FISH reporting in a referral laboratory: The benefits of BioView

Jemma Shead[1], David Allen[1]
1. Health Services Laboratories - Advanced Diagnostics (HSL-AD). 60 Whitfield Street, London W1T 4EU

What is FISH?

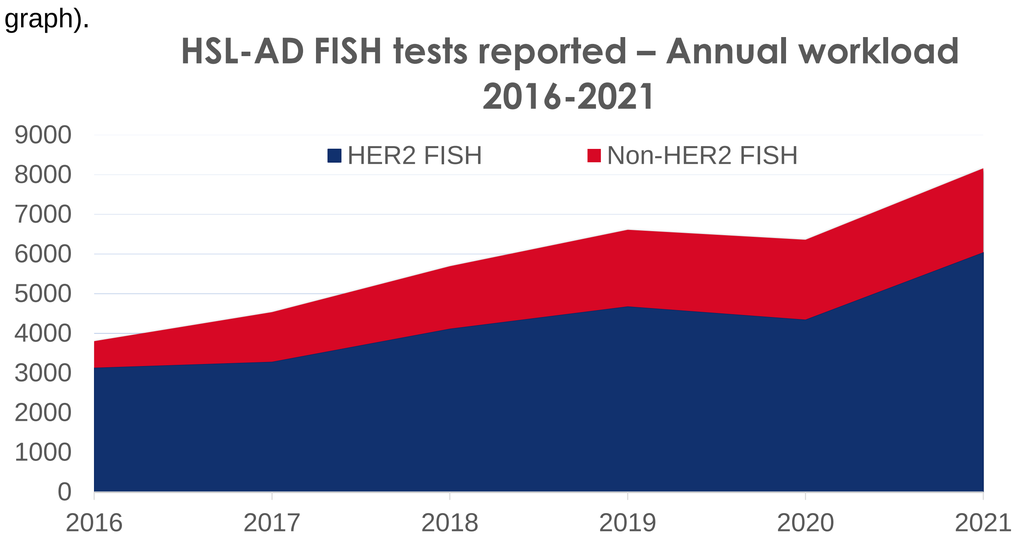
- FISH is a powerful tool routinely employed in the diagnosis, prognosis and treatment of many different types of cancer.
- Fluorescence in-situ hybridisation (FISH) is the hybridisation of labelled fluorescent probes to specific DNA or RNA sequences to detect target molecules.
- During analysis, target molecules are visualised where they are located within the cell.
- HSL-AD (formally UCL-AD) provides a high-volume reference FISH processing and reporting service to over 100 laboratories, covering our local pathology partnership in North Central London (NCL) and external clients including NHS, non-NHS within and outside of the UK.

Why is FISH testing used for patients?

FISH TEST	GENE ABBERATION	TISSUE TYPES	CLINICAL UTILITY AND SIGNIFICANCE OF RESULT
HER2 (ERBB2)	Amplification	Breast and Gastric	Prognostic and indicates potential therapeutic benefit from anti-HER-2 therapies e.g. HERCEPTIN® (trastuzumab)
ALK / ROS	Translocation	Lung	Potential therapeutic benefit from tyrosine kinase inhibitor drugs
MDM2 / CDK4	Amplification	Lipoma or Liposarcoma	Diagnostic: discriminate lipomas (non-amplified) from atypical lipomatous tumour / well-differentiated or dedifferentiated liposarcoma (amplified)
BCL2 / BCL6 / MYC	Translocation	Lymphoma	Subclassification of disease type and prognosis (e.g. Identify patients with double or triple hit gene alterations)

Why is the BioView system used in FISH reporting at HSL-AD?

- BioView is an FDA approved imaging and analysis system that can be used to:
  - Digitise FISH slides that have undergone processing.
  - Replicate a microscope environment in a digital setting, allowing users to focus through nuclei to assess cell morphology and signal location. Nuclei selection is entirely user driven.
  - Software allows automatic assessment of signals in tumour nuclei, capture of areas examined and rule based algorithms only allow appropriate nuclei to be assessed.
- It is compatible with, and aids in the analysis of most FISH probes available including amplification, break-apart and multiplex type probe analysis.
- Implementation of BioView at HSL-AD was essential to overcome limitations encountered with manual-only FISH reporting. following consistent yearly ISH workload growth (see below graph).



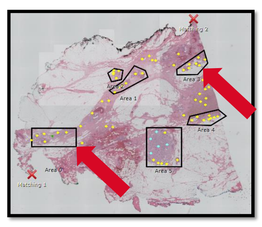
BIOVIEW SCANNING STEPS (Using the Encore Duet-3)

BRIGHFIELD SCAN (Day 1)
- Brightfield (BF) = A stained slide → Representative of the tissue to be analysed
- Ideally the IHC slide for Her-2 (If available) or Haematoxylin & Eosin (H&E).
- SMM/p63 or double IHC staining requested internally for complex cases.

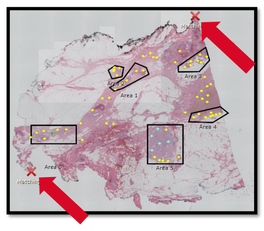
DAPI SCAN (Day 2)
- DAPI is a blue nuclear counterstain applied after FISH hybridisation is complete, allowing visualisation of nuclei and overall tumour morphology.
- Basis for 'tissue matching' (Brightfield and DAPI slide anchoring).
- Provides foundation for morphological assessment during field of view (FOV) selection at tissue matching (see 'Tissue matching steps' diagram).

DEEP SCAN (Overnight)
- System automatically re-calibrates its position in relation to distinctive morphological features present in the DAPI scan.
- Full Z-stack scanning of FOV replicating manual microscopy.
- Exposure to visualise probe signals is optimised by the system on a slide by slide basis.

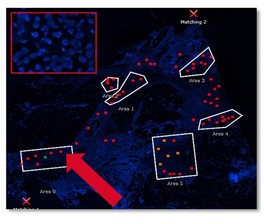
2. TISSUE MATCHING STEPS (Using multiple Solo Workstations simultaneously) – Must be performed prior to deep scanning FISH slide



Tissue morphology is assessed on BF slide:
- Outline(s) are drawn to highlight specific features to include (e.g. high grade disease morphology) or exclude (e.g. *in-situ* disease) for FOV selection and guide effective anchoring.
- Specific selection is dependent on FISH test & tissue type and morphology.



Two points are selected to anchor BF to DAPI
- Distinct structures present in BF and DAPI slides are ideal (e.g. vessels).
- Aligns the two slides so specific BF correlation with DAPI FOV selection is possible during analysis (see arrows).



FOV selection from (red markers) for areas to FISH scan
- This is a critical step in insuring accurate representation of the morphology present (tumour cells must be selected on DAPI) (red markers).
- Poor quality tissue matching is a primary cause for BioView reporting to fail, necessitating manual assessment instead or repeat scanning.

The benefits of BioView – How does it improve The HSL-AD FISH reporting service compared to manual reporting?

TASK	TIME (mins)	ADDITIONAL FACTORS
Manual FISH Reporting	Mean 23 (range 5-45)	Full BF slide review, full FISH slide review (across 2 different microscopes), manual signal counting, transcribing, calculation and reporting. Limited by number of fluorescent microscopes.
BioView FISH Reporting	Mean 6.5 (range 1-20)	Scanning time (walk-away), tissue matching and case analysis (semi-automated). Each step independent of the others (LEAN workflow). Limited by number of Solo Workstations on network.

- Reduced user fatigue: Mentally and ergonomically →BioView simplifies and streamlines the time-consuming task of reporting cases resulting in a more efficient FISH reporting service.
- Increased capacity for reporting - BioView system implementation for semi-automated reporting was essential for upscaling demand as demonstrated by multi-year workload increases.
- For complex cases, the presentation of tissue morphology alongside the FISH slide for analysis ensures complete confidence and accuracy of assessment.
- The potential for remote working – A reduced demand for staff daily physical presence – And an attractive feature for future potential employees more flexible workload management
- Tissue morphology and enumerated cells are easily visualised by multiple staff concurrently, enabling effective collaboration, teaching, training, and review of complex cases.
- One of the most significant drawbacks of FISH over other ISH methods such as DDISH is the lack of retained material, due to signal loss over time.
  - The use of BioView scanning overcomes the main disadvantage of FISH through routine large-scale image capture and storage - emphasising the benefit of using the more sensitive FISH method, whilst maintaining case records as archived material and allowing for historic audit.
- In addition, research and teaching are feasible when cases are archived.

Disadvantages and limitations in using BioView for FISH reporting:

- Scanning incorporates additional sample handling steps and increases time to reporting.
- Set-up and maintenance costs prohibits usefulness except in very high-volume settings.
- Service may become over-dependent on digital systems, contingency is required.
- Staff training on software use is essential, and like microscopy reporting, a deep understanding of tissue and tumour morphology and reporting are required.
- Does not replace manual reporting → some morphology encumbers scanning.
- Not all tissue / tumour types are suitable – MDM2/CDK4 FISH is not recommended for use.
- High quality technical preparation is essential for successful imaging.
- Highly dependent on the quality of tissue matching performed on each case.
- Significant bench space and IT requirements including servers and storage.

Expected and potential developments using BioView at HSL-AD:

- Full integration with Leica Cerebro sample tracking system (expected 2022).
- Utilisation of the open, adaptable platform for assimilation of new tests or workflows, including research projects to contribution to medical research through collaboration.
- Expansion of multiplex prognostic & diagnostics probes offered.