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Technical Note

HER2 FISH for Breast Cancer: Advances in Quantitative Image Analysis and Automation

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Abstract

Quantitative image analysis of the status of human epidermal growth factor receptor 2 (HER2) by both immunohistochemistry staining and fluorescent in situ hybridization (FISH) is important for the treatment of breast cancer. Guidelines of the American Society for Clinical Oncology and College of American Pathologists, for HER2 FISH, have evolved over time to improve test accuracy, and efforts have been made to better address the problems with the interpretation that are encountered with borderline-positive cases. Standardization and automation of HER2 sample preparation, processing, and digital quantitation are being considered. We compared the manual quantitation of HER2 FISH with automated scoring and reviewed the history and current status of automated scoring of HER2 FISH. We

explored areas for the possible automation of the process of HER2 FISH and discussed the latest improvements in quantitative image analysis. We conclude that an integrated review of hematoxylin and eosin staining, immunohistochemistry, and FISH by digital image analysis technology would help pathologists to readily identify tumor areas, differentiate invasive from *in situ* carcinoma, and to recognize HER2 signal patterns (even in clustered heterogeneity). An integrated system would also allow automatic alerts for discrepancies in results for FISH versus immunohistochemistry, and for tumor histology and grade.

Keywords
Breast cancer; HER2; FISH quantitative image analysis; digital pathology; whole-slide scanning; ASCO/CAP guideline.

1. Introduction

The latest cancer statistics indicate that breast cancer is still the most common cancer among women, accounting for nearly 30% of the new cancer cases and 15% of all cancer deaths [1]. Targeted therapies for invasive breast cancer rely on the accurate detection of the human epidermal growth factor receptor 2 (*HER2* or *ERBB2*) gene amplification by fluorescent *in situ* hybridization (FISH), chromogenic *in situ* hybridization (CISH), and protein overexpression by immunohistochemical staining (IHC) [2-4]. These tests are often used simultaneously, especially for the borderline-positive cases, to determine HER2 status accurately. Manual evaluation of HER2 status by FISH and IHC on paraffin-embedded tissue sections is inherently subjective [5], compromising test accuracy. Efforts to improve test accuracy include standardization and increased automation of laboratory procedures, including image analysis. In this article, the development of automated scoring of HER2 FISH over the past two decades, and the latest improvements in quantitative image analysis (QIA), have been reviewed and discussed. The possibility of developing an image analysis pipeline, that might improve the test accuracy and eventually assist pathologists in their daily workflow, has also been explored.

2. Current Guidelines for Clinical HER2 FISH Test

2.1 ASCO/CAP Guidelines for HER2 FISH Test

After an expert panel from the American Society for Clinical Oncology (ASCO) and College of American Pathologists (CAP) determined that the standardization of HER2 testing was needed, the panel published guidelines for HER2 testing for breast cancer in 2007 [6]. The 2007 ASCO/CAP guidelines provided detailed recommendations regarding laboratory testing, including specimen selection, sample preparation, interpretation, and reporting of test results. These guidelines were updated in 2013 in an effort to promote the accurate identification of breast cancers that were potentially eligible for HER2-targeted therapy [7]. Issues related to HER2 FISH testing, which were addressed in these guidelines, included validation, implementation, and continuous performance evaluation; FISH scoring for tumor samples with aneuploidy or genetic heterogeneity; detection of false-positive or false-negative findings; use of alternative control probes on chromosome-17 for

certain subtypes of tumor samples; and handling of ambiguous results. The latest ASCO/CAP guidelines, published in 2018, provide more specific recommendations on five groups of FISH results based on the 2013 guidelines [2, 7]. The 2018 guidelines have recommended additional workup for rare clinical scenarios with unknown biological or clinical significance, and have also recommended against the use of alternative chromosome-17 probes. The 2018 guidelines are aimed to improve further the accurate interpretation of IHC and FISH testing. The expert panel has made detailed recommendations about the classification of less common FISH signal patterns to help clinicians make decisions about targeted therapies for rarer breast cancers. However, the new guidelines have also triggered a review of previous HER2 FISH cases, which were evaluated according to the 2013 guidelines, and reclassification of some of these cases has been done according to the 2018 guidelines. The impact of the 2018 guidelines is currently being evaluated. Among factors that might contribute to testing accuracy, the automated or algorithmic classification of IHC and FISH results has not been addressed in the ASCO/CAP guidelines.

2.2 CAP Checklist for HER2 FISH Testing

Under the Clinical Laboratories Improvement Amendments (CLIA) Program, CAP requires all the accredited cytogenetics laboratories to participate in and meet the minimal requirements of proficiency testing. Accredited cytogenetic laboratories must follow all the requirements for HER2 FISH testing, which are specified on the 2018 CAP Cytogenetic Checklist for assay validation (CYG.48399), sample fixation (CYG.48932), and sample scoring (CYG.49465). CAP is stringent about fixation reagents and the duration of sample fixation prior to FISH testing. This standardization is necessary to improve the reproducibility of testing results across different clinical laboratories. CAP also requires the accredited laboratories to note their scoring method (manual or automated) on all reports and to cite the specific HER2 testing guideline (2018 ASCO/CAP or U.S. Food and Drug Administration, FDA) followed by the laboratory. The most recent CAP survey showed that about 5% laboratories use only automated scoring methods, and approximately 11% laboratories use both manual and automated scoring methods [8]. About 84% laboratories use only manual scoring methods. Despite the fact that most clinical cytogenetic laboratories surveyed still use manual scoring methods for HER2 FISH testing, laboratories are increasingly showing an interest in automated scoring options (Table 1).

Table 1 FISH for *HER2* gene amplification summary of scoring methods (US CAP/ACMG)*.

Survey Mailing	Manual	Automated	Manual and Automated
2017A	233 (85.6%)	10 (3.7%)	29 (10.7%)
2017B	199 (84.0%)	11 (3.7%)	29 (10.7%)
2018A	188 (81.4%)	13 (5.6%)	30 (13.0%)
2018B	215 (84.0%)	13 (5.1%)	28 (11.0%)
2019 A	219 (83.0%)	10 (3.8%)	35 (13.3%)
2019 B	206 (82.7%)	12 (4.8%)	31 (12.5%)
2020 A	214 (83.6%)	13 (5.1%)	29 (11.3%)

* Participant Summary Report data use approved by CAP in April 2020.

2.3 HER2 FISH Manual Quantitation

2.3.1 General Criteria for the Manual Quantitation of HER2 FISH

Manual quantitation of HER2 FISH tests should follow the guidelines of ASCO/CAP [2, 6, 7]. The 2007 guidelines have summarized the sources of test variation and emphasized the need for validation of the test by individual laboratories, with more stringent requirements for laboratory-developed tests (LDTs) than for FDA-approved tests. The ASCO/CAP 2007 guidelines for FISH had recommended counting at least 20 non-overlapping cells in at least two separate areas of invasive breast cancer [6]. The ASCO/CAP guidelines have also identified conditions where cells should be rejected for quantitation. However, the cutoff values for HER2/CEP17 ratio and signal patterns have evolved over time. The 2018 guidelines have reclassified signal patterns into five groups and eliminated the equivocal category. All the cases falling into the newly defined Group-2 (HER2/CEP17 ratio ≥ 2 with average HER2 copy number < 4 signals/cell), Group-3 (HER2/CEP17 ratio < 2 with average HER2 copy number ≥ 6), and Group-4 (HER2/CEP17 ratio < 2 with HER2 copy number > 4 and ≤ 6 signals/cell) need additional workup using IHC. This new guideline is expected to facilitate the interpretation of borderline-positive cases or cases with intra-tumor heterogeneity.

2.3.2 Challenges in the Manual Quantitation of HER2 FISH

Manual quantitation of HER2 FISH can be a huge challenge if sample preparation is suboptimal with high background noise and weak signals. Manual signal counts could be inaccurate if samples with *HER2* amplification do not have countable signals or display cluster amplification. In the case of weak-positive samples, inter-observer variation in manual counting can be an issue. The manual counting of amplified samples may result in underestimated signal counts with an even greater inter-observer variation.

2.3.3 Review and Reclassification of HER2 FISH Results Following the 2018 Guidelines

We searched the literature for studies with reclassified HER2 FISH cases based on the 2013 ASCO/CAP guidelines and identified eight studies [9-16] with 11987 HER2 FISH cases from various groups. Among the 11987 cases reviewed, 1080 (9.0%) equivocal cases were identified based on the 2013 guidelines. Using the 2013 guidelines as a baseline, reclassification based on the 2018 ASCO/CAP guidelines resulted in a 10.7% increase in negative HER2 FISH cases and a 1.7% decrease in positive HER2 FISH cases (Table 2). These data demonstrated that the elimination of equivocal results mainly contributed to an increase in negative results according to the 2018 guidelines.

Table 2 Review of HER2 FISH result reclassification with the updates of 2018 ASCO/CAP guideline for invasive breast cancer.

Studies	Positive cases per 2013 guideline	Positive cases per 2018 guideline	Negative cases per 2013 guideline	Negative cases per 2018 guideline	Equivocal cases per 2013 guideline	Total cases reviewed
Liu, 2019 [8]	534 (24.0%)	528 (23.6%)	1524 (68.2%)	1705 (76.4%)	175 (7.8%)	2233
Gordian-Arroyo, 2019 [9]	180 (13.0%)	174 (13.0%)	1092 (81.0%)	1176 (87.0%)	78 (6.0%)	1350
Xu, 2019 [10]	80 (24.2%)	80 (24.2%)	207 (62.5%)	251 (75.8%)	44 (13.3%)	331
Zare, 2019 [11]	269 (17.4%)	203 (13.2%)	1178 (76.4%)	1339 (86.8%)	95 (6.2%)	1542
Lin, 2019 [12]	154 (19.0%)	142 (17.5%)	506 (62.3%)	670 (82.5%)	152 (18.7%)	812
Murray, 2019 [13]	182 (17.4%)	107 (10.2%)	824 (79.0%)	937 (89.8%)	38 (3.6%)	1044
Martin, 2019 [14]	250 (28.4%)	243 (27.6%)	533 (60.6%)	637 (72.4%)	97 (11%)	880
Yang, 2019 [15]	1001 (26.4%)	973 (25.6%)	2393 (63.1%)	2822 (74.4%)	401 (10.5%)	3795
Total	2650 (22.1%)	2450 (20.4%)	8257 (68.9%)	9537 (79.6%)	1080 (9%)	11987

2.4 Automated Scoring of HER2 FISH Test

2.4.1 History and Current Status of the Automated Scoring of HER2 FISH

Klijanienko *et al.* (1999) demonstrated that automated spot counting worked well with non-amplified samples, in a study on HER2 FISH QIA using an automated image analyzer. However, inaccurate automated spot counts were observed when samples with HER2 amplification contained signal clusters without countable dots [17]. The researchers offered a solution by calculating the integrated fluorescence ratio, defined as the integrated mean fluorescence of the tumor cells divided by the integrated mean fluorescence of the normal epithelial cells. Another index used by these authors was the percentage of positive nuclear area, defined as the total area of HER2 spot fluorescence divided by the total nuclear area of DAPI fluorescence. In 2005, Ellis *et al.* developed a system that could perform both HER2 IHC and FISH quantitation automatically. The QIA showed a significant correlation between the manual visual exam and image analysis for both non-amplified and amplified HER2 FISH samples [18]. In 2006, Tubbs *et al.* conducted a study using automated wet lab processing and image analysis. Their results showed a very good correlation [19]. They used an automated scanning microscope with Z-stacking capability and captured images from both IHC and FISH preparation for automated QIA. Positive and negative controls were used to train the system for the range of IHC staining intensities. The FISH analysis used automated spot counting to calculate the HER2/CEP17 ratio. Their comparison indicated that FISH was more precise and consistent than IHC scoring. IHC and FISH QIA were more reliable and consistent than manual analysis. Moerland *et al.* (2006) compared manual counting with automated spot counting and observed 100% correlation [20]. They manually captured three representative areas from each sample and analyzed the images automatically. They reported that the time used for the automated analysis reduced by half compared to that required for manual analysis. They used the HER2/CEP17 ratio and observed that the ratio from automated spot counting of amplified samples was more likely to be underestimated, especially in samples with

high-level cluster or overlapping HER2 signals. They further acknowledged that the manual counting of these highly amplified samples was also likely to be underestimated. A multicenter blinded study that aimed to improve HER2 FISH efficiency was conducted in 2007 [21]. This was an early effort to develop a fully automated image analyzer for HER2 FISH testing. The researchers used a fully automated fluorescent microscope with multi-slide scanning and Z-stacking capability. Technologists used slides with previously marked tumor areas and confirmed HER2 signal quality before setting them up for automated scanning. The prototypical software program could fit cells into boxes or "tiles" with fixed dimensions for automated counting. Results were compared to those from manual counting of the same tumor areas, and showed a high correlation. The study also concluded that the average hands-on time per case could be reduced, which could be important for a high-volume laboratory. In 2007, Theodosiou *et al.* developed a software program that could be used for the quantitation of HER2 FISH [22]. This program had three modes that allowed users to perform fully automated, semi-automated, and manual image analysis. In the fully automated mode, the program recognized signals and calculated the HER2/CEP17 ratio. In the semi-automated mode, the program recognized signals but did not calculate the HER2/CEP17 ratio until users manually corrected the mistakes. In the manual mode, the program neither identified the signals nor calculated the HER2/CEP17 ratio. The researchers reported that the semi-automated and manual modes were extremely useful when the quality of HER2 FISH was sub-optimal, and that their automated analysis performed consistently in a multicenter test. Konsti *et al.* (2008) reported the use of an open source image processing tool called 'imageJ' to develop a system for the automated analysis of HER2 FISH samples [23]. Although image capturing and selection of the region of interest (ROI) were done manually, the authors' image analysis showed high agreement between the manual and automated counts. As more commercial imaging systems became available, Furrer *et al.* (2013) validated a new classifier with fully automated capturing and scoring modules [24]. The system used was an FDA-approved image analyzer, and the program fitted cells into tiles with fixed dimensions for automated counting, as mentioned previously by Stevens *et al.* [21]. The scoring algorithm was later customized to meet the special requirements of HER2 FISH. For the small portion of amplified HER2 cases without countable signals, the software evaluated clusters of HER2 by quantitating the area in the HER2 channel. The agreement between manual and automated counts was high and could be further improved by manual review with considerably less effort. Although the determination of the ROI before analysis is important, it poses challenges to automated FISH scoring. The selection of ROI by pathologists using manual screening can be tedious, and this might be an area for automation with artificial intelligence (AI) technology. There are commercial cytogenetic imaging systems that can perform the co-localization of hematoxylin and eosin (H&E) staining and FISH imaging [25, 26]. Such a system was employed in a study in 2017 by Radziuviene *et al.*, who manually selected the ROI for image acquisition, and used automated analysis for signal counting [27]. The automated analysis excluded cells with only one HER2 or CEP17 signal. Manual counting results were considered to be the gold standard for comparison. Among the > 36,000 nuclei detected by the automated imaging program from 50 patients, 15% were mistakenly segmented, and 9% were not detected. These errors resulted in an underestimation of *HER2* amplification. However, the automated analysis was capable of generating indicators of HER2 intra-tumoral heterogeneity, which was important to improve the testing accuracy. The use of tiles or boxed regions for HER2 FISH has recently been largely replaced by cell segmentation, where the software automatically

detects the nuclei based on DAPI labeling and identifies the perimeters and area of the nuclei to enable more accurate signal counts per cell. This cell-based method of FISH scoring is more relevant to the 2013 and 2018 ASCO/CAP guidelines, that have defined cutoffs based on signal counts per cell, for both single-probe and dual-probe FISH assays.

The ASCO/CAP guidelines have not provided recommendations on the quantification of HER2 amplified cluster signals, that are not suitable for conventional automated spot counting. Höfener *et al.* proposed an automated density-based count of amplified HER2 FISH signals in terms of the integral, over a density map predicted by deep learning (DL) [28]. Their results indicated that the approach performed better than the detection-based and area-based methods. With the recent development of automated spot counting, Zakrzewski *et al.* introduced a DL pipeline system that attempted to mimic the work of a pathologist diagnosing breast cancer using HER2 FISH results [29]. Although this AI technology is still far away from implementation in the clinical testing environment, the preliminary data appears promising and shows the ability to accurately grade breast cancers based on morphological and HER2 FISH information.

2.4.2 QIA

Manual quantitation of HER2 IHC and FISH in breast cancer is subjective, which results in inaccuracy and poor reproducibility. QIA for HER2 testing in breast cancer, if used properly, could address these issues. Although CAP published its first guideline for HER2 IHC QIA in 2019 [30], a similar practice guideline for HER2 FISH QIA is presently lacking. The 2018 CAP Cytogenetics Checklist items that are relevant to the HER2 FISH test do not include QIA validation and continuous quality improvement. With over ten years of advancements in digital imaging and cytogenetic FISH spot counting technology, it is not difficult to achieve accurate scoring results for samples with optimized signal intensity and well-separated cell boundaries. However, QIA for HER2 FISH amplification on tissue sections with a high degree of cell overlap remains challenging. With the control over pre-analytic variables, the validation and revalidation of QIA should be emphasized once the imaging system and quantitative algorithms are selected. It is highly likely that quantitative algorithms for FISH will be modified over time to comply with guideline updates, technology advancements, and/or new regulatory oversight of digital imaging software, possibly making the repeat evaluation and revalidation of test results necessary.

2.4.3 Strategies for Automated Scoring of HER2 FISH

Although manual signal counting is currently the gold standard for HER2 FISH testing, it is labor-intensive and subjective. Fully automated algorithms for scoring could be adapted if accuracy and reproducibility could be ensured. However, not all HER2 FISH slide preparations are suitable for fully automated scoring. Quality control checks must be incorporated into the process. Fully automated image capture and counting systems are available through different vendors [24, 26, 31], and individual laboratories can validate these platforms by following the established guidelines. A fully automated scoring system, upon validation, could be used as the first scorer, while a technologist counts as the second scorer. Another approach could be fully automated scoring by a validated system, with manual review and reclassification of captured cells by a certified technologist. Regardless of the automated scoring strategy used, there should be a quality control system in place to check and correct cell selection, segmentation, and counting

errors from automated systems. Ideally, the time-consuming steps of manual quality control could be replaced by better programming and AI technology in the near future. Although more than 80% of the US CAP-accredited laboratories performing HER2 FISH still use a more labor-intensive manual scoring method [8], a transition to increased automated scoring is anticipated with technical advances in QIA.

3. Exploring the Possibility of Automation for the HER2 FISH Process

3.1 Pre-Analytic Automation

Although pre-analytic automation is beyond the scope of this chapter, automation in tissue slide processing, including slide pre-treatment, application, and hybridization of FISH probes, slide washing post-hybridization, and image acquisition could greatly reduce the variations observed in subsequent steps, and could facilitate standardization. Validated commercial systems are routinely used for these pre-analytical steps in clinical FISH testing [31-33].

3.2 Analytic Automation

Automation of image processing and quantitation is a crucial part of the QIA process. The goal of automation is to improve efficiency without compromising the accuracy and precision of the test (Figure 1).

	Possible Automation		Manual Processing
Pre-analytic	Automated tissue processing for fixation		Manual fixation
	Automated HER2 FISH denaturation and hybridization		Manual FISH denaturation and hybridization
	Automated post-wash with a programmable auto wash system		Manual post-wash with water bath
	Automated identification of ROI		Manual review by the pathologist
	Automated image acquisition (whole slide imaging)		Manual capturing
Analytic	Automated cell segmentation	Manual review and correction if necessary	Manual screening and exclusion of cells that are not qualified
	Automated signal counting	Manual review and correction if necessary	Manual counting
Post-analytic	Automated calculation of ratios		Manual calculation of ratios
	Classification of cells using deep learning and artificial intelligence technologies		Review by pathologist or director

Figure 1 Possible areas that are suitable for automation in HER2 FISH.

Some laboratories have adopted algorithms of full automation, while others have applied semi-automated algorithms with manual control of certain steps. A laboratory could develop a system to carry out automated segmentation, scoring, and ratio calculation, with a pause for quality

control and checking between steps, through computer programming. Automated segmentation could be followed by immediate automated cell rejection, using computational criteria such as size and shape of the cells, cells failing to be segmented correctly, minimum numbers of signals for each color probe used, samples with poor signals, and samples with high non-specific background noise, before automated signal counting. As ASCO/CAP guidelines for cell rejection criteria change over time, application developers should keep them in mind when programming. Several commercial systems allow user-defined parameters to be entered before the analysis. Fixed-dimension boxes displaying segmented cells from the above steps could also be reviewed manually to ensure the accuracy of cell selection. Manually performing the quality control check at this step could significantly reduce the chances of computing overload caused by dumping a large quantity of useless data to the central processing unit and causing users to experience significant slowdown when analyzing samples. Automated signal counting could be challenging when the signal size and intensity barely pass the inclusion threshold but are still suboptimal, as these factors can be a major source of analytical variation. Defining thresholds for minimum signal size and intensity during program calibration and validation is essential to reduce variations in quantitation, but samples containing high background noise might not be suitable for automated analysis. A spot counting program, which allows individual cells to be displayed for manual reclassification, is recommended. Increased automation often requires more manual quality control checks, which will reduce the efficiency of the process. There is always an issue of finding a balance between automation and efficiency, without compromising quality. Studies have demonstrated that automated counting could reduce the time needed for analysis of each case, provided that a large number of samples were analyzed [19, 34].

3.3 Post-Analytic Automation

Automated calculation of the HER2/CEP17 ratio should be acceptable if the results from image processing and quantitation are accurate. Ratios calculated following the 2013 ASCO/CAP guidelines could be re-programmed so that samples could be sorted into the five new HER2 FISH groups defined by the 2018 ASCO/CAP guidelines. For groups 2–4, where additional workup is required, the development of an automated imaging system to co-localize or match parallel H&E, IHC, and FISH tissue sections would be beneficial. This approach could improve the quality of testing and identify candidates for targeted therapies with higher efficiency and accuracy.

4. Future Direction

4.1 Computer DL and AI

Mimicking the sophisticated workflow of breast cancer pathologists to resolve issues of discordant, borderline, or unusual results by a computer is not an easy task [35]. Protein overexpression detected by IHC and gene amplification revealed by FISH are two essential components of the diagnostic process, and samples could be digitized for analysis by whole-slide scanning technology [36, 37]. Hekler *et al.* used a pre-trained convolutional neural network analyzer to classify skin lesions from whole-slide scanning images, and observed that the machine had better sensitivity, specificity, and accuracy than that reported by board-certified pathologists [38]. Although DL-based QIA for digital pathology has been tested on H&E and IHC samples,

including breast cancer tissues [39, 40], QIA for FISH analysis using DL algorithms on breast cancer tissues has not been explored until recently. Liu *et al.* performed a study using a DL-based analyzer to detect metastatic breast cancer in sentinel lymph node biopsies, and the results appeared promising with respect to assisting the pathologist's workflow [41]. A large-scale DL framework, that combined convolutional neural network with recurrent neural network approaches and focused on clinical-grade decisions, was recently explored [42]. The study indicated that clinical-grade decisions could be reached without pixel-level manual annotations. Another proposed model tried to integrate information from mammography, histology, and even phenotype for computerized DL-based diagnosis [43]. However, given the limited literature on quantitating HER2 FISH using DL and AI, our review of these methods is still basic and needs to be updated over time.

4.2 Analyzer Combines both Automated IHC and FISH Digital Image Analysis

Although there are CAP guidelines for breast cancer HER2 IHC QIA, similar guidelines for breast cancer HER2 FISH QIA need to be developed. The ideal all-in-one system for testing of breast cancer biomarkers would be capable of performing whole-slide or ROI scanning from H&E, IHC, and FISH in a low-power but the high-resolution format, to allow digital magnification for detailed visual examination and confirmation of results. The system should have the option of incorporating IHC scanning for other antibodies, such as ER/PR/Ki–67, on parallel tissue sections. The new analyzer should be capable of co-localizing H&E, IHC, and FISH images from the same area of a slide, and should display multiple images side-by-side using a previously described approach [26]. ROI will be determined by either manual screening by a pathologist or automated algorithms employing methods such as AI technology. Pathologists could digitally annotate any ROI on the H&E image, and the analyzer could automatically display semi-quantitative IHC and FISH counting data (including the HER2/CEP17 ratio) simultaneously. FISH counting data should use a measurement that linearizes the spot counting and amplification quantitation. A recently published paper discussed possible solutions to the problem of weak IHC staining of the adjacent normal epithelium in breast cancer samples, which contributes to the false-positive rate, and was not addressed by the 2018 ASCO/CAP guidelines [44]. Although FISH is considered to be more precise than IHC in evaluating *HER2* amplification for targeted therapies [45-47], a significant amount of manual review is still required to resolve the discrepancy between the two tests. An all-in-one analyzer could make FISH confirmation and pathologists' review more efficient. We hope to see a system that could mimic a human reviewer's ability to correct segmentation and counting errors, both of which still largely rely on manual review and correction.

5. Conclusions

With the availability of IHC and FISH scanning data, we hope to see an integrated system that is capable of classifying the challenging breast cancer cases and predicting treatment outcomes using DL and AI technology, in the near future.

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Author Contributions

All authors participated in writing and editing this manuscript.

Competing Interests

The authors have declared that no competing interests exist.

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