An AI-based, Automated Workflow for Identification and Scoring of Invasive Tumor in Ki-67 Stained Breast Cancer Specimens

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ABSTRACT

Understanding the rate of tumor cell growth in breast cancer specimens may be indicative of disease aggressiveness, a tumor characteristic which can be used to make an informed treatment decision. The nuclear protein Ki-67 is increased in cells as they prepare to divide, or proliferate, and is therefore widely used as a proliferation marker for tumor progression. This degree of tumor cell proliferation, or the proliferative index, is commonly detailed in pathology reports shared with the patient care team.

In this study, we utilized the Ki-67 [K2] immunohistochemistry (IHC) assay to stain 10 breast cancer specimens. Stained slides were imaged using the AT2 scanner (Leica Biosystems, Buffalo Grove, IL) and analyzed using the Visiopharm Image Analysis platform. Previous efforts to assess Ki-67 positivity utilizing image analysis have relied on the use of a secondary stain or manual effort by the pathologist to exclude non-invasive tumor regions. These antiquated methods are costly to the lab as they require additional materials or valuable pathologist time. Our novel image analysis approach utilizes artificial intelligence (AI) to automatically denote non-invasive verses invasive tumor regions, which can then be used to quantify the Ki-67 proliferative index. This valuable tool will allow for greater accuracy, cost-savings, and time efficiency when analyzing breast cancer samples compared to traditional methods.

STANDARDIZED SPECIMEN PROCESSING

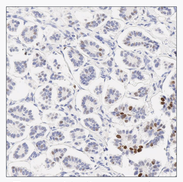
Immunohistochemical Staining and Imaging

Formalin-fixed, paraffin-embedded (FFPE) breast cancer specimens were obtained and stained using the Ki-67 [K2] monoclonal antibody. Staining was performed on the Leica BOND RX™ automated stainer using pre-determined conditions. Brightfield 20X whole-slide imaging was performed using the AT2 scanner with pre-optimized acquisition parameters. All instruments were calibrated and validated for use according to applicable Lanterne Dx SOPs and quality standards. All work was performed at Lanterne Dx's single-site CLIA-certified and CAP-accredited laboratory.

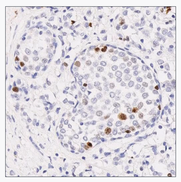
Manual Interpretation

All breast cancer blocks were initially assessed by a Lanterne Dx board certified MD pathologist who has been trained to grade these slides. Assessments included a qualitative review by the Lanterne Dx pathologist for tumor content, appropriate tissue type, overall tissue quality, and tissue artifacts such as folding, necrosis and hemorrhages. All stained slides underwent blinded manual scoring by a board-certified MD pathologist. The manual scoring included a proliferation index (% positivity) for Ki-67 within identified invasive tumor nest regions. Scores provided by the pathologist were used as the gold standard to validate digitally-derived scores.

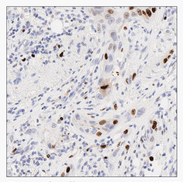
Lanterne Dx's quality control process is designed to provide quality services and accurate histopathology data that meet or exceed the expectations and requirements of the study.



Tumor with diverse nuclear staining, demonstrating light expression sensitivity.



Non-invasive tumor region expressing Ki-67 that should not be included in scoring.



Stromal cells expressing Ki-67 which should not be included in scoring, intermixed with tumor cells.

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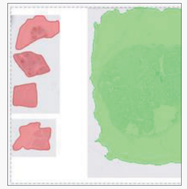
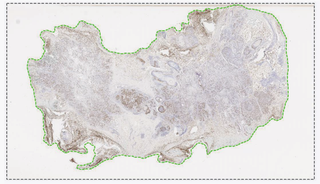
AUTOMATED, AI BREAST WORKFLOW

Patient Sample Distinction

A deep learning network was trained to identify patient tissue on the image and place a region of interest (ROI) around it for further analysis. This required the differentiation of background glass, on-slide controls, and patient samples by defining these image classes. To generate this algorithm, a teach-by-example approach was utilized, and examples of each image class were given as ground truth annotations. These annotations were then used to train a deep learning network, and post-processing modifications were added to discount all classes but the patient sample. If no on-slide controls exist, the network instead discriminates the patient sample from background glass.



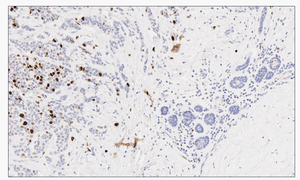
AI-based distinction between control tissues (red) and the patient sample (green).



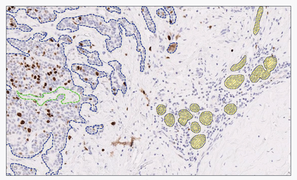
Region of Interest (ROI) automatically placed around patient sample for further analysis, histology and scanning artifacts disregarded.

Invasive Tumor Detection

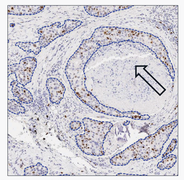
To match manual scoring methods, it was necessary to develop an algorithm that differentiates invasive tumor from other tissue features, including cells within the tumor microenvironment (TME), necrotic areas, and non-invasive tumor. A deep learning network was trained using ground truth annotations for invasive tumor, non-invasive tumor, necrotic areas, and TME to classify all pixels within the tissue. Invasive tumor regions are automatically circled for further Ki-67 analysis. Non-invasive tumor regions are highlighted for pathologist confirmation and then automatically excluded from subsequent analysis. The identification of invasive and non-invasive regions does not require the use of additional staining, utilizing only the Ki-67 IHC staining.



Native image, breast cancer specimen stained for Ki-67.



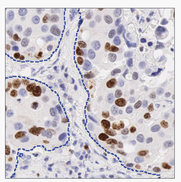
Automatic detection of invasive tumor regions (blue outline) and non-invasive tumor regions (yellow fill) to be excluded scoring.



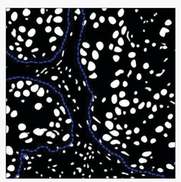
Detected Invasive tumor regions (blue outline) with the automatic exclusion of necrosis (black arrow).

Nuclear Segmentation and Ki-67 Quantification

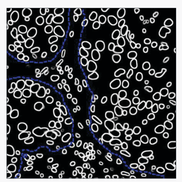
Following the invasive tumor detection, an algorithm was designed to precisely segment nuclei and quantify them for Ki-67 staining to produce a proliferation index (% positivity). Again, a deep learning network was utilized to classify the image data into a nucleus, background [tissue], and nucleus boundary class. By teasing out a nucleus boundary class, accurate nuclei segmentation with varying nuclear morphology and hematoxylin or DAB staining intensity was able to be performed without relying solely on watershed or high intensity peaks for segmentation. Assessment of Ki-67 expression could then be controlled by stain intensity, as well as overall object requirements for stain presence.



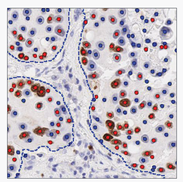
Native image with detected invasive tumor regions outlined.



Deep learning "Nucleus" class, where white represents high probability.



Deep learning "Boundary" class, where white represents high probability.

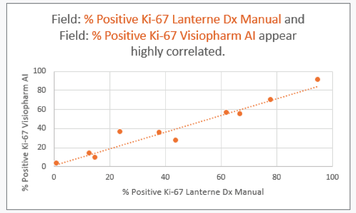


Ki-67 quantification, where each nucleus centroid is marked positive (red) or negative (blue).

PERFORMANCE RESULTS

Manual and AI Score Comparisons

Visiopharm's deep learning networks described in this research were not previously exposed to or trained on the sample set obtained and processed by Lanterne Dx. These algorithms were used in their default, or "off the shelf", state to produce a proliferation index for each sample. The manually determined proliferation index by an MD pathologist was then compared to the AI-based results and an r-value was calculated.



Correlation between Lanterne Dx's MD pathologist manual proliferation index scoring and Visiopharm's AI-based score

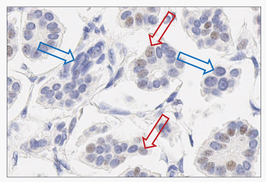
r = 0.969

Value ranges of r and the associated relationship interpretation

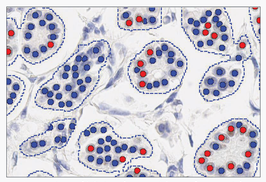
Absolute Value of r	Strength of Relationship
r < 0.25	No relationship
0.25 < r < 0.5	Weak relationship
0.5 < r < 0.75	Moderate relationship
r > 0.75	Strong relationship

Visual Assessments of Sensitivity and Specificity

The range of Ki-67 staining intensity noted on the specimens yielded the need for a solution which was capable of picking up light, granular DAB positive staining (sensitive) without generating false positive nuclei (specific). We designed our algorithm to allow for both intensity requirements and the amount of pixels meeting these requirements on a per-nuclei basis to be considered, intending to provide greater control over positive nuclei detection.



Native image of specimen with light Ki-67 expression noted as positive by Lanterne Dx's MD pathologist.



Associated Visiopharm AI markup, with invasive tumor circled and nuclei denoted as positive (red) or negative (blue).

Denotes positive nuclei with light, granular staining, also found as positive by AI

Denotes negative nuclei with dark hematoxylin staining also found as negative by AI

CONCLUSIONS

We developed an automated, AI workflow to accurately assess Ki-67 proliferation in breast cancer specimens that have undergone quality-controlled processing.

- The use of instruments calibrated and validated in a CLIA-certified, CAP-accredited laboratory advocates for quality standards across specimens undergoing IHC staining and whole-slide scanning.
- The elimination of manual interaction points allows for time-saving automation. These include the identification of the patient sample, detection of invasive tumor and exclusion of non-invasive regions, and precise nuclear segmentation with Ki-67 quantification.
- Due to the calculated r-value, a strong relationship between Lanterne Dx's manual scores and Visiopharm's AI scores was suggested.
- Greater control over requirements for staining positivity produces results which appear to visually agree with manual notes, suggesting a sensitive and specific stain detection method.