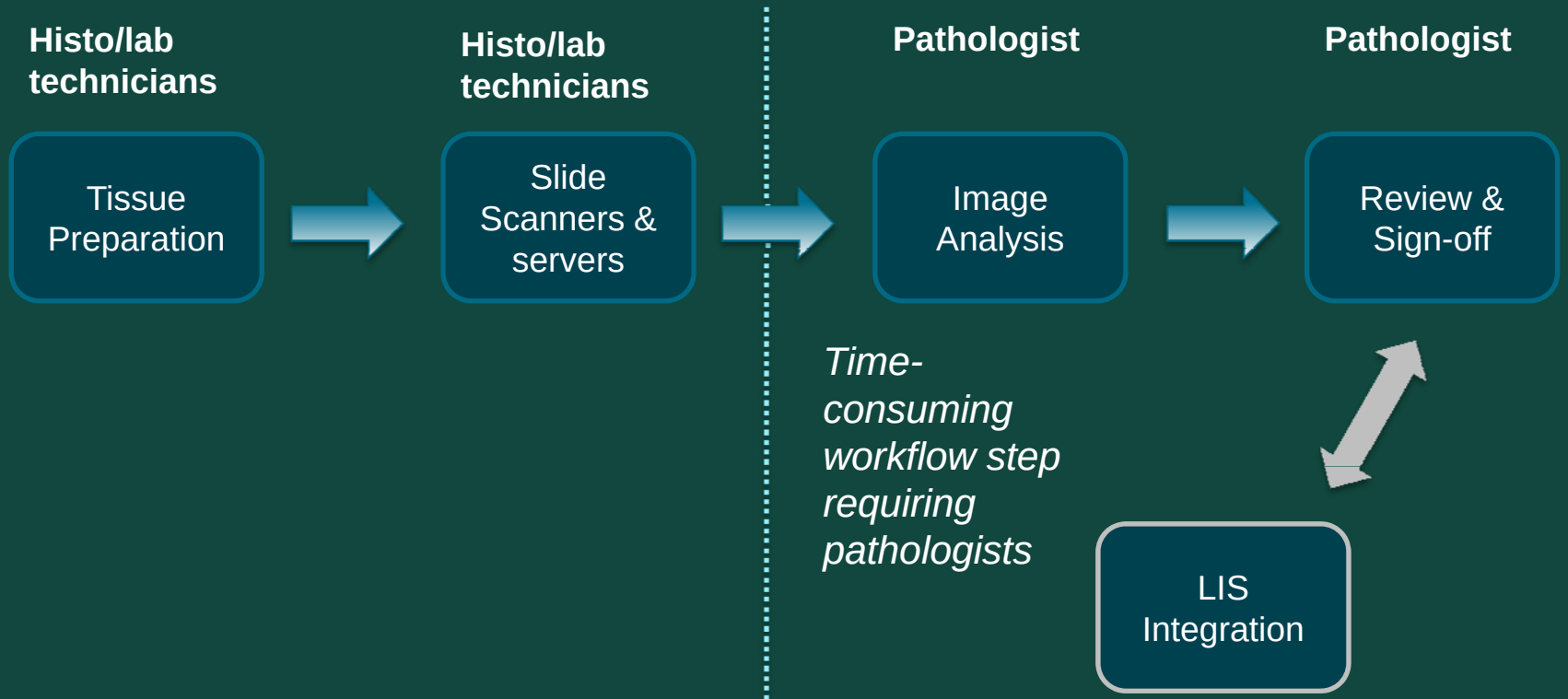
- Image analysis using Virtual Double Staining (VDS) Ki67 and PHH3 APPs
- Ki67/PHH3 positive and negative cells are identified, excluding all non-tumor cells based on the positivity of the tumour cell marker



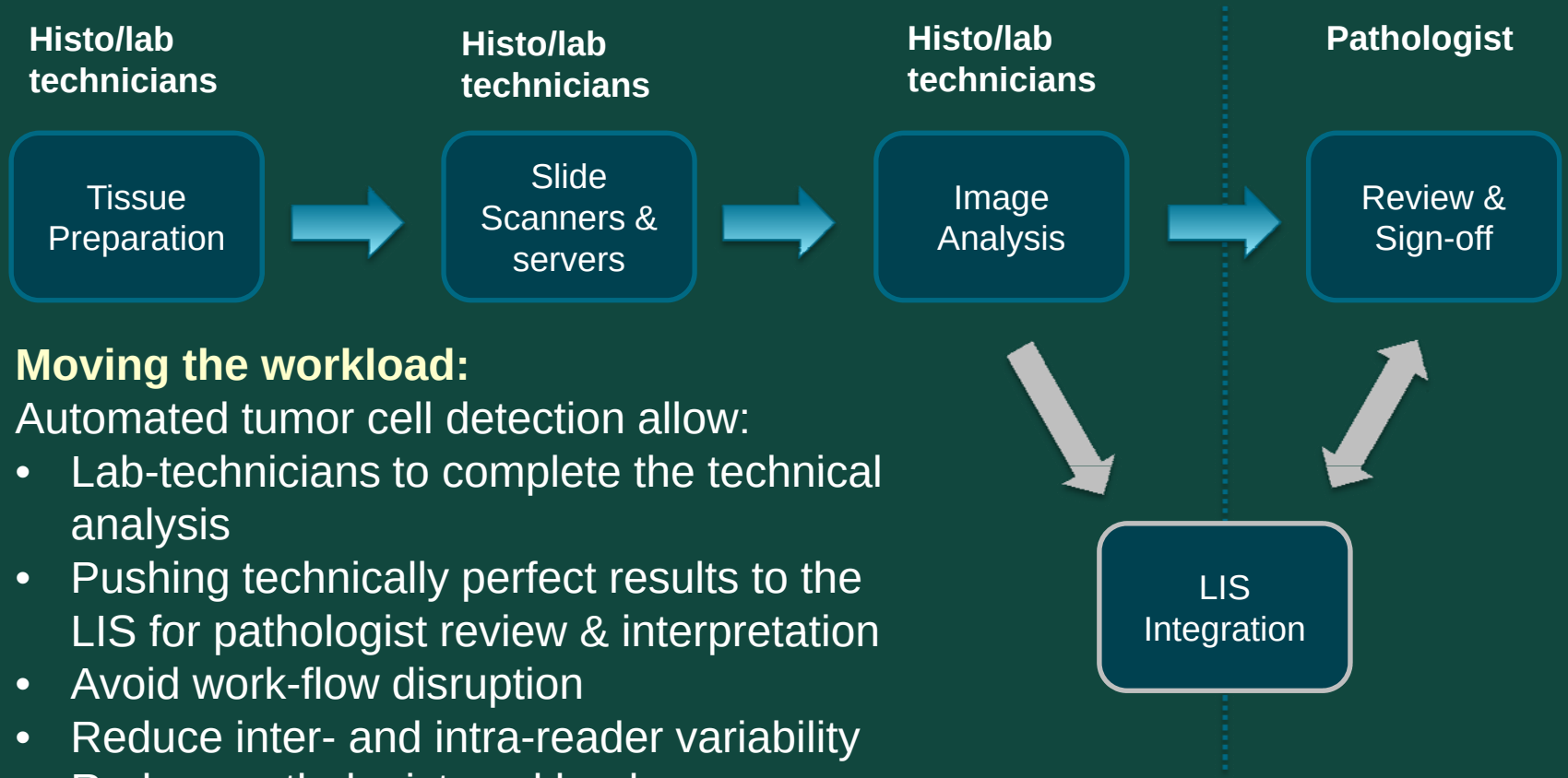
Ki67 vs. Phosphohistone H3



Digital Workflow ”Today”



Digital Workflow ”Tomorrow”



Moving the workload:

Automated tumor cell detection allow:

- Lab-technicians to complete the technical analysis
- Pushing technically perfect results to the LIS for pathologist review & interpretation
- Avoid work-flow disruption
- Reduce inter- and intra-reader variability
- Reduce pathologist workload

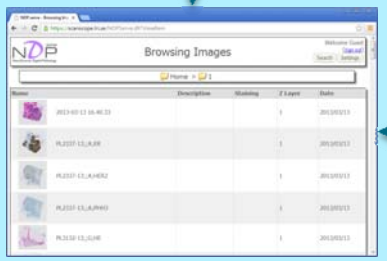
New standards in pathology

NordiQC

Fully integrated workflows

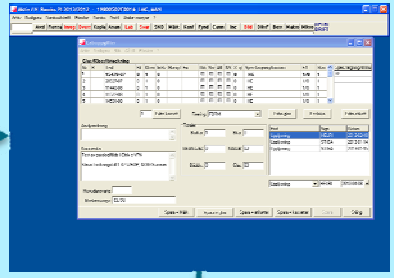
Scanning, storage & retrieval

Scanned slides to slide server



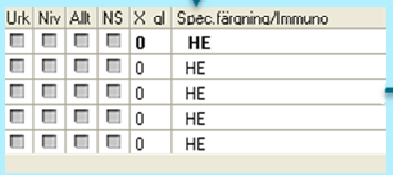
Histotech

Data management and LIS integration



Selecte slides for analysis

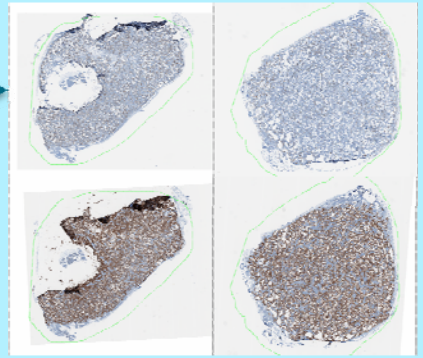
LIS communicates with Slide Server



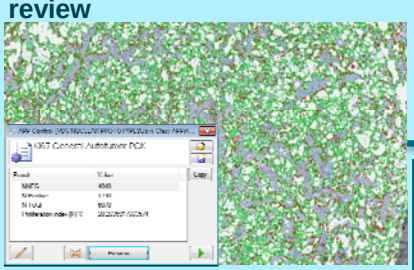
Select slides for analysis

Histotech

Image analysis & initial review



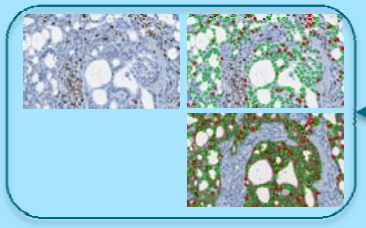
The analysis is started from LIS: Tumor detection, batch processing, preliminary review

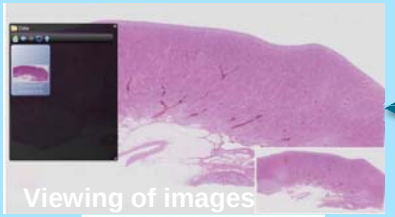


Histotech

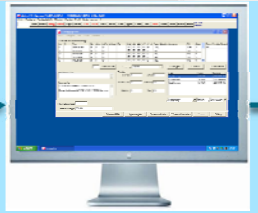
Pathologist review

Review of IA results





Push results to LIS



Select action in LIS

Pathologist