

# Fluorescent in situ hybridization: Utility and limitations for the analysis of melanocytic lesions

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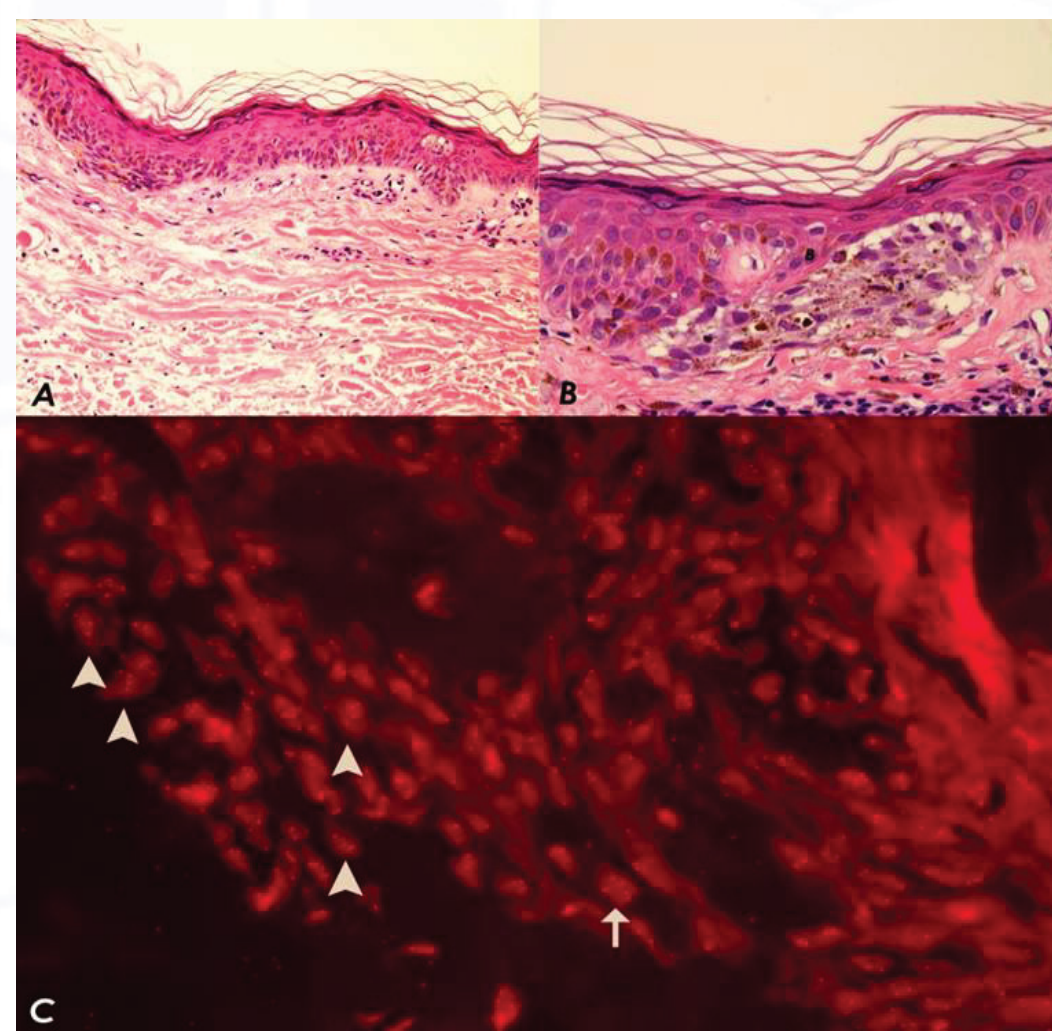
## Introduction:

Fluorescent in situ hybridization is a common diagnostic tool for the determination of malignancy, and Sub-typing of solid tumors (i.e., invasive breast carcinoma, prostate, bladder cancer, etc.) and leukemias (i.e., CML, ALL, CLL, myeloma, etc.). Recently, (FISH) has been utilized to identify atypical melanocytic lesions with gross chromosomal aberrations, as a surrogate marker for progression to melanoma. From a technical perspective, performing FISH for the analysis of melanocytic lesions is particularly challenging. Melanocytes are long lived, subject to constant UV light exposure, and undergo periods where they exhibit a high rate of cell division coupled to replicative senescence. They congregate in small nests and under a DAPI stain look morphologically very similar to several co-localizing cell types. Analysis is technically demanding with the scoring technologist requiring training in both melanocytic histology and FISH analysis. Scoring is compounded by the intrinsic challenges associate with detecting deletions from thin sectioned material, and further complicated by the observation that genetic aberrations are not always found in the most phenotypically worrisome region. Additionally, several authors, including this study have identified a disparately high proportion of tetraploid melanocytic cells via melanocytic FISH analysis.

## Discussion:

Determining the invasive and metastatic potential of a melanocyte by FISH while technically challenging, is of significant and diagnostic value in the analysis of dysplastic melanocytic lesions. Phenotypically, melanocytes form nests which are difficult to identify under DAPI staining. In contrast to analysis of other solid tumors, dysplastic melanocytes are a normal skin complement, which necessitates the scanning and analysis of the entire biopsy, and analysis of several representative clusters to ensure accuracy. Tetraploidy (i.e., 4N) is a common occurrence, and has not been associated with increased risk of invasion or metastatic potential. Inclusion of a tetraploid component leads to false positives and/or high cutoff values, and in our hands, lower assay sensitivity. Analysis of a 4-fluor FISH assay from sectioned material is technically complex, and has required extensive procedural development from the development, pathology and clinical laboratory teams to ensure consistency, and to account for the many variations of possible results.

### Identifying Melanocytes:



Melanocytes congregate in small nests, and under DAPI stain look very similar to several co-localized cell types. The dermatopathologist specified areas are localized using a DAPI filter at low magnification. The tumor area was then thoroughly inspected for the presence of nuclei harboring abnormal copy numbers of any probe. Areas with the most significant copy number changes are manually selected for automated enumeration

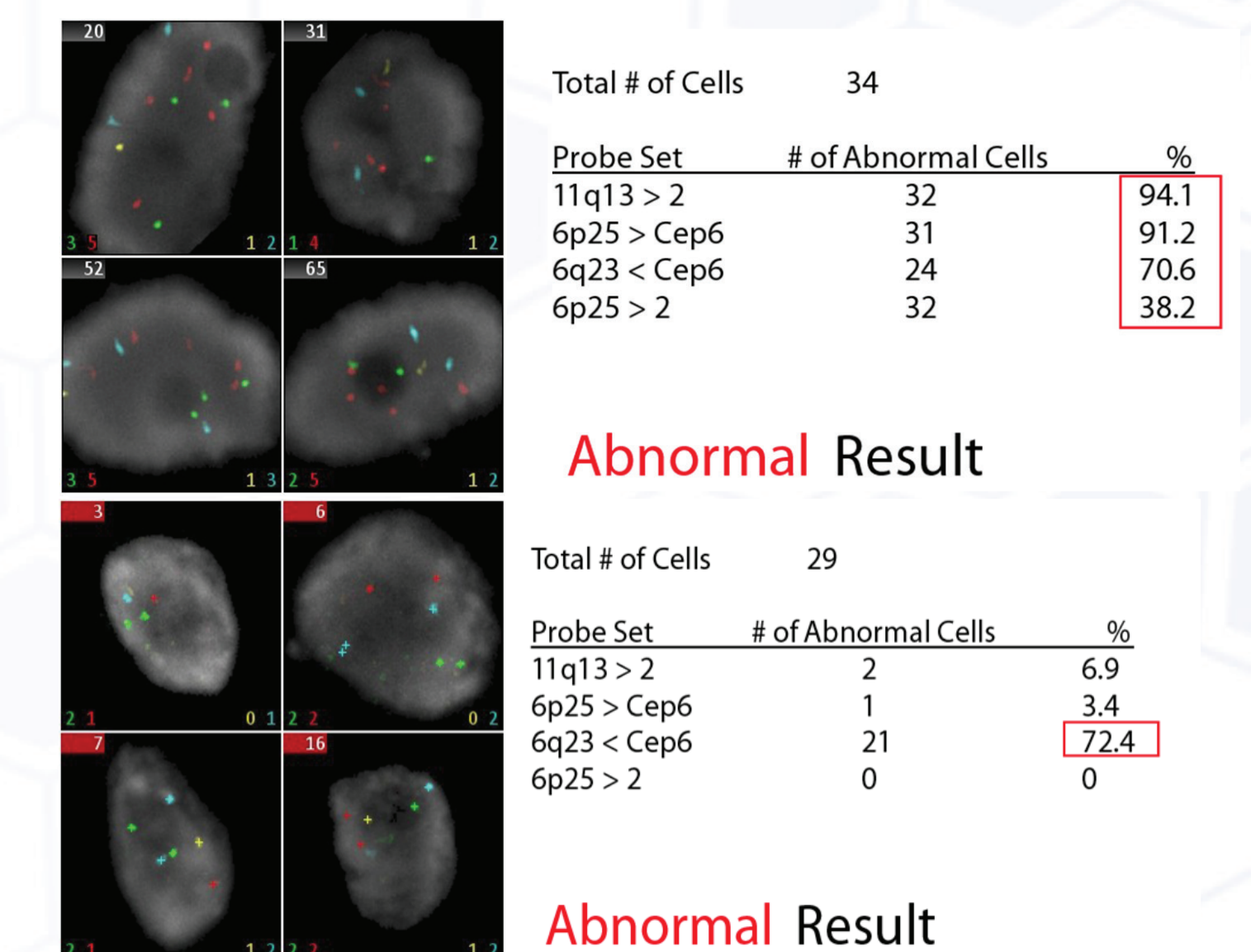
While Dermatopathologists will always provide a scribed H&E, the FISH technologists must be expertly trained to recognize the equivalent melanocyte cells on a DAPI stained FISH slide under 63 to 100 x magnification.

### Scoring the Melanocytes

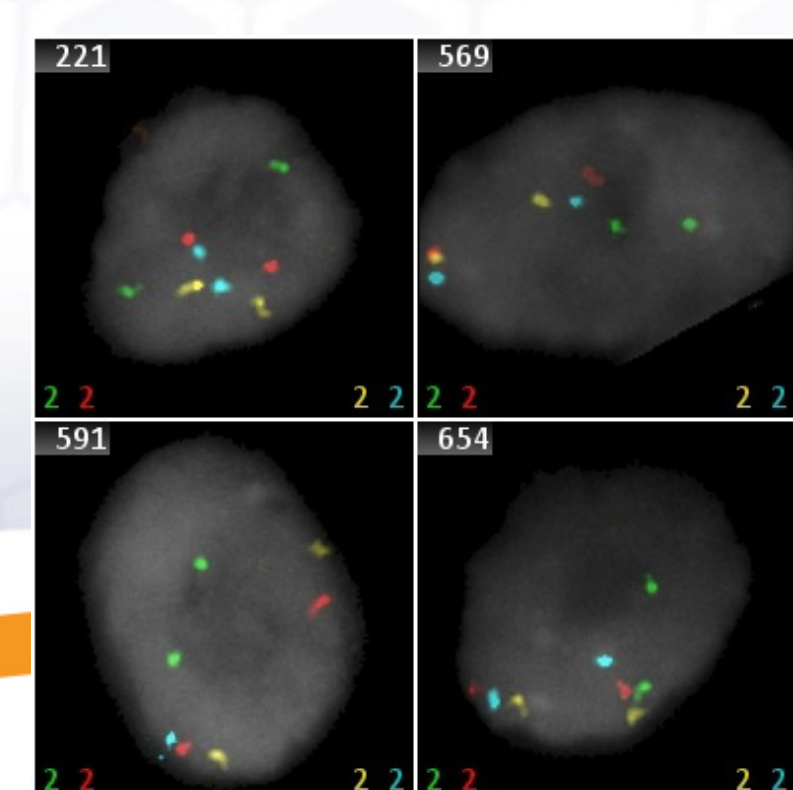


Automated imaging systems are utilized to score several melanocytic nests. Tumor heterogeneity is often observed, with genetic aneuploidy only detected in a proportion of the analyzed nests. Unlike solid tumors which have a clonal origin, melanocytic proliferations are a normal component of the skin structure, and the cells exhibiting the highest phenotypic dysplasia do not always correlate with a positive FISH finding. Heterogeneity between melanocytic nests is expected, necessitating the scanning of the entire slide section, and the optimization of the scoring algorithms to focus on positive nests once identified. Additionally, melanocytes with a tetraploid complement have been shown in a number of studies to have no increased risk of progression to melanoma. Therefore, after a thorough evaluation, tetraploid cells are discarded from analysis.

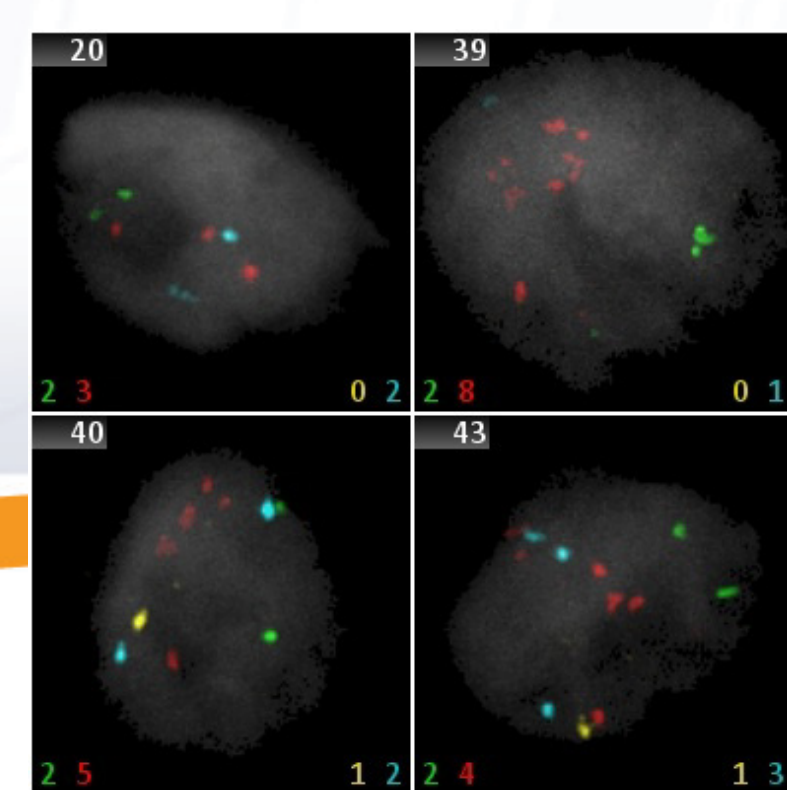
### Result Analysis



Each scored cell is analyzed for a number of quality criteria prior to scoring. Cells are analyzed by nest, and regions with an aneuploid genotype are preferentially scored. Typically a minimum of 40 cells will be analyzed, although the automated FISH instrument routinely includes over 100 cells in the analysis. Even with the assistance of automated systems, a new technologist will require several weeks of training to score this assay consistently.

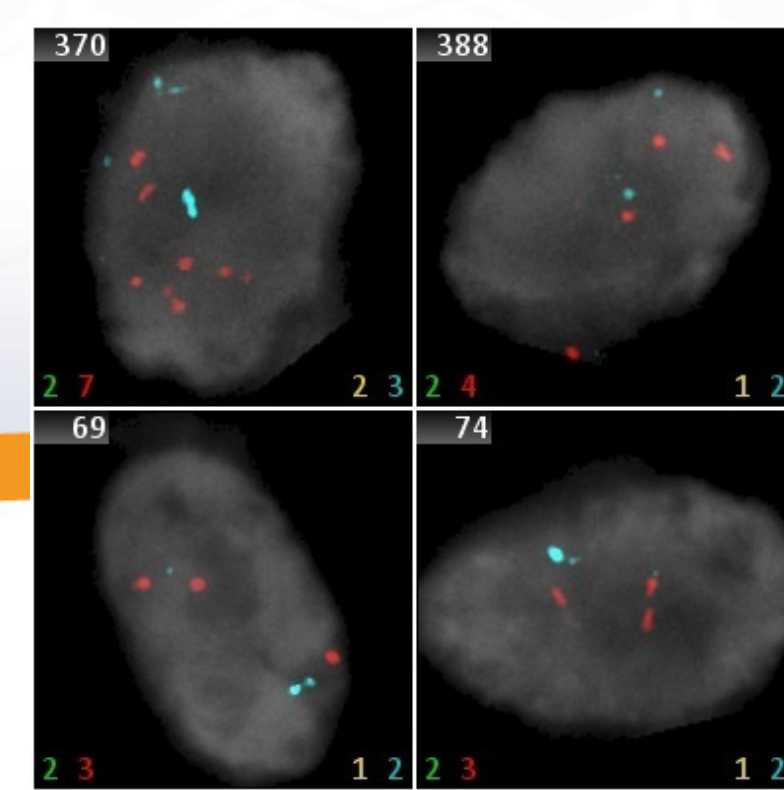


Normal FISH Pattern



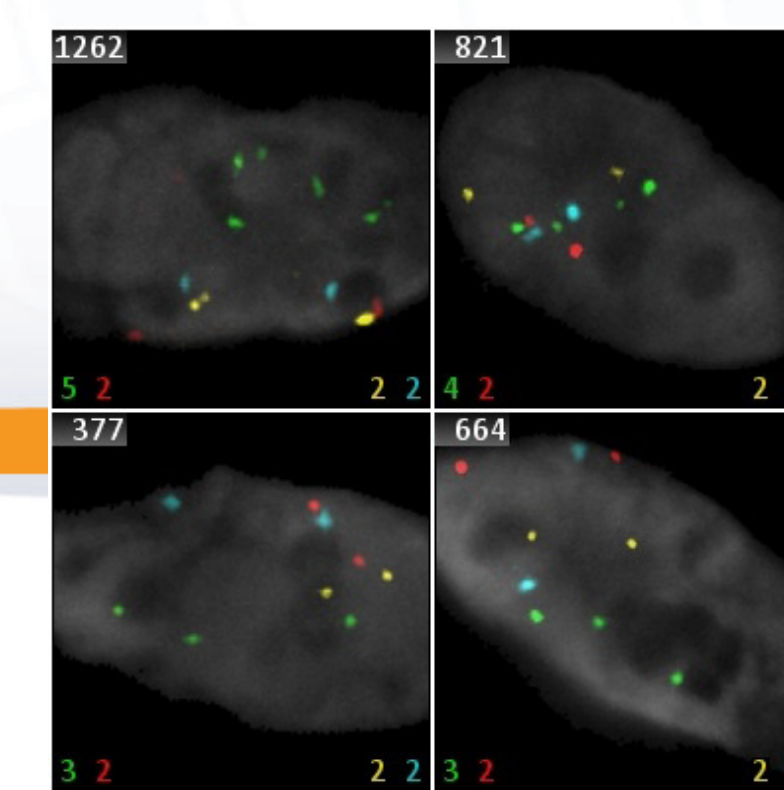
Abnormal FISH Pattern

Increase of 6p25 (red) copies  
+ Decrease of 6q23 (gold):  
Cep6 (aqua) ratio



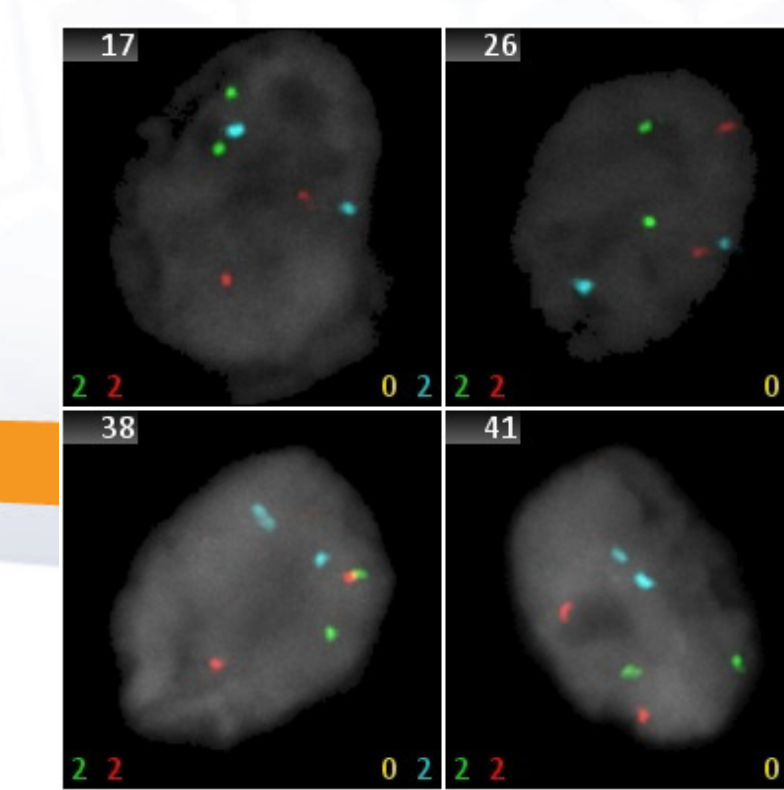
Abnormal FISH Pattern

Increase of 6p25 (red) copies  
+ Increase of 6q25 (red):  
Cep6 (aqua) ratio +  
Decrease of 6q23 (gold):  
Cep6(aqua) ratio



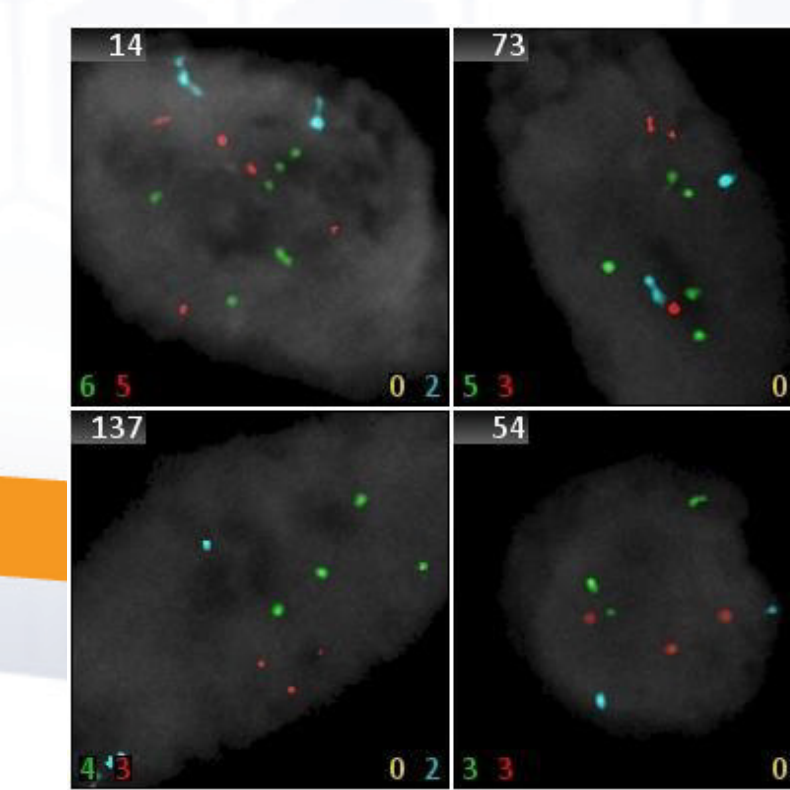
Abnormal FISH Pattern

Increase of 11q13  
(green) copies



Abnormal FISH Pattern

Decrease of 6q23 (gold):  
Cep6 (aqua) ratio



Abnormal FISH Pattern

Increase of 6p25 (red) copies  
+ Decrease of 6q23 (gold):  
Cep6 (aqua) ratio +  
Increase of 11q23 (green) copies