USCAP'24
Poster 1160

Improving interoperability in digital HER2 FISH enumeration - a pilot evaluation

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Disclosures: AJSS, RA and RI have no disclosure, CS and YG are employees of Applied Spectral Imaging, DW is an employee of Motic Digital Pathology

Background & Introduction

- Tissue matching between H&E or IHC slide and HER2 FISH specimen allows to specifically target tumor regions with highest protein expression when selecting areas for high magnification scanning of the FISH slide.
- While this workflow was established when performed on the same scanning platform in the FISH lab[1,2], the goal of the present evaluation was to assess the feasibility to match a brightfield image acquired on one scanning platform with the FISH image acquired in a different scanning system, therefore allowing to use the region of interest marked by the pathologist on the H&E or IHC image without having to rescan it.

Design & Methods

- Core biopsy specimens from breast cancer patients were included in this evaluation. H&E and HER2 IHC slides were scanned with MoticEasyScan Infinity at 40X resolution (0.26 um/px) and saved in SVS format.
- Slides were examined by certified pathologists using both conventional microscopy and digitalized imaging. FISH was requested in case of equivocal reporting, and analysis was performed manually.
- The FISH slides were then scanned and analyzed using the PathFusion system (Applied Spectral Imaging). The brightfield images, acquired on the MoticEasyScan and marked by the pathologist when requesting FISH, were registered to the FISH images matched on the ASI system in the FISH lab.
- Regions of interest were automatically transferred from the brightfield image to the FISH scan and frames were selected in these marked areas for scanning at high magnification (Figure 1). Results of digital FISH enumeration were compared to manual results.

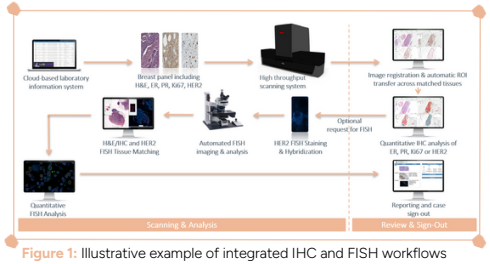

Figure 1: Illustrative example of integrated IHC and FISH workflows

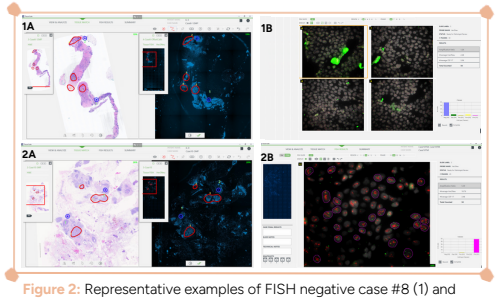

Figure 2: Representative examples of FISH negative case #8 (1) and FISH positive case #10 (2) featuring tissue matching between MoticEasyScan brightfield image and ASI FISH scan (A) and computer-aided FISH analysis results (B)

Case #	Manual HER2 FISH	Computer-aided HER2 FISH
1	1.1 NEG	1.1 NEG
4	1.1 NEG	1.1 NEG
5	1.0 NEG	1.3 NEG
8	1.3 NEG	1.2 NEG
10	4.9 POS	5.4 POS
17	3.8 POS	2.8 POS
18	3.5 POS	*Not available (faded signals)*

Table 1: Compared results of manual and computerized HER2/CEN17 FISH amplification ratio

Results

- Twenty biopsy specimens from 20 patients were included in this evaluation. 19 samples had a diagnosis of invasive ductal or lobular carcinoma, and one of metaplastic carcinoma with chondroid differentiation.
- Among the 20 samples, 7 were diagnosed as HER2 IHC equivocal (2+). Manual HER2 FISH enumeration performed on these cases confirmed 3 as HER2 positive and 4 as HER2 negative.
- FISH specimens were then scanned in the FISH lab and high magnification frames were acquired in regions of interest marked by the pathologist on the brightfield image (Figure 2), eliminating the need to review the FISH slide under the microscope.
- Comparison to manual process showed that digital FISH enumeration provided equivalent results for 6 slides (Table 1). In one specimen, FISH signals were faded and therefore unusable for digital enumeration.

Conclusions

- Interoperability in digital FISH enumeration allows to use regions of interest marked by the pathologist on the H&E or IHC image when requesting FISH.
- This integrated workflow is envisioned to enhance both accuracy and efficiency when performing digital HER2 FISH enumeration.

1.Wilcock et al, Hum Pathol (2022) 126: 129-135
2.Wilcock et al, Arch Pathol Lab Med (2023) 147 (12): 1402–1412

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Combined manual reading and computer-aided quantitative analysis for the standardization of HER2 IHC scoring

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Background & Introduction

- Computer-aided image analyses are gaining growing adoption, particularly when seamlessly integrated in the digital pathology workflow.
- These computerized methods, when combined with manual reading, have been shown to help standardize the reporting of IHC specimens[1], specially in cases where the tumor has variable intensity of expression.
- The aim of this evaluation is to further assess the usefulness of computer-aided quantitative analysis when integrated in the laboratory workflow, focusing on its role as a second opinion for the standardization of HER2 IHC scoring on breast cancer cases.

Design & Methods

- Five slides prepared for each breast cancer core biopsy specimen were stained with H&E, ER, PR, Ki67 and HER2.
- All slides were scanned with MoticEasyScan Infinity (Motic Digital Pathology) at 40X (0.26 um/px) and saved in SVS format. They were examined by certified pathologists using both conventional microscopy and digital imaging. HER2 FISH was requested to complete the evaluation of equivocal HER2 IHC cases.
- HER2 IHC images were further analyzed using the HiPath Pro scanner-agnostic software (Applied Spectral Imaging). Regions of interest marked on the H&E images were automatically transferred to HER2 specimens following tissue matching. Cells automatically identified as tumor cells were segmented and classified using a color-coded overlay.
- Computerized results were compared to manual readings. In case of discrepancy, a second manual reading was performed.

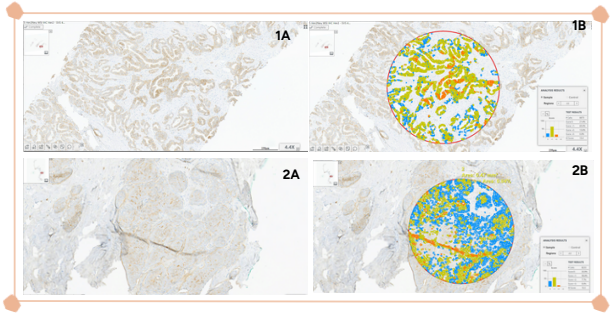

Figure 1: Representative examples of concordant HER2 case #5 (1) and discordant HER2 case #8 (2) featuring IHC staining (A) and computer-aided analysis (B)

Case #	ER	PR	Ki67	HER2 Read 1	HER2 Aided	HER2 Read 2	HER2 FISH
1	+	+	<14%	2+	2+		NEG
2	-	-	≥14%	0	0		
3	+	+	<14%	0	1+	1+	
4	+	+	≥14%	2+	2+		NEG
5	+	+	≥14%	2+	2+		NEG
6	+	-	≥14%	3+	3+		
7	+	+	≥14%	0	0		
8	+	+	≥14%	2+	1+	2+	NEG
9	+	+	≥14%	3+	*No focus*		
10	+	+	≥14%	2+	3+	3+	POS
11	-	-	≥14%	0	1+	1+	
12	+	+	≥14%	1+	1+		
13	+	+	<14%	0	0		
14	-	-	≥14%	0	0		
15	+	+	≥14%	1+	0	1+	
16	+	+	≥14%	3+	2+	3+	
17	-	+	≥14%	2+	2+		POS
18	-	-	≥14%	2+	2+		POS
19	+	+	≥14%	0	0		
20	-	-	≥14%	3+	3+		

Table 1: IHC profile of study cases including manual reading of ER, PR, Ki67 and HER2 (reading 1 and repeated reading 2 for discrepant cases), as well as computer-aided HER2 IHC scoring. HER2 FISH results are provided for all equivocal cases.

Results

- Twenty biopsy specimens from 20 patients were included in this evaluation. 19 samples had a diagnosis of invasive ductal or lobular carcinoma, and one of metaplastic carcinoma with chondroid differentiation.
- The ER, PR, Ki67 and HER2 IHC profiles of the cases are detailed in Table 1. As shown in this Table, the first manual reading of HER2 IHC diagnosed 4 cases as positive (3+), 9 as negative (0 or 1+) and 7 as equivocal (2+). FISH was performed on all equivocal cases, confirming 3 as HER2 positive and 4 as HER2 negative.
- Computer-aided HER2 scoring was obtained for 19 out of 20 images as one image out of focus was removed from analysis. Concordance with first manual reading was observed in 13 cases.
- During second manual reading following computerized analysis, two cases scored as HER2 (0) were re-scored as HER2 (1+), matching software assessment. A third case diagnosed as equivocal (2+) was re-scored as (3+) following the review of the computerized analysis. This case was later confirmed as FISH positive.
- The diagnosis of the remaining 3 cases was unchanged after review of computerized results.

Conclusions

- This evaluation exemplifies the potential usefulness of computer-aided scoring as second opinion when reporting HER2 IHC in breast cancer, particularly in cases of low HER2 expression.
- This further illustrates that the combined use of manual reading and computerized analysis may help standardize IHC assessment and potentially reduce inter-observer variability.

1. Glickman et al, European Journal of Pathology (2023) 483 (S1): E-PS-01-026