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 workflow 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 software and validation study

<table>
<thead>
<tr>
<th>Study</th>
<th>HER2 IHC</th>
<th>FISH status of samples used in the validation and evaluation study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verify</td>
<td>45</td>
<td>15 (2+), 22 (3+), 8 (4+)</td>
</tr>
<tr>
<td>Validate</td>
<td>22</td>
<td>8 (2+), 14 (3+)</td>
</tr>
</tbody>
</table>

Materials and methods
The Visiopharm HER2 IHC membrane connectivity algorithm includes:

Pre-processing:
- Identifying brown pixels in linear structures

Segmentation:
- Statistical rules define intensity of brown and dimensional criteria to classify the relevant pixels

Post-processing:
- Statistical rules for membrane, morphological segments, and eliminate small segments by a user-specified cut-off ($A_{\text{min}}$)

Results

<table>
<thead>
<tr>
<th>Concordance</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verification</td>
<td>89.8%</td>
</tr>
<tr>
<td>Validation</td>
<td>94.9%</td>
</tr>
</tbody>
</table>

Conclusion
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. The algorithm worked for the two FDA-approved HER2 IHC staining methods, and is applicable to digital images acquired by all types of digital microscopes and numerous microscopic cameras. The output of ROIs is fast and simple.