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 (formerly 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?

<table>
<thead>
<tr>
<th>FISH TEST</th>
<th>GENE ABERRATION</th>
<th>TISSUE TYPES</th>
<th>CLINICAL UTILITY AND SIGNIFICANCE OF RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2</td>
<td>Amplification</td>
<td>Breast and</td>
<td>Prognostic and indicates potential therapeutic benefit from anti-HER-2 therapies e.g. HERCEPTIN® (trastuzumab)</td>
</tr>
<tr>
<td>ALK</td>
<td>ROE</td>
<td>Lung</td>
<td>Potential therapeutic benefit from tyrosine kinase inhibitor drugs</td>
</tr>
<tr>
<td>MDM2/C/CDK4</td>
<td>Amplification</td>
<td>Lipoma or Liposarcoma</td>
<td>Diagnostic: discriminate lipomas (non-amplified) from atypical lipomatous tumour / well-differentiated or dedifferentiated liposarcoma (amplified)</td>
</tr>
<tr>
<td>BCL2/L</td>
<td>Translocation</td>
<td>Lymphoma</td>
<td>Subclassification of disease type and prognosis (e.g. identify patients with double or triple hit gene alterations)</td>
</tr>
</tbody>
</table>

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 IGH workload growth (see below graph).

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).

FISH reporting in a referral laboratory: The benefits of BioView

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

<table>
<thead>
<tr>
<th>TASK</th>
<th>TIME (mins)</th>
<th>ADDITIONAL FACTORS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual FISH Reporting</td>
<td>Mean 23</td>
<td>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.</td>
</tr>
<tr>
<td>BioView FISH Reporting</td>
<td>Mean 6.5</td>
<td>Scanning time (walk-away), tissue matching and case analysis (semi-automated). Each step independent of the others (LEAN workflow).</td>
</tr>
</tbody>
</table>

- Reduced user fatigue: Mentally and ergonomically →BioView simplifies and streamlines the time-consuming task of reporting cases resulting in 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 – 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.