USCAP'24
Poster 1160

Improving interoperability in digital HER2 FISH enumeration - a pilot evaluation

Alma J Sanchez-Salazar, MD[1] , Ruben Alvarado, BSc, MBA[1] , Rabiu Ibraheem, BSc [1], Cristina Steele, MBA, CG(ASCP)[2], Dan Walker, BA[3] , Yael Glickman, PhD [2]
1 CorePath Laboratories, San Antonio, TX, USA; 2 Applied Spectral Imaging, Carlsbad, CA, USA; 3 Motic Digital Pathology, Emeryville, CA, USA
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.

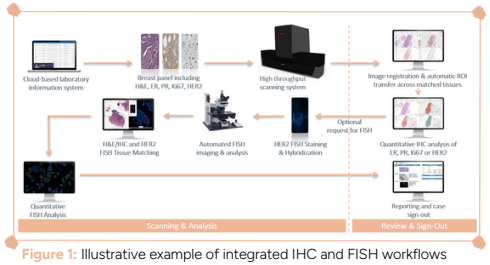

Figure 1: Illustrative example of integrated IHC and FISH workflows

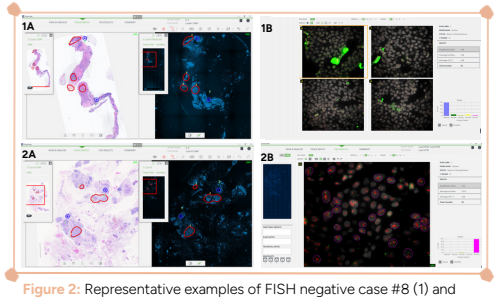

Figure 2: Representative examples of FISH negative case #8 (1) and FISH positive case #10 (2) featuring tissue matching between MoticEasyScan brightfield image and ASI FISH scan (A) and computer-aided FISH analysis results (B)

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