Automatic quantification of HER2 amplification in invasive breast cancer using CISH and computational pathology

Hossain Md Shakhawat\textsuperscript{1,2}, Tomoya Nakamura\textsuperscript{1}, Matthew Hanna\textsuperscript{2}, Noahiro Uraoka\textsuperscript{2}, Dara S. Ross\textsuperscript{2}, Meera R. Hameed\textsuperscript{2}, Masahiro Yamaguchi\textsuperscript{1}, Yukako Yagi\textsuperscript{2}

1. Tokyo Institute of Technology, Japan
2. Memorial Sloan Kettering Cancer Center, USA
Background

- **15-20% of invasive breast cancers (IBC) are HER2+** due to *ERBB2* gene amplification and subsequent HER2 protein overexpression.

- Targeted therapy like HERCEPTIN/Trastuzumab enables effective diagnosis for HER2+ patients by targeting the **HER2 overamplification**.

**HER2 is routine test for all IBC patients to decide on the prognosis & treatment.**
Methods for HER2 Test

- In practice, IHC is test performed firstly.
- But IHC test is not conclusive for all cases

ASCO/CAP 2018 guide:
If IHC status 2+ => perform ISH

Primary focus

Immunohistochemistry (IHC)

Fluorescence ISH (FISH)
Chromogenic ISH (CISH)
## FISH vs CISH

<table>
<thead>
<tr>
<th></th>
<th>FISH</th>
<th>CISH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Technology</strong></td>
<td>Fluorescence</td>
<td>Bright field</td>
</tr>
<tr>
<td><strong>Setup</strong></td>
<td>FISH Setup: dark room</td>
<td>CISH Setup: usual work place</td>
</tr>
<tr>
<td><strong>Requirement</strong></td>
<td>Special training &amp; setup</td>
<td>No training &amp; usual setup</td>
</tr>
<tr>
<td><strong>Cost of dye</strong></td>
<td>Expensive</td>
<td>Cheap</td>
</tr>
<tr>
<td><strong>Archive sample</strong></td>
<td>Cant; signal fades quickly</td>
<td>Archive for long period</td>
</tr>
<tr>
<td><strong>Morphological assessment</strong></td>
<td>Not possible</td>
<td>Possible</td>
</tr>
<tr>
<td><strong>Quantification method</strong></td>
<td>Manual/ Semi-automated/automated</td>
<td>Manual</td>
</tr>
</tbody>
</table>

**Propose:** To develop an automated system that can quantify the CISH whole slide images to determine the HER2 amplification status.

- Digital image processing
- Artificial intelligence

Picture: <https://bionanolab.ca/laboratory/facilities>
Proposed system

- System relies on breast pathologists to annotate tumors on H&E WSI
- Proposed method evaluates the regions automatically and determine HER2 status
Nuclei, HER2 and CEP17 Detection

1. Separate Background
2. Remove Noise
3. Partition Joints
4. Score & Select

Nuclei and signals are detected in 4 phases:

1. Separate background: Color unmixing
2. Remove outliers: Hue and intensity of HSV color space
3. Partition: Distance transform + watershed
4. Score & select: nuclei \( \Rightarrow \) shape features & HER2 and CEP17 \( \Rightarrow \) likelihood score

**Machine learning selected singular nuclei from candidates**

- SVM detected *untruncated and non-overlapped nuclei* using 9 shape features
- Features were selected using the sequential feature selection method
- Accuracy: 90% in 4-fold cross validation (average)
Counted HER2 and CEP17 signals for each singular nuclei, if a signal falls inside the boundary of a nucleus

Sort nuclei based on HER2-CEP17 difference value and select top 20 nuclei with highest difference

Estimate HER2/CEP17 ratio and average HER2 copy per nuclei from the selected nuclei

Then the HER2 status is determined based on ASCO/CAP 2018 guideline

Additionally another 20 nuclei are quantified for the equivocal cases
Result: Sample Quantified Image

MEMORIAL HOSPITAL FOR CANCER & ALLIED DISEASES
DEPARTMENT OF PATHOLOGY
2273 YORK AVENUE, NEW YORK, NY 10029
TEL: (212) 935-5858 FAX: (212) 935-5870
SURGICAL PATHOLOGY REPORT

DIAGNOSTIC INTERPRETATION

HER2/NEU GENE STATUS: AMPLIFICATION IS DETECTED (see note)
HER2/CEP17 ratio: 3.5227, Average HER2 copy number: 7.581

Note:
The HER2 status is interpreted as amplified, based on the combined presence of HER2/CEP17 ratio >2.0 and average HER2 copy number > 4.0 signals per cell. Correlation with the corresponding H&E and REPORTED HER2 immunohistochemically stains has been performed.

Test and Methodology:
Chromogenic in situ Hybridization (CISH) is performed using FDA-approved HER2 and CEP17 probes. Assessment of HER2 (ERBB2) status by chromogenic in situ hybridization (CISH) is performed using the HER2 and CEP17 probes. This is an FDA-approved method for assessment of HER2 gene amplification in breast and gastric cancer specimens. The HER2 test results are reported in accordance with the ASCO/CAP guideline recommendations for HER2 testing in breast cancer.

Laboratory Notes:
Part: 1
Specimen type: Formalin
Adequate number of invasive tumor cells present: 125
Observer: Shimarin, PACQ
Number of invasive tumor cells counted: 125
Average number of HER2 probe signals per nucleus: 7.381
Average number of CEP17 probe signals per nucleus: 2.6852
Average HER2/CEP17 ratio: 3.5227

Diagnostic Report

Shimarin: Pathologist's Assistant for Automatic CISH Quantification
Result: Evaluation of Proposed Method

- 96.5% correlation with FISH and 98.5% with manual CISH in terms of HER2 ratio
- 92% correlation with FISH and 88.5% with manual CISH in terms of HER2 copy number
- Quantified 22 cases, selected randomly. 12 positive and 10 negative
- In our experiment, we quantified cases with all types of IHC status to ensure efficacy
Summary and Future Works

Advantages
+ Proposed quantification saves time and reduce labor
+ It allows to assess more nuclei
+ Allows to test HER2 status for all cases regardless its IHC status as it is cheaper than FISH.

Limitations
- Depends on manual tumor annotation by pathologists
- Poor quality image could affect the quantification result
- Necessary to evaluate more cases

Tumor detection using CNN (convoloutional Neural Network) to automate the annotation
Automatic image quality evaluation before quantification
Evaluate at least 40 cases for clinical validation using CAP guidelines
Questions and Suggestions?