The correlation between a manual count and an automated system of estimation of Ki67 in breast cancers in Danbury Hospital

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Introduction

- Immunohistochemical characterization of breast cancers with biomarkers including Ki67 are required for contemporary oncologic care.

- Manual estimation of Ki67 expression in breast cancer by Pathologists may be prone to intra- and inter-observer variability.

- Automated methods minimize variability and time spent with manual estimation with the hypothesis that correlation between a manual estimate and automated count is acceptable.

Materials and methods

- Breast cancer cases diagnosed between March, 2020 and August, 2021 were retrospectively reviewed.
- We randomly selected 40 biopsy cases.
- The H&E and Ki67 slides of the selected cases were retrieved from the archives.
- Images of the Ki67 slides were taken with Olympus camera (U-TV0.63XC).
- The score was estimated by two residents and subsequently, a scoring application (QuPath, 0.2.3) was used on the same images.

Materials and methods

- Manual estimation was calculated as the average of the scores from the two residents.

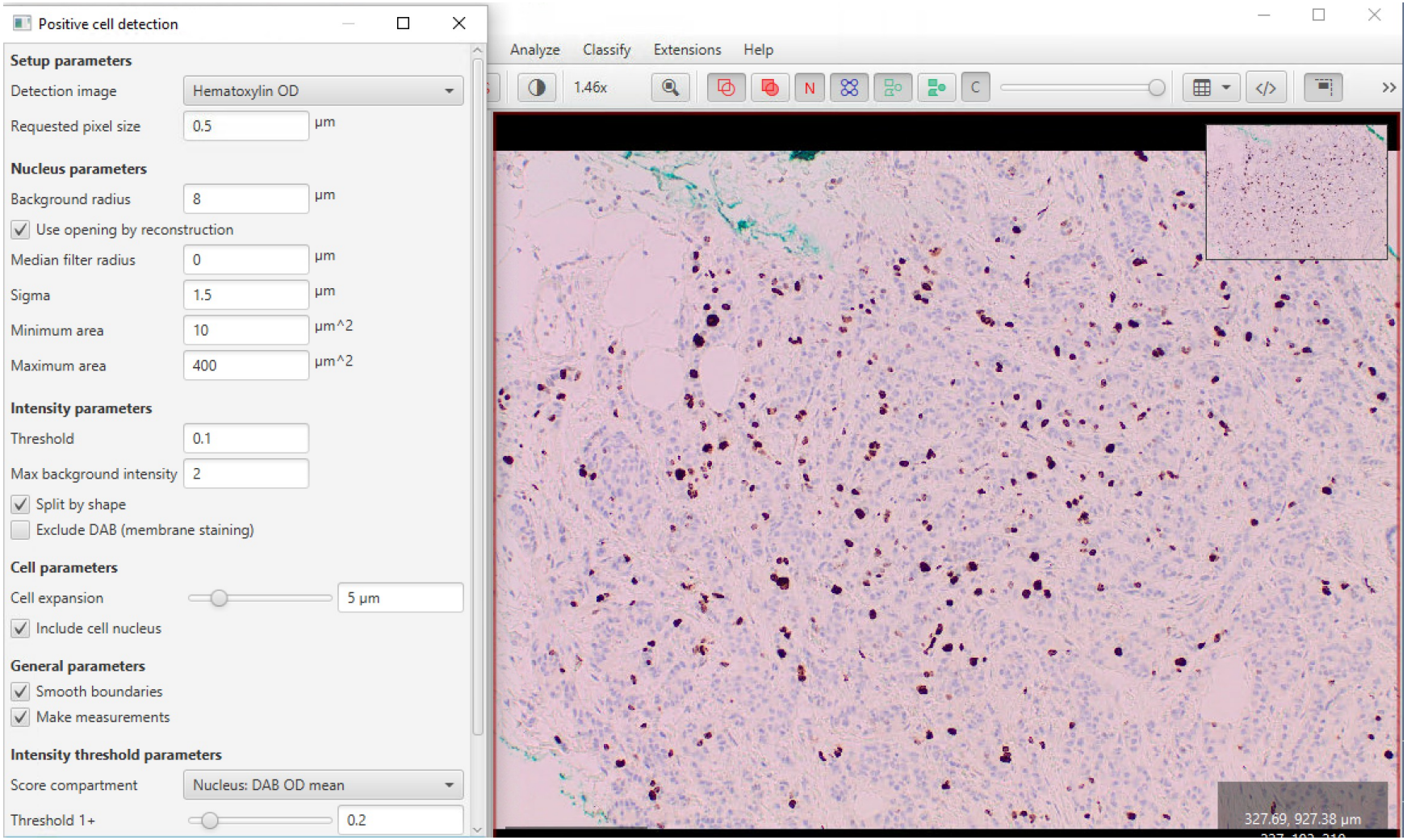
- The QuPath application settings were made to minimize interference of tumor Ki67 staining estimation by stromal and inflammatory cell Ki67 staining.

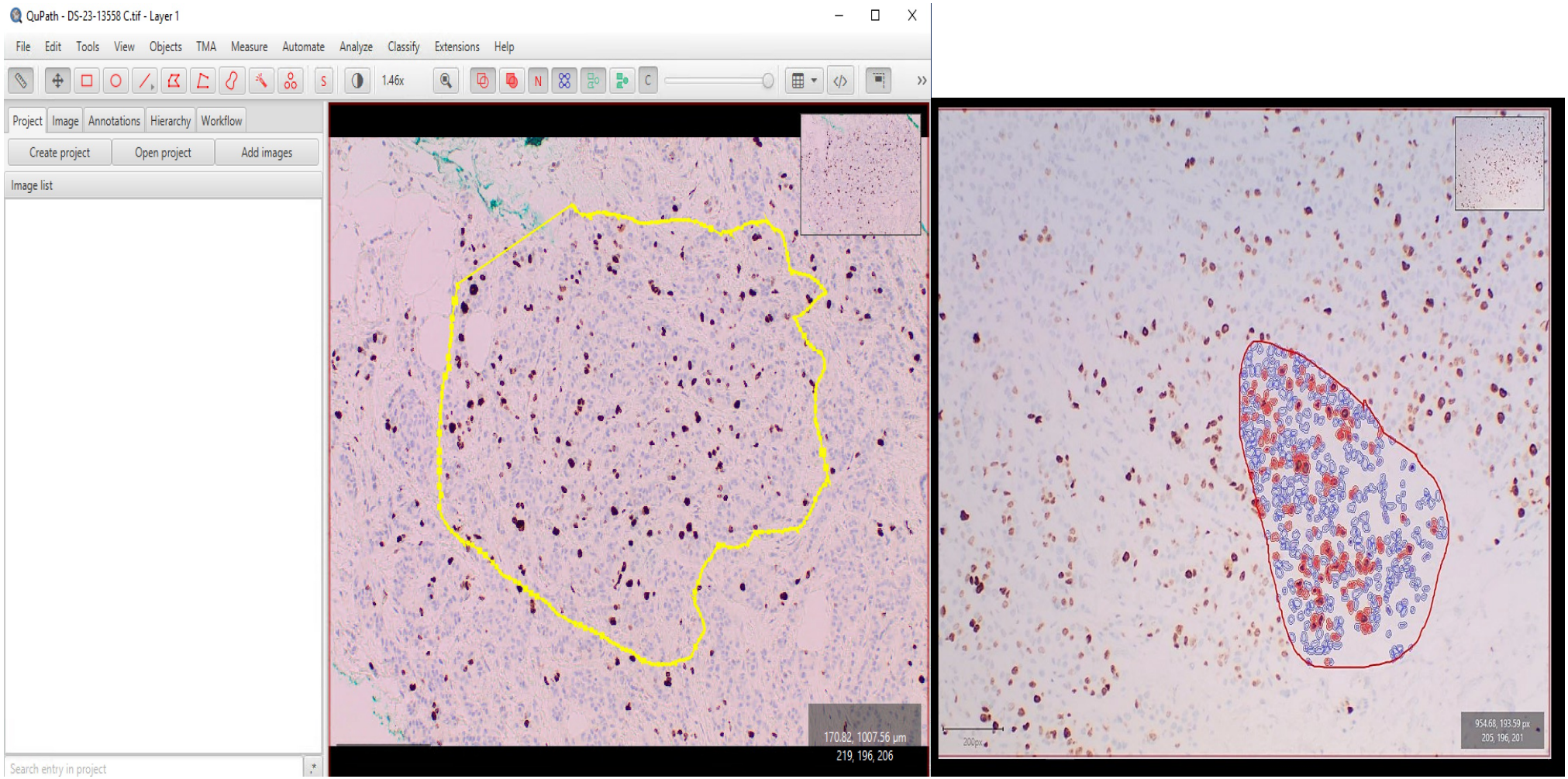
- This was done by selecting the hotspot of tumor cell staining and irregular polygon annotation of hotspots.

Material and methods

- Manual method estimation was performed prior to automated count for all cases.

- Correlation of tumor grades as well as manual and automated scoring systems was performed (Linear regression analysis, Excel 2016).


Positive cell detection
Setup parameters
Detection image
Hematoxylin OD
Requested pixel size
0.5
µm
Nucleus parameters
Background radius
8
µm
Use opening by reconstruction
Median filter radius
0
µm
Sigma
1.5
µm
Minimum area
10
µm^2
Maximum area
400
µm^2
Intensity parameters
Threshold
0.1
Max background intensity
2
Split by shape
Exclude DAB (membrane staining)
Cell parameters
Cell expansion
5 µm
Include cell nucleus
General parameters
Smooth boundaries
Make measurements
Intensity threshold parameters
Score compartment
Nucleus: DAB OD mean
Threshold 1+
0.2
Analyze
Classify
Extensions
Help
1.46x
327.69, 927.38 µm


QuPath - DS-23-13558 C.tif - Layer 1
File Edit Tools View Objects TMA Measure Automate Analyze Classify Extensions Help
1.46x
Project Image Annotations Hierarchy Workflow
Create project Open project Add images
Image list
Search entry in project
170.82, 1007.56 µm
219, 196, 206
200px
954.68, 193.59 px
205, 196, 201

Results

- Forty cases were reviewed for the study
- All but one case were females.
- About 70% of the breast cancer cases studied were invasive ductal carcinoma (70%) followed by Invasive lobular carcinoma (19%)
- Majority of the cases (37.5%) were grade 1 carcinomas.

Results

- There was a good level of correlation between the manual methods of Ki67 estimation compared to the automated method with a R2 of 0.86 (Fig. 1).

- Grade 1 and 2 tumors were found to have a higher level of correlation (R2 of 0.88 and 0.84 respectively) compared to grade 3 tumors with a R2 of 0.69 (Fig. 2).

All grades and histologic types.

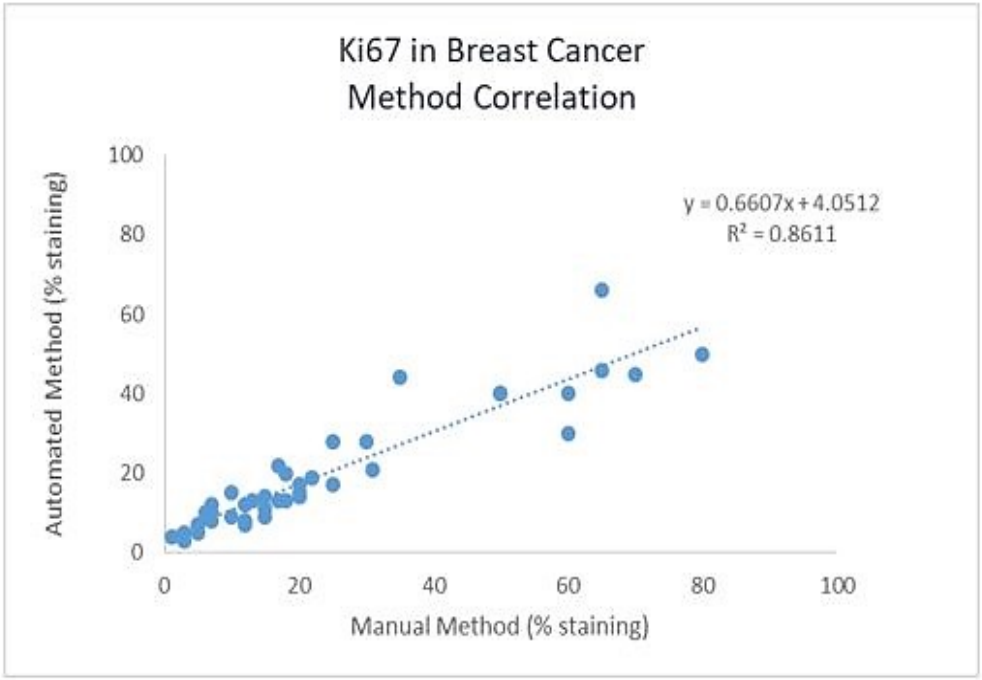


Figure 1: Correlation between manual estimation of Ki67 and automated calculation of Ki67.

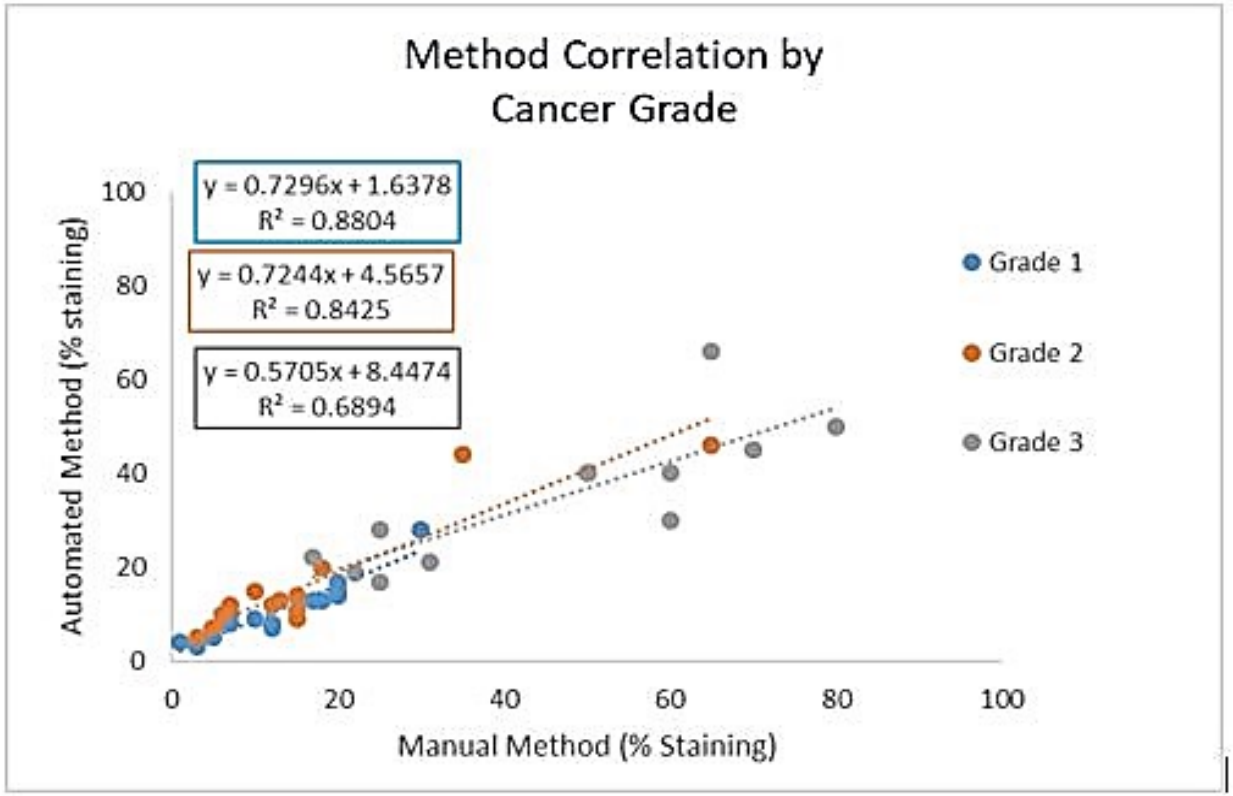


Figure 2: Correlation between manual estimation of Ki67 and automated estimation of Ki67 between the different grades of tumor.

Grade 3 carcinomas with significant difference in both scoring methods

Invasive ductal carcinoma (NST)	3
Metaplastic carcinoma	1
Invasive carcinoma with medullary features	1
Poorly differentiated invasive mammary carcinoma	1

Conclusion

- Automated methods such as QuPath are helpful in reducing the intra and inter-observer variability in Ki67 scoring.
- We noticed a higher correlation in cases that had lower Ki67 scores than those with higher scores.
- Line plots of correlation show a slope less than 1 for all tumor classifications.
- This indicates that Ki67 may be over-estimated with manual method compared with the automated method even when an average count was taken between two observers.

- REFERENCES:
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- 2. Boyaci C, Sun W, Robertson S, Acs B, Hartman J. Independent Clinical validation of automated Ki67 scoring guideline from the International Ki67 on Breast Cancer working group. Biomolecules. 2021; 11(11): 1612. https://doi.org/10.3390/biom 11111612.
- 3. Bankhead P, Fernandez J, McArt D, Boyle D, Li G, Loughrey M, Irwin G, Harkin D, James J, McQuaid S, Salto-Tellez M, Hamilton P. Integrated tumor identification and automated scoring minimizes Pathologist involvement and provides new insights to key biomarkers in breast cancer. Laboratory investigation. 2018; 98(1): 15-26.