

# Membrane connectivity as a robust measure for the HER2 IHC score

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## Introduction

Several software algorithms for HER2 IHC scoring are already on the market, and some are approved by the regulatory authorities for diagnostic use. Still, there is room for improvement, when it comes to increasing the concordance with manual reading by experts. Also the workload associated with the use of software-based procedures should be further reduced.

## Objectives

The objective of the present study was to develop an automated software algorithm for HER2 IHC scoring with the following characteristics:

- 1) A high correlation to the manual readings by experienced assessors
- 2) A high specificity and sensitivity when compared to HER2 FISH
- 3) Robustness to:
  - Variations in staining by different FDA-approved HER2 IHC reagents
  - Variations in digital imaging by different whole slide scanners and cameras
  - Simple outlining of regions of interest (ROI)

## Materials and methods

### Clinical verification and validation study

**Table 1. HER2 IHC and FISH status of samples used in the verification and validation study**

Study	Pts	Cores	ROIs	HercepTest (Dako)				HER2 Pathway (Roche)						
				Digital Reading (5 assessors)				Digital Reading (5 assessors)						
				0/1+	2+	3+	Total	0/1+	2+	3+	Total			
Verification	45	84 (H) 83 (P)	420 (H) 415 (P)	FISH	Neg	209	45	11	265	Neg	220	32	8	260
					Pos	0	1	139	140	Pos	0	9	131	140
					N.A.	15	0	0	15	N.A.	15	0	0	15
					Total	224	46	150	420	Total	235	41	139	415
Validation	22	43 (H) 43 (P)	215(H) 215(P)	FISH	Neg	110	22	3	135	Neg	125	10	0	135
					Pos	0	7	73	80	Pos	0	5	75	80
					Total	110	29	76	215	Total	125	15	75	215

Patients: Women with ER-negative invasive breast cancer (72 consecutive cases)  
 Tissue: Formalin-fixed, paraffin-embedded breast tumor tissue  
 Sections: 2 TMA cores per patient (2 mm diameter, 4 µm serial sections)  
 IHC: H = HercepTest (Dako) and P = HER2 Pathway (Roche)  
 Scanning: Hamamatsu Nanozoomer HT (20x objective, single layer)  
 Scoring: Individual ROI outlining and digital reading on computer monitors by 5 expert assessors  
 FISH: HER2 FISH pharmDx (Dako) evaluated by fluorescence microscopy.

### The HER2 IHC algorithm

The Visiopharm HER2 IHC membrane connectivity algorithm includes:

### Pre-processing:

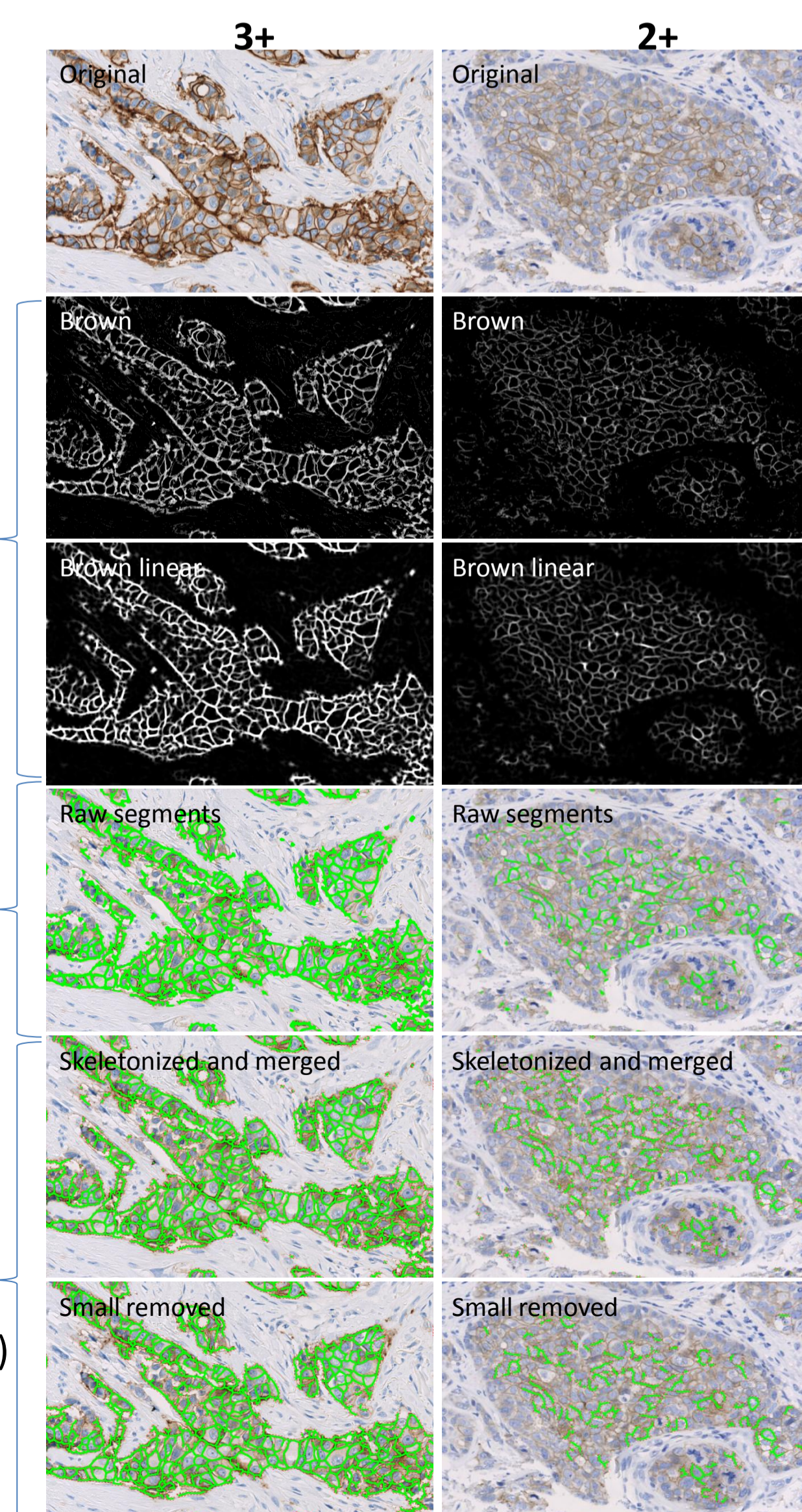
Identifies brown pixels in linear structures

### Segmentation:

Statistical rules define intensity of brown and dimensions of linearity to classify the relevant pixels.

### Post-processing:

Skeletonize the membrane, merge membrane segments, and eliminate small segments by a user-specified cut-off ( $A_{min}$ )



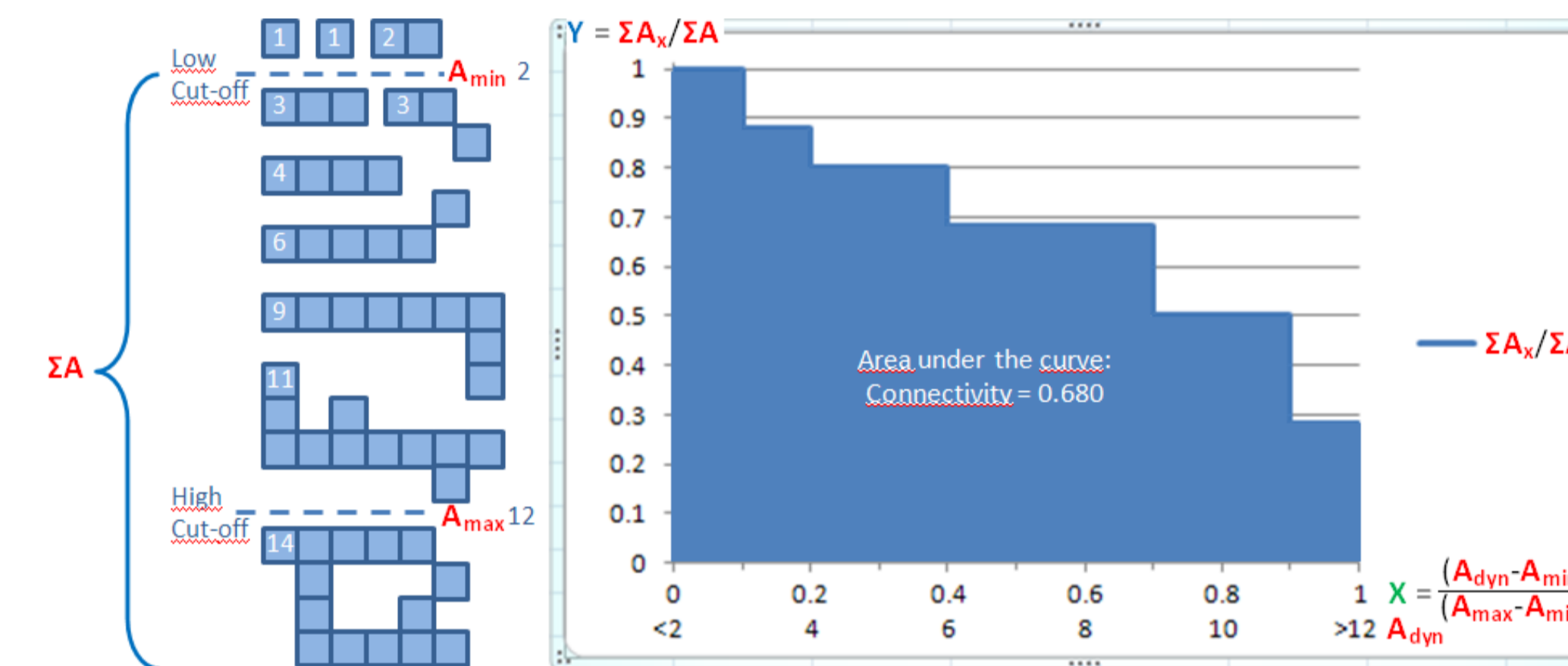
## Materials and methods (cont'd)

### Connectivity

The connectivity is calculated from the size distribution of all membrane segments remaining after post-processing. The size of a membrane segment is defined as the area ( $A$ ) of pixels composing its skeleton. The connectivity is the area under the curve in the following graphic representation:

$X = (A_{dyn} - A_{min}) / (A_{max} - A_{min})$ , where  $A_{max}$  is a user-specified cut-off value defining large membrane segments, and  $A_{dyn}$  is a dynamic cut-off varying between  $A_{min}$  and  $A_{max}$ , thereby ensuring  $X$ -values in the interval 0 to 1.

$Y = \sum A_x / \sum A$ , where  $\sum A$  is the total area of all membrane segments after post-processing, and  $\sum A_x$  is the total area of all membrane segments with an area higher than the variable  $A_{dyn}$ , thereby ensuring  $Y$ -values in the interval 0 to 1.



**Figure 1. Schematic example of the calculation of connectivity.**

Of the identified 10 skeletonized membrane segments, 3 segments are eliminated by the algorithm's post-processing as too small for inclusion (here:  $A_{min} = 2$ ). The total area of the remaining 7 segments is 50 ( $\sum A = 3+3+4+6+9+11+14$ ). A maximum cut-off for large segments is defined (here:  $A_{max} = 12$  pixels). The dynamic cut-off ( $A_{dyn}$ ) is a variable between 2 and 12, thereby defining  $X$  in the interval 0-1. The total area of membrane segments with a size above  $A_{dyn}$  is calculated for all  $x$ -values, and expressed relative to the total area of all relevant membrane segments. For example for  $A_{dyn} = 5$ :  $X = (5-2)/(12-2) = 0.3$ , and  $Y = (6+9+11+14)/50 = 0.8$ . The membrane connectivity is determined as the area under the curve for this plot:  $\int_0^1 (\sum A_x / \sum A) dx$

### Conversion of connectivity to classical HER2 score

For  $0.12 < \text{Connectivity} \leq 0.56$  HER2 IHC is 2+, and for  $0.56 < \text{Connectivity} \leq 1$  it is 3+.

## Brief discussion

The practical advantages of using a membrane connectivity measure, includes:

- No contribution by cytoplasmic staining in the tumor cells.
- No requirement for defining whether a stained membrane belongs to a particular cell or its neighbor cells in a dense region of invasive tumor.
- No contribution by stromal cells within the outlined ROI, thereby greatly facilitating its outlining.

However, quantification of connectivity of HER2-stained membrane deviates from the guideline of ASCO/CAP<sup>1</sup>, according to which the HER2 IHC score must be based on individual cell analysis. Thus, concordance studies are required to determine the applicability of the Visiopharm HER2 IHC algorithm.

## Results

### Concordance

**Table 2. Concordance results for HER2 IHC scoring by manual digital reading and image analysis**

Study	Pts	Cores	ROIs	HercepTest (Dako)				HER2 Pathway (Roche)						
				Digital Reading (5 assessors)				Digital Reading (5 assessors)						
				0/1+	2+	3+	Total	0/1+	2+	3+	Total			
Verification	45	84 (H) 83 (P)	420 (H) 415 (P)	Image analysis	0/1+	214	19	0	233	0/1+	221	16	0	237
					2+	10	26	2	38	2+	14	22	1	37
					3+	0	1	148	149	3+	0	3	138	139
					Total	224	46	150	420	Total	235	41	139	415
Validation	22	43 (H) 43 (P)	215 (H) 215 (P)	Image analysis	0/1+	110	10	0	120	0/1+	116	0	0	116
					2+	0	10	3	13	2+	9	14	1	24
					3+	0	9	73	82	3+	0	1	74	75
					Total	110	29	76	215	Total	125	15	75	215

### Verification study:

Percentage agreement: 92.1% (HercepTest: 92.4% and Pathway: 91.8%)  
 Cohen's kappa: 0.859 (HercepTest: 0.865 and Pathway: 0.852)  
 Specificity\* vs. FISH: 95.5%  
 Sensitivity\* vs. FISH: 100%

\*Excluding 70 of 805 samples: 8.7% (2+). According to principles in the ASCO/CAP guideline<sup>1</sup> and used by Dobson et al<sup>2</sup>.

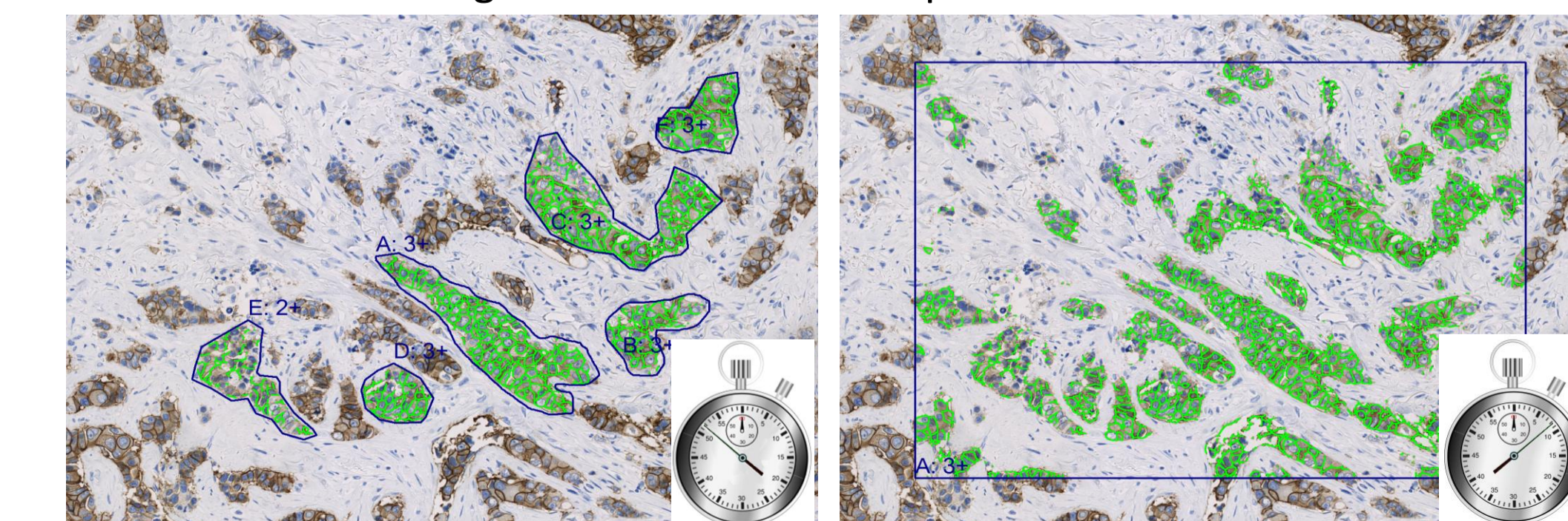
### Validation study:

Percentage agreement: 92.3% (HercepTest: 89.8% and Pathway: 94.9%)  
 Cohen's kappa: 0.864 (HercepTest: 0.821 and Pathway: 0.908)  
 Specificity\* vs. FISH: 99.2%  
 Sensitivity\* vs. FISH: 100%

\*Excluding 37 of 430 samples: 8.6% (2+). According to principles in the ASCO/CAP guideline<sup>1</sup> and used by Dobson et al<sup>2</sup>.

## Conclusions

The concordance to manual scorings by HER2 expert assessors was higher for the membrane connectivity-based Visiopharm HER2 IHC algorithm than for any previously reported image analysis method<sup>2</sup>. The algorithm worked for the two FDA-approved HER2 IHC staining methods, and is applicable to digital images acquired from almost any whole slide scanner and numerous microscope cameras. The outlining of ROIs is fast and simple.



Tumor outlining as required by classical HER2 IHC image analysis and ROI outlining as required by the Visiopharm HER2 IHC algorithm

## References

1. Wolff AC et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for HER2 testing in breast cancer. J Clin Oncol 25:118-45, 2007.
2. Dobson L et al. Image analysis as an adjunct to manual HER-2 immunohistochemical review: a diagnostic tool to standardize interpretation. Histopathology 57:27-38, 2010.

**Abstract**  
 The use of digital image analysis for HER2 IHC evaluation is encouraged by ASCO/CAP and reimbursement policies. By individually analyzing the invasive tumor cells and determining the ratio of invasive tumor cells with positive membrane staining, the commercially available classical HER2 IHC algorithms for image analysis are compatible with the scoring principles of the ASCO/CAP guidelines.  
 Several practical limitations and controversial aspects are associated with individual cell evaluation and counting. Whether by microscopy or digital reading on a monitor, manual scoring of HER2 IHC is therefore typically performed as an overall assessment of the degree of membrane staining within the invasive tumor region of interest (ROI). Counting principles are more readily implemented if based on image analysis, but the controversial aspects of individual cell evaluation remain. It is complicated for algorithms to identify individual invasive tumor cells, since their nucleus and/or cell membrane may not be clearly defined after HER2 IHC staining. Also, it is not obvious if a cell membrane belongs to a particular cell and/or adjacent cells in a dense region of invasive tumor. Furthermore, the classical algorithms and cell ratio quantification require a meticulous outlining of the ROI to avoid contributions from e.g., stroma and ductal carcinoma in situ.  
 In this study, we present a novel HER2 IHC algorithm, which uses principles that are not direct translations of the ASCO/CAP and reagent manufacturer's guidelines. Rather than estimating cell ratio, the present algorithm exploits the unique capacity of computerized image analysis to quantify the characteristic "chicken wire" pattern of HER2 IHC by measuring the connectivity and size distribution of stained membranes. The validation study included 430 ROI's easily outlined in 86 scanned images of 43 invasive breast carcinoma specimens stained by HercepTest and Pathway HER2/neu, and showed a 92.3% agreement with independent manual reading by 5 experienced assessors.