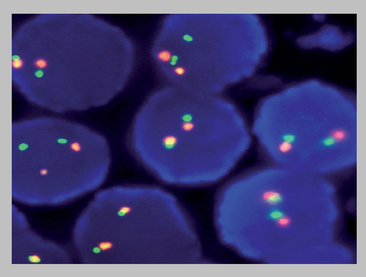
Rigshospitalet

PAT-03



Introducing Digital Pathology in Fluorescent in Situ Hybridization on Tissue by Validation of the Bioview Imaging System

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Introduction

One of the most important tasks at the Department of Pathology at Rigshospitalet is cancer diagnostics, which is based on the examination of cells and tissue samples from the patients. Here FISH has a special role, as the method used is fast, inexpensive and easy in execution.

The analysis principle relies on the use of a probe - a synthetic DNA sequence for hybridization on a particular gene. The probes are marked with the fluorochromes. After hybridization, one can read the signals by fluorescence microscopy and perform cells counting with gene translocation, amplification and deletion.

Microscopy of slides takes place in the dark and is associated with challenging ergonomic conditions. In addition, the counting result may be influenced by the bioanalyst's subjective perception of the signal's size and distance between the signals.

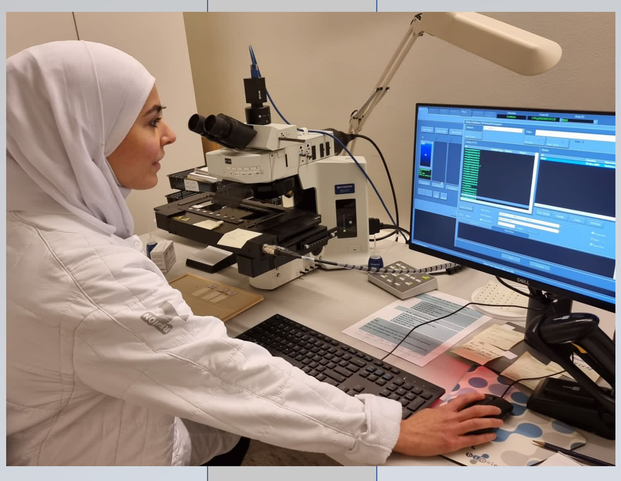
Therefore, department wanted to improve the ergonomic conditions and working environment when performing this function, as well as to standardize and automate FISH analysis.

Results

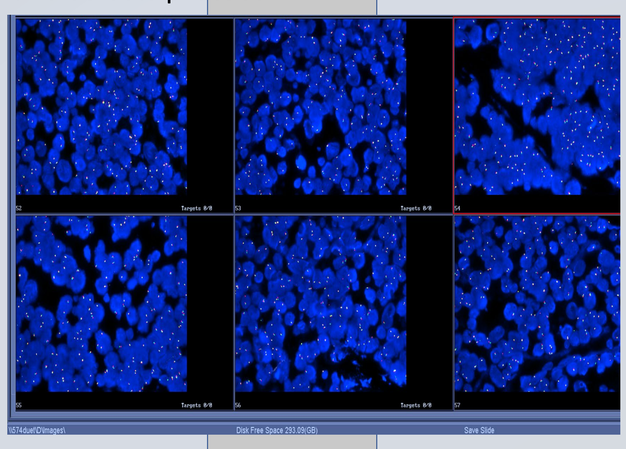
Tests results included 206 slides, where 15 discrepancy cases between manual and machine counting occurred. After troubleshooting and recounting, 6 slides were consistently matched with manual counting. Furthermore, 9 slides out of 206 still showed discrepancy when counting.

Results revealed 4.36% of deviation, while consistency of counting results on the Bioview and using conventional methodology were 95.64% . When applying Cohen's Kappa statistical test, a kappa value of 0.72 was obtained, confirming a substantive correspondence.

Borderline group displayed 4.36% results' uncertainty, which indicate deviation in the counts. It is recommended to retest the cuts from this group again with manual counting.



Bioanalyst puts glass in the machine and selects protocol



Scanned images are saved in image gallery

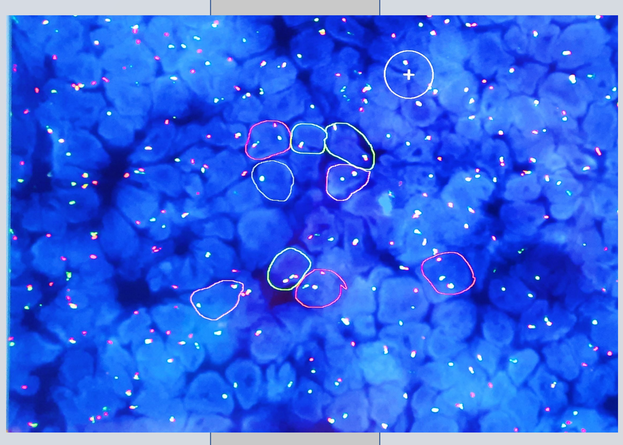
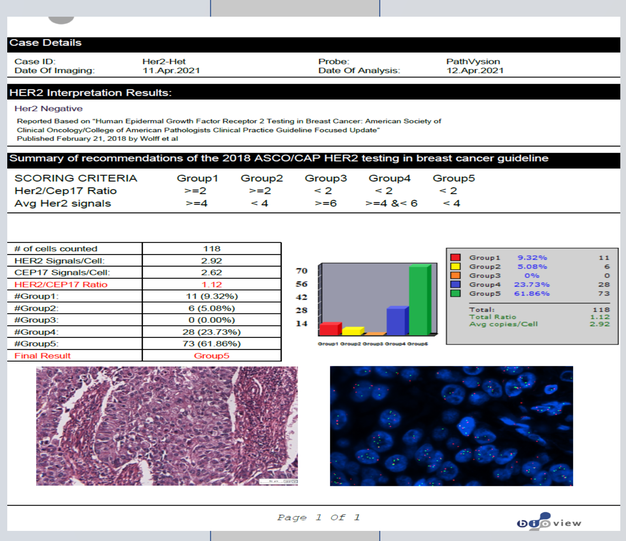


Image processing : bioanalyst selects cells for counting and the machine counts number of signals per nucleus.



The machine prepares a report after the end of the count. The report indicates the number of counted nucleus, ratio and result.

Material and methods

In the Bioview evaluation, we compared the counted results from the conventional fluorescence microscopy with the ones counted on the Bioview Image Analyzer.

We tested on the most widespread FISH probes in our routine BCL2, BCL6, MYCBR, which breaks apart probes and HER2, applied for the diagnosis of HER2 gene amplification.

Signal counting was performed on the Olympus microscope, 100x magnification. Subsequently, the cuts were read on the Bioview and results from the manual- and machine counting were registered. Signal reading took place according to a certain reading criteria and cut-off values.

When discrepancy between the manual and the machine test results occurred, another recount was performed by a different bioanalyst then the one, who carried out the signal counting on the Olympus microscope.

Conclusion

Ultimately, when using the Bioview together with the conventional fluorescence microscopy with a kappa value of 0.72, the counting results were consistent- 95.64%.
Thus, it is concluded that the Bioview is a reliable instrument for digital counting of FISH.

Slides recounting at borderline values is recommended to be perform by an experienced bioanalyst.

Perspectives

In the future, we will apply Bioview for further probes, such as EWSR1, SS18,DDIT3,USP6,NMYC, CMYC, CMET.
We will perform FISH counting on cytological material : smears, MGG stain cuts.