



# Next generation image analysis for immunohistochemistry quantitation

Ben Vainer  
Department of Pathology, Rigshospitalet  
University of Copenhagen Medical Center



Men are only so good as their technical developments allows them to be.

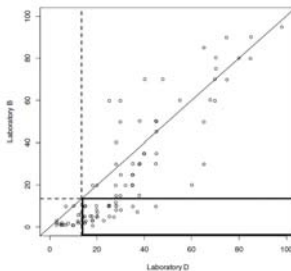
George Orwell



## An International Ki67 Reproducibility Study

Mai-Yin C. Polley, Samuel C. Y. Leung, Lisa M. McShane, Dongxia Gao, Judith C. Hugh, Mauro G. Mastropasqua, Giuseppe Viale, Lila A. Zabaglo, Frédérique Penault-Llorca, John M. S. Bartlett, Allen M. Gown, W. Fraser Symmans, Tammy Piper, Erika Mehl, Rebecca A. Enos, Daniel F. Hayes, Mitch Dowsett, Torsten O. Nielsen, on behalf of the International Ki67 in Breast Cancer Working Group of the Breast International Group and North American Breast Cancer Group  
J Natl Cancer Inst.2013;105:1897-1906

Inter-laboratory variation



Eight North American and European laboratories.

"At a hypothetical 13.5% cutoff, there are 32.3% cases that Laboratory D would call high Ki67 but Laboratory B would call low Ki67."

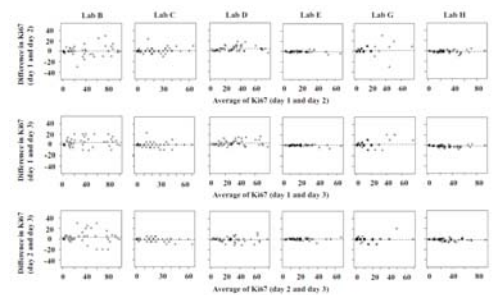
"Our data suggest that even if a common Ki67 cutoff is agreed upon, lack of interlaboratory reproducibility in Ki67 measurements represents a major obstacle to confident use of Ki67 for clinical decisions."



## An International Ki67 Reproducibility Study

Mai-Yin C. Polley, Samuel C. Y. Leung, Lisa M. McShane, Dongxia Gao, Judith C. Hugh, Mauro G. Mastropasqua, Giuseppe Viale, Lila A. Zabaglo, Frédérique Penault-Llorca, John M. S. Bartlett, Allen M. Gown, W. Fraser Symmans, Tammy Piper, Erika Mehl, Rebecca A. Enos, Daniel F. Hayes, Mitch Dowsett, Torsten O. Nielsen, on behalf of the International Ki67 in Breast Cancer Working Group of the Breast International Group and North American Breast Cancer Group  
J Natl Cancer Inst.2013;105:1897-1906

Intralaboratory variation



Same 50 breast cancer cases (two 1 mm-cores from each) assessed on three consecutive days.

Numerical differences.

**Rigshospitalet** FACULTY OF HEALTH AND MEDICAL SCIENCES UNIVERSITY OF COPENHAGEN

Pathobiology Original Paper

**An Intra- and Interobserver Reproducibility Analysis of the Ki-67 Proliferation Marker Assessment on Core Biopsies of Breast Cancer Patients and Its Potential Clinical Implications**

András Vörös<sup>a</sup> Erika Csörgő<sup>a</sup> Tibor Nyári<sup>b</sup> Gábor Cserni<sup>a,c</sup>

Three pathologists, using their own scoring method

Interval < 2 months

**Conclusion:** Although reproducibility is better in the clinically more relevant distinction of high versus low proliferation, without standardization, the current practice of Ki-67 assessment in many laboratories does not allow proper and consistent therapeutic decision-making.

2nd NordiQC Conference on Applied Immunohistochemistry Rigshospitalet, University of Copenhagen

**Table 2.**  $\kappa$  values reflecting intraobserver reproducibility of the classification of Ki-67 LIs into 4 or 3 categories

**a. Quarter categories**

	0-25%	26-50%	51-75%	76-100%	Overall
Observer 1	0.76	0.34	0.13	0.51	0.42
Observer 2	0.4	0.01	0	0.61	0.26
Observer 3	0.55	0.32	0.3	0.51	0.41

**b. Categories determined by cutoff values**

	<16%	16-30%	>30%	Overall
Observer 1	0.64	0.5	0.69	0.61
Observer 2	0.37	0.67	0.57	0.56
Observer 3	0.45	0.32	0.64	0.5

**Rigshospitalet** FACULTY OF HEALTH AND MEDICAL SCIENCES UNIVERSITY OF COPENHAGEN

**Laboratory variation - Ki67 in breast cancer**

Which counting method used?

100% Eyeballing, 12% Image analysis, 88% Manual counting

300 cells (...) accepted as the absolute minimum International Ki67 in Breast Cancer Working Group

Vyberg & Røge, NordiQC

2nd NordiQC Conference on Applied Immunohistochemistry

**Rigshospitalet** FACULTY OF HEALTH AND MEDICAL SCIENCES UNIVERSITY OF COPENHAGEN

**Explanations...?**

Counting method Experience

Vyberg & Røge, NordiQC

2nd NordiQC Conference on Applied Immunohistochemistry Rigshospitalet, University of Copenhagen

**Rigshospitalet** FACULTY OF HEALTH AND MEDICAL SCIENCES UNIVERSITY OF COPENHAGEN

**How can we improve the assessment?**

2nd NordiQC Conference on Applied Immunohistochemistry Rigshospitalet, University of Copenhagen

**Rigshospitalet** FACULTY OF HEALTH AND MEDICAL SCIENCES UNIVERSITY OF COPENHAGEN

### Histopathology

Comparison of the effect of different techniques for measurement of Ki67 proliferation on reproducibility and prognosis prediction accuracy in breast cancer

Elmar Gullingsgaard,<sup>1</sup> Inar Skaland,<sup>1</sup> Eivind A M Jansen,<sup>2</sup> Rune Smadland,<sup>2</sup> Zhiming Shao,<sup>3</sup> Anam Malikova,<sup>4</sup> Freja Voerhies,<sup>5</sup> & Jan P A Bank,<sup>1,4</sup>

<sup>1</sup>Department of Pathology, Rigshospitalet University Hospital, Copenhagen, Norway; <sup>2</sup>Oslo, Stange University Hospital, Stange, Norway; <sup>3</sup>Department of Breast Surgery, Fudan University Cancer Hospital, Shanghai, China; <sup>4</sup>Department of Pathology and Laboratory Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA and <sup>5</sup>Department of Epidemiology and Biostatistics, Vrije Universiteit Medical Center, Amsterdam, the Netherlands

**Table 2.** Agreement between the subjective Ki67 counts of the two pathologists. With a threshold of 15% Ki67 positivity, the therapeutic decision differs in 28 = 8 / 31/237 = 13% of cases.

%Ki67 Pathologist 1	%Ki67 pathologist 2		Total
	≤15	>15	
≤15	162	28	190
>15	3	44	47
<b>Total</b>	<b>165</b>	<b>72</b>	<b>237</b>

Visiopharm software

2nd NordIQ Conference on Applied Immunohistochemistry Rigshospitalet, University of Copenhagen

**Conclusion:** In node-negative breast cancer without adjuvant systemic treatment, Ki67% by DIA, but not subjective counts, is reproducible and prognostically strong. This casts serious doubt on therapeutic guidelines using subjective counts of Ki67.

**Rigshospitalet** FACULTY OF HEALTH AND MEDICAL SCIENCES UNIVERSITY OF COPENHAGEN

### Neuroendocrine tumours in the gastrointestinal tract

Cutoff-values in neuroendocrine tumours

- G1: <2 mitoses per HPF and Ki67 ≤2%
- G2: 2-20 mitoses per HPF and/or Ki67 2.1-20%
- G3: >20 mitoses per HPF and/or Ki67 >20%

- Mitosis count in 50 HPF (2 mm<sup>2</sup>)
- Ki67 proliferation index in hot spots (500-2000 cells)

ENETS Consensus Guidelines. Neuroendocrinology 2012; 95.

2nd NordIQ Conference on Applied Immunohistochemistry Rigshospitalet, University of Copenhagen

**Rigshospitalet** FACULTY OF HEALTH AND MEDICAL SCIENCES UNIVERSITY OF COPENHAGEN

### Grading of Neuroendocrine Tumors With Ki-67 Requires High-quality Assessment Practices

Sara Maria Remes, MSc,<sup>1</sup> Filipa J. Tuomimäki, MSc,<sup>1</sup> Heikki Heikin, MD, PhD,<sup>2</sup> Jorma Isola, MD, PhD,<sup>1</sup> and Johanna Arola, MD, PhD,<sup>3</sup>

(Am J Surg Pathol 2012;36:1359-1363)

Digital photos  
ImmuRatio software

Original proliferation indices (PI): From pathology reports  
Re-assessment PI: One "qualified" pathologist

2nd NordIQ Conference on Applied Immunohistochemistry Rigshospitalet, University of Copenhagen

**Rigshospitalet** FACULTY OF HEALTH AND MEDICAL SCIENCES UNIVERSITY OF COPENHAGEN

### Objective Quantification of the Ki67 Proliferative Index in Neuroendocrine Tumors of the Gastroenteropancreatic System

A Comparison of Digital Image Analysis With Manual Methods

Laura H. Tang, MD, PhD,<sup>1</sup> Mihai Gonen, PhD,<sup>1</sup> Cyrus Hebut, MD, PhD,<sup>2</sup> Irvin M. Modlin, MD, PhD,<sup>2</sup> and David S. Klimstra, MD<sup>3</sup>

(Am J Surg Pathol 2012;36:1761-1770)

22 cases from Memorial Sloan-Kettering Cancer Center

Eyeball-estimate (EE)  
Manual counting (MC) of 2000 cells (!)  
Digital image analysis (Aperio's QIA-algorithm)

Manual selection of hot-spots  
Manual exclusion of stained non-tumour cells

**TABLE 3. Incorrect Ki67 Assessment by EE and Resultant Tumor Grade Change (DIA as Standard)**

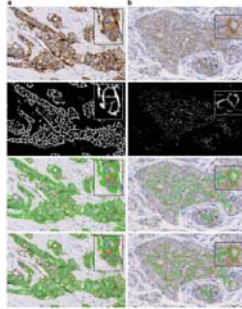
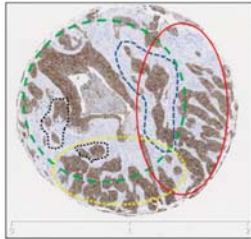
	%Ki67 by DIA	%Ki67 by EE
WHO G1 (n = 14)	1.9 ± 0.2	4.5 ± 2.0
WHO G2 (n = 27)	7.8 ± 1.0	13.7 ± 3.0
2.1%-2% (n = 11)	3.5 ± 0.2	1.0 ± 0.1
5.1%-20% (n = 14)	10.0 ± 1.0	22 ± 4.0

2nd NordIQ Conference on Applied Immunohistochemistry Rigshospitalet, University of Copenhagen

## HER2-CONNECT in breast cancer

Digital image analysis of membrane connectivity is a robust measure of HER2 immunostains

Anja Brügmann · Mikkel Eld · Giedrius Leikalis · Søren Nielsen · Michael Grunkin · Johan D. Hansen · Nick T. Foged · Mogens Vyborg  
Breast Cancer Res Treat 2012; 132: 41-9.



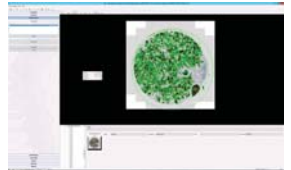
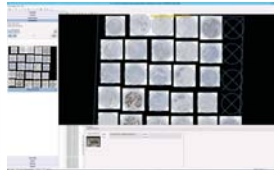
99.5 % konkordans med FISH

## The Rigshospitalet study

Holten-Rossing H, Talman MLM, Kristensson M, Vainer B: Optimizing HER2 assessment in breast cancer - application of automated image analysis. *Breast Cancer Research and Treatment* 2015



- 462 breast cancer samples
- TMA with automated dearraying
- Zeiss Axio Scan Z.1
- Visiopharm's cloud-based software

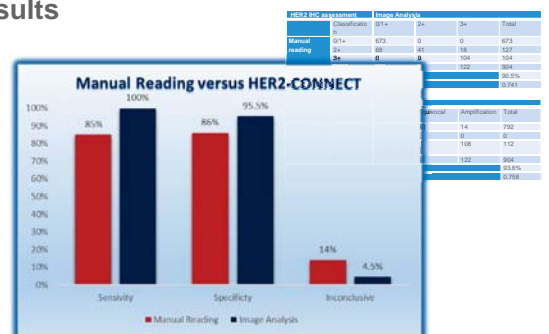


## Results

HER2 IHC assessment		Image Analysis			Total
Classification	0/1+	2+	3+		
Manual reading	0/1+	673	0	0	673
	2+	68	41	18	127
	3+	0	0	104	104
	Total	741	41	122	904
Agreement					90.5%
Cohen's kappa					0.741

HER2 FISH assessment		Image Analysis			Total
Classification	Negative	Equivocal	Amplification		
Manual Reading	Negative	738	40	14	792
	Equivocal	0	0	0	0
	Amplification	3	1	108	112
	Total	741	41	122	904
Agreement					93.6%
Cohen's kappa					0.758

## Results



67% reduced need for reflex testing (FISH)

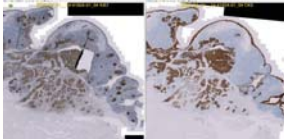




## Using automated image analysis to prognosticate HPV+ oropharyngeal carcinomas

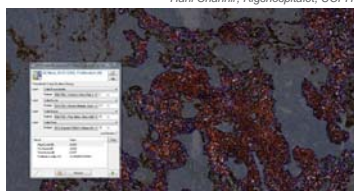
Virtual double staining with serial sections of CK5 and marker of interest (Visiopharm)

- Ki67
- PHH3
- PD-L1 (T-cell modulator)



Ki67

CK5



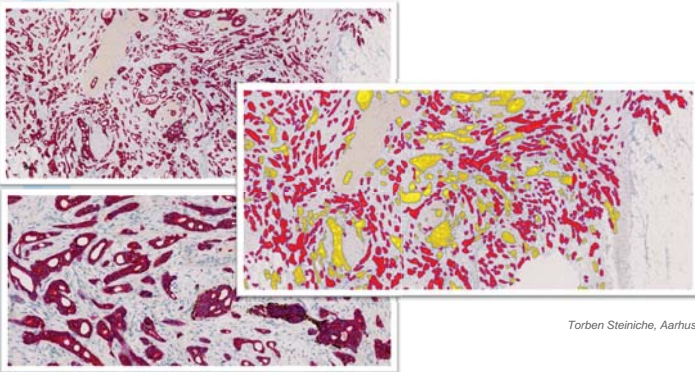
Hani Channir, Rigshospitalet, UCPH



## Challenges



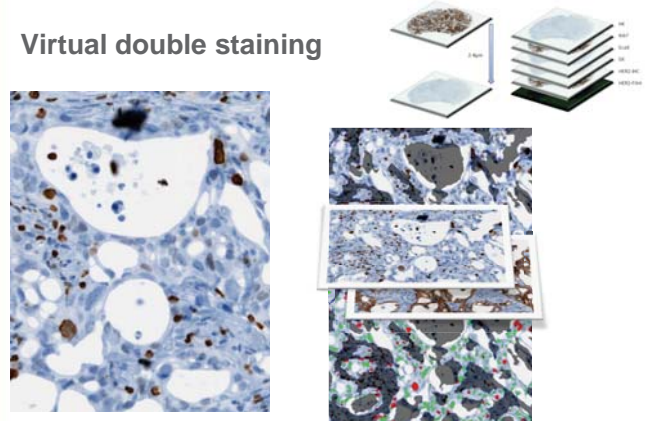
## Selection of invasive areas in breast cancer – automated elimination of DCIS



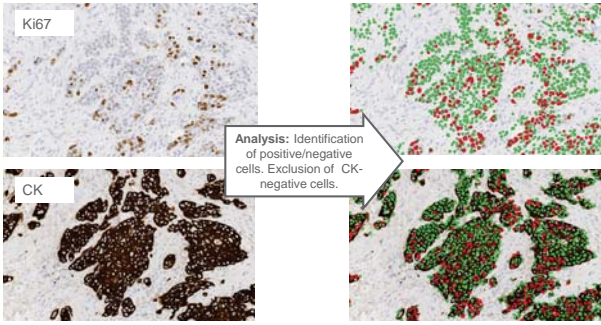
Torben Steiniche, Aarhus



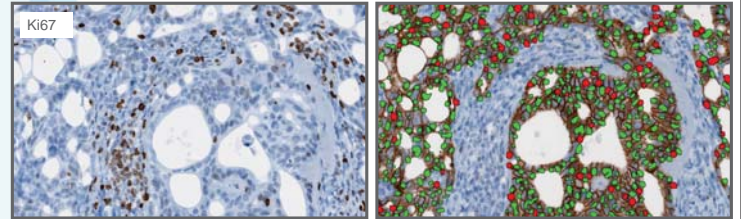
## Virtual double staining



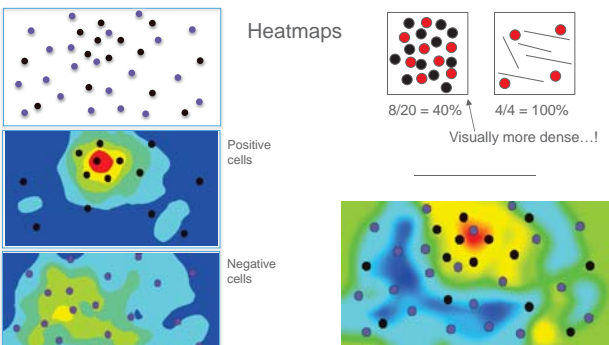
### Alignment and analysis



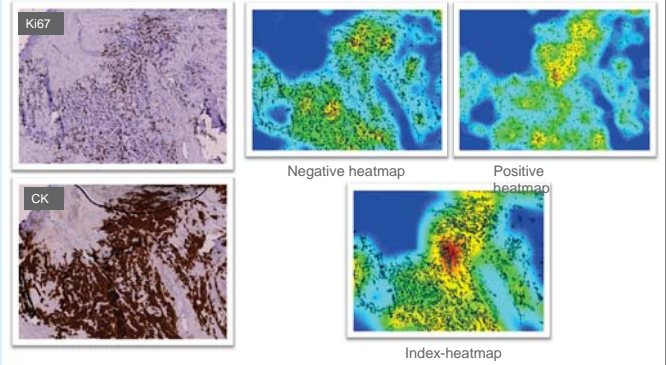
### Example: Ki67 in breast cancer

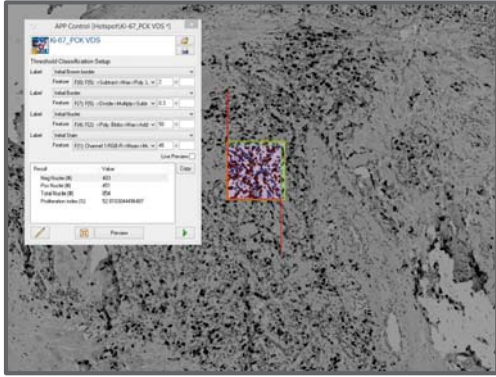


### Automated detection of hot spots (Visiopharm)



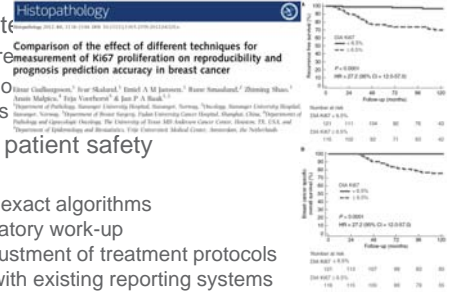
### Digital hot spot-detection – example (Visiopharm)





## Next generation immunoassessment - Why?

- Minimal intervention
  - Same reproducibility
  - Likely for high patient safety
- Increased patient safety
  - Correct and exact algorithms
  - Stable laboratory work-up
  - Possibly adjustment of treatment protocols
  - Integration with existing reporting systems and databases



*When the winds of change blows, some people build walls, while others build windmills.*

Chinese proverb