



**Your Partner in
Personalized
Medicine**

Utilization of Innovative Web Based FISH Review and Analysis Solution in Daily Clinical Practice

**Nasdaq
(CGIX)**

V Subhadra Nandula, PhD, FACMG

Associate Director of Cytogenetics
and Molecular Diagnostics



Agenda

- Clinical laboratory, a challenging environment
 - Routine, technical, Scientific
- Addressing the lab challenges using the web based system
- Telepathology
- Addressing Telepathology requirements
- Overview of Bioview Implementation in our Lab

Clinical Laboratory, a Challenging Environment

Routine Challenges

- TAT major challenge in medium sized laboratories
- Resources for a medium size laboratory
 - Between different lab sites
 - Availability of pathologist
- Time consuming analysis

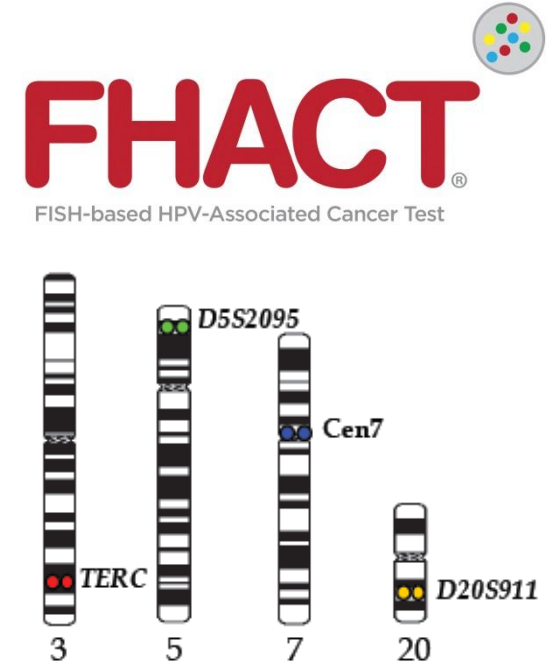
Solutions to Routine Challenges Using Bioview

- TAT major challenge in medium sized laboratories
 - 50 slides loaded to scan during off hours
 - All cases ready for analysis as techs come in
- Resources for a medium size laboratory
 - Resources available across different sites
 - Scanned case allotted across sites
 - Availability of pathologist any time as the case is available for online review
- Time consuming analysis
 - FISH for cervical cancer needs 2,000 cells to be analyzed
 - Case study

New Biomarkers in Cervical Cancer

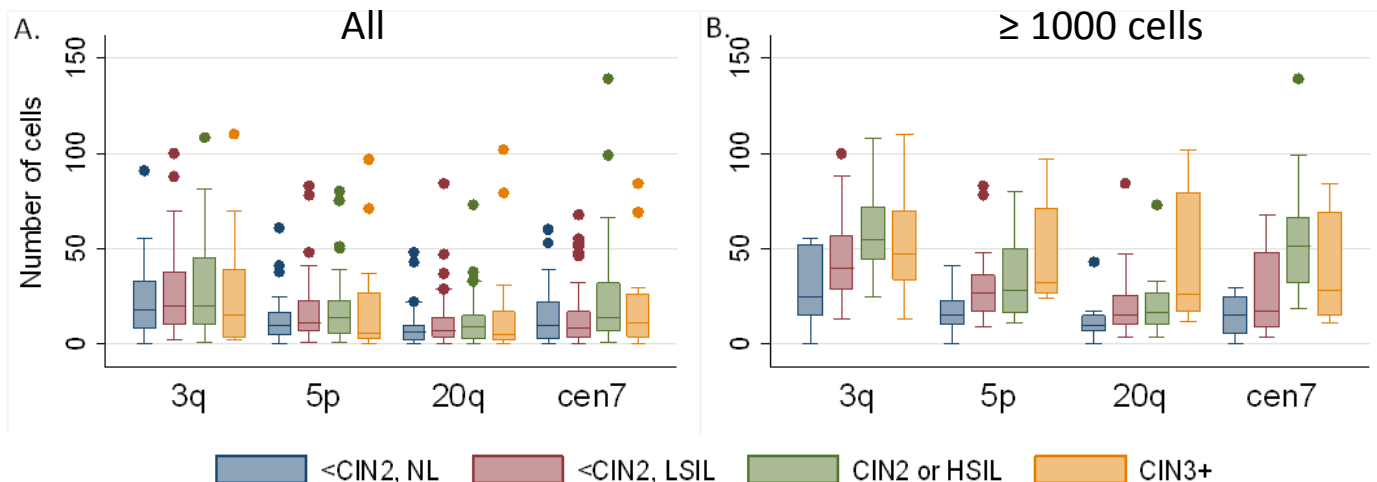
FISH-based HPV-Associated Cancer Test, FHACT®

- **3q26 gain** (red)
- **Cen7** (aqua)
- **5p15 gain** (green) and
- **20q13 gain** (gold)



Impact of Number of Cells Scored on Correlation of Copy Number Changes with Lesion Severity

≥ 3 signals per cell = positive for gain, irrespective of pattern



Differences in distributions and trends in the number of cells with chromosomal abnormalities across disease categories were determined using Kruskal-Wallis tests and nptrend, respectively.

At least 1,000 cells should be scored

Overview of Cervical Cancer FISH Analysis

Manual Analysis

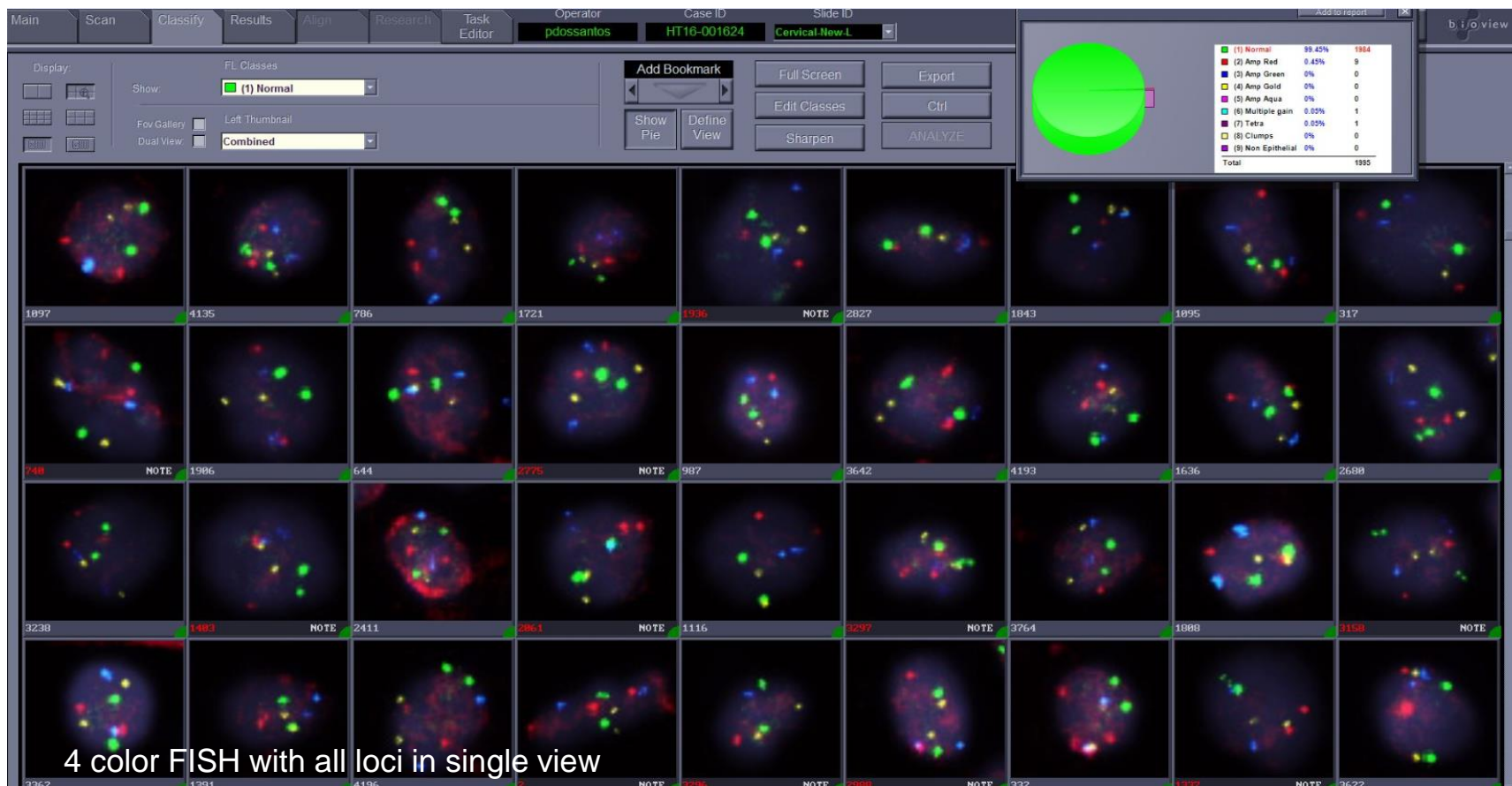
- 2000 cells scored
- 4 loci scored
- 2 techs needed
- 1.5-2hrs per case
- Low level gains additional cells reviewed or the entire slide reviewed
- Case analysis limited to the number of microscopes

Automated Analysis

- 2000 analyzed cells scored
- 4 loci present in the same view
- Cells categorized based on signal patterns
- 1 tech needed
- 30 mins per case
- Additional non analyzed cells are reviewed as a part of regular analysis
- Case analysis limited to the number of review stations or unlimited with a terminal server

Case Study 1

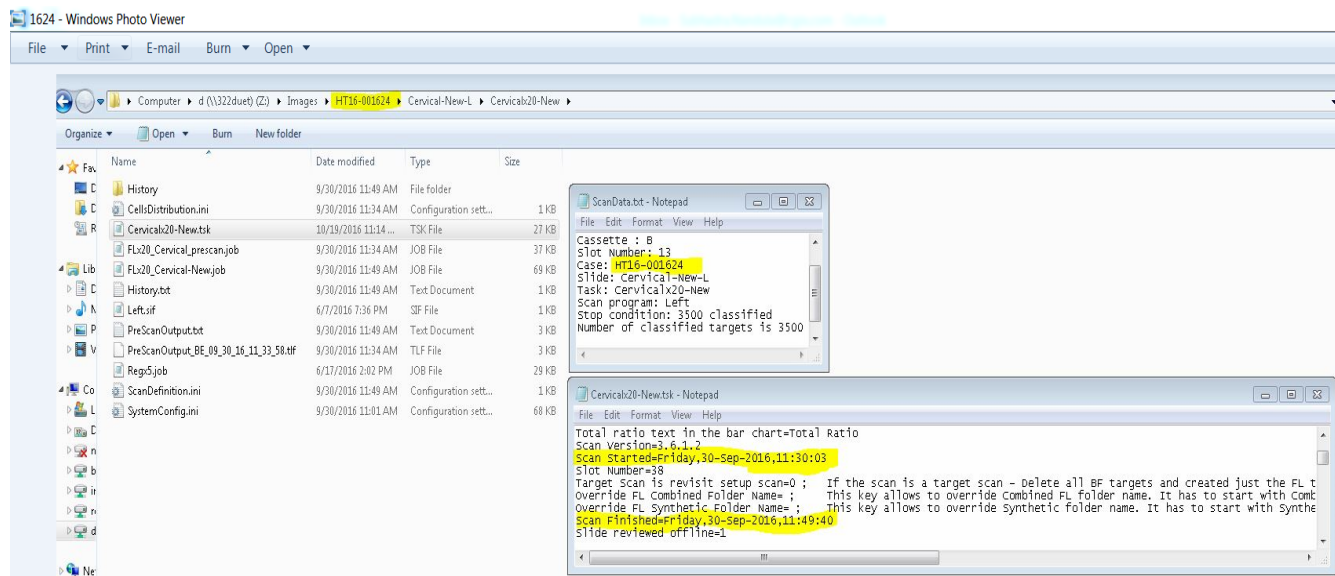
Cervical Cancer FISH (Normal)



Case Study 1

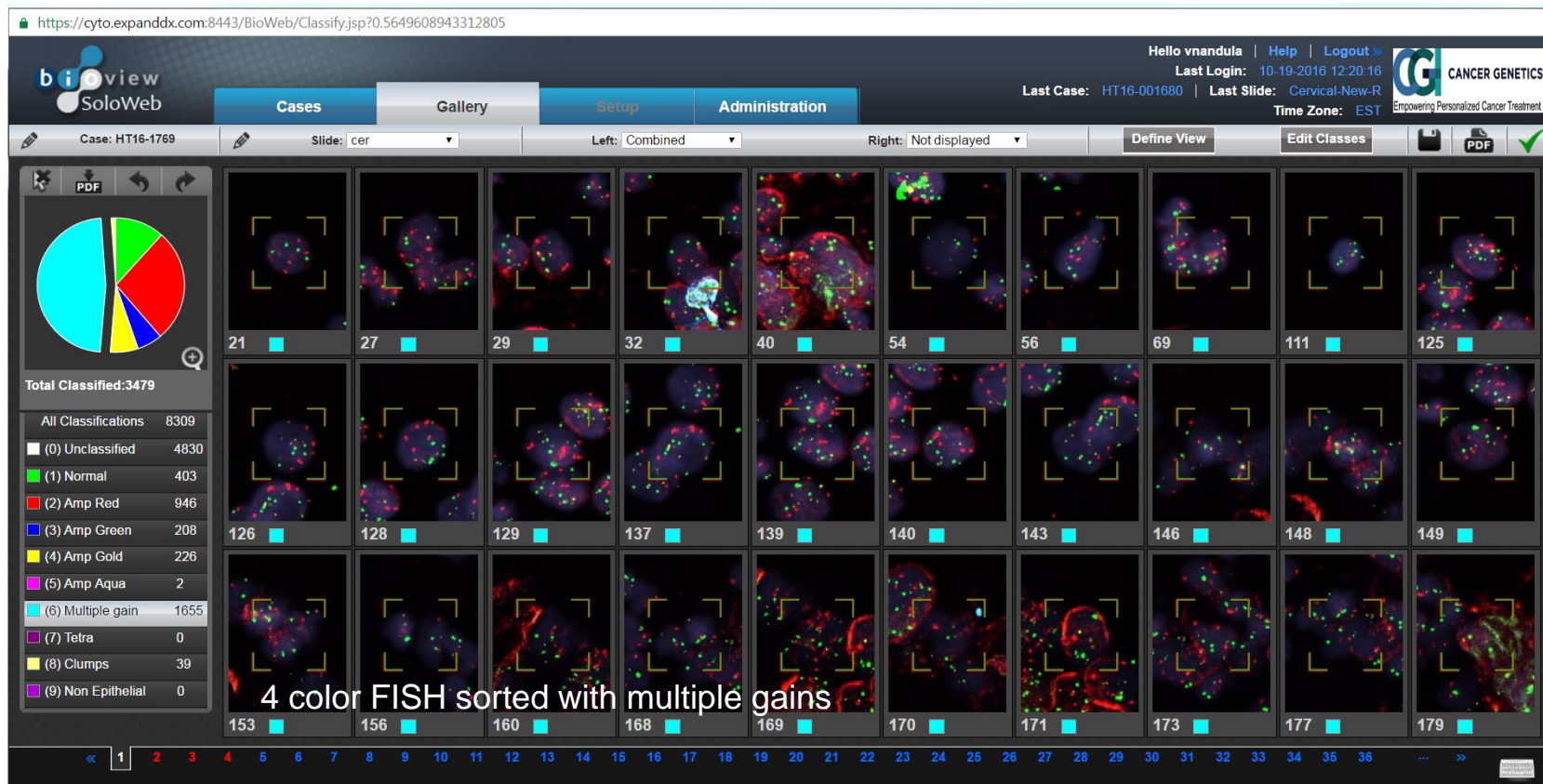
Cervical Cancer FISH

Scan and analysis time



Case Study 2

Cervical Cancer FISH (Abnormal)



Case Study 2

Cervical Cancer FISH

Scan and analysis time

The screenshot shows a Windows File Explorer window with the address bar set to `d:\322duet\Z:\Images\HT 16-001769\cer`. The file list includes folders like Aqua, Cervicalx20-New, Combined, Gold, Green, Red, Synthetic, and Target, as well as files like CellsDistribution.ini, CurrentTask.txt, Graphs.txt, rdnl.sys, SlideNotes.txt, TargetList.tif, and various TargetList_*.tif files. Two Notepad windows are open. The first, titled 'ScanData.txt - Notepad', contains the following text:

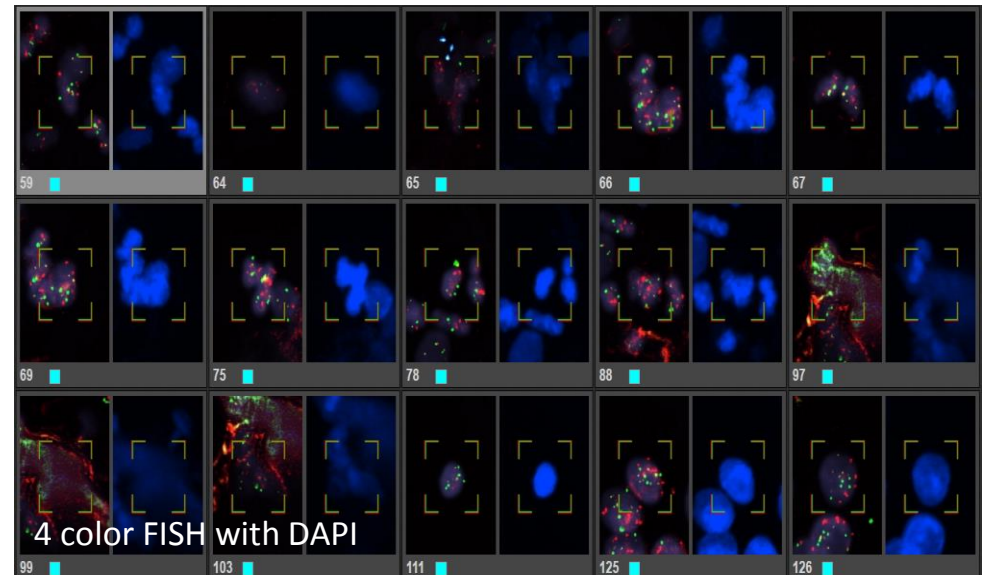
```
1. Manual Slide
Template: 99
Case: MDsexample
Slide: Cer
Task: Cervicalx20-New
Scan program: cerv
Stop condition: 3500 classified
Number of classified targets is 3500
Total number of targets is 6136
```

The second Notepad window, titled 'Cervicalx20-New.tsk - Notepad', contains the following text:

```
Show comments On Pie=0 ; comments are added in the Classification section in the format - classify1 comment
Sort History List By Numbers=0 ; Sort the history list by numbers. Combined are 1st and synthetic are last. Default = 0
Show total percentage instead of total ratio=0 ; In the bar chart show total percentage instead of total ratio - default 0
Total ratio text in the bar chart=Total Ratio
Scan version=3.6.1.2
Scan started=Monday,17-Oct-2016,13:39:21
Target Scan is revisit setup scan=0 ; If the scan is a target scan - Delete all BF targets and created just the FL targets in the BF tar
Override FL Combined Folder Name= ; This key allows to override Combined FL folder name. It has to start with Combined (case sensitive)
Override FL Synthetic Folder Name= ; This key allows to override Synthetic folder name. It has to start with Synthetic (case sensitive)
Scan Finished=Monday,17-Oct-2016,13:55:49
```

In Summary Bioview Brings Solutions to time consuming analysis

- Reduction in scan time
- Sort cells based on gains/quality/size of the cells
- Viewing all the four loci on the same plane
- Ease to view multiple loci in a single view increasing sensitivity for low level multiple gain
- Ease to eliminate Clusters/overlapping cells from analysis
 - DAPI image to identify overlapping cells



Clinical Laboratory, a Challenging Environment

Technical Challenges

- Improve sensitivity to improve patient outcome
- Background noise vs the signal strength
- Choosing the right regions for FFPE FISH
 - Reviewing the images corresponding to a region of interest

Solutions for Technical Challenges using Bioview

H&E scan and identifying the regions of interest

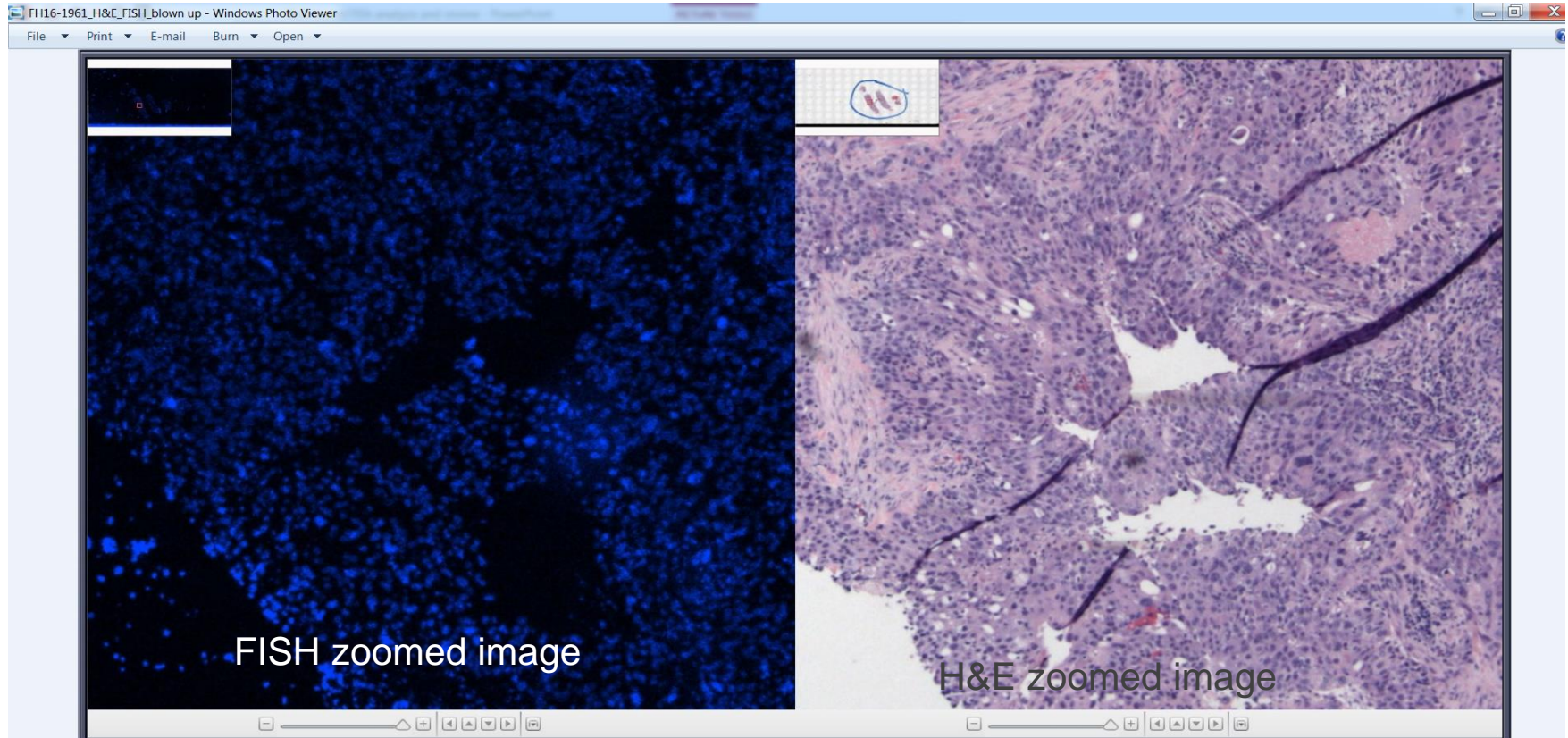
The screenshot displays the Bioview software interface. On the left, a large H&E scan is shown with a green outline and a black rectangle indicating a region of interest. Below the scan, a classification legend is visible:

- Total Classified: 60
- Total Ratio: 4.08
- Avg copies/Cell: 9.93
- All Classifications: 60
- (0) Unclassified: 0
- (1) 0-1.8: 0
- (2) 1.8-2.0: 0
- (3) 2.0-4.0: 45
- (4) 4.0-: 15

The main area shows a grid of 15 small images, numbered 1 to 15, each displaying a different region of interest. The interface includes a top navigation bar with tabs for Cases, Gallery, Setup, and Administration. The top right corner shows user information: Hello vnanula | Help | Logout. The bottom right corner shows the last case: Sales-Her2_B-abn | Last Slide: 1342HER2 | Time Zone: EST. The bottom left corner shows the slide number: 1.

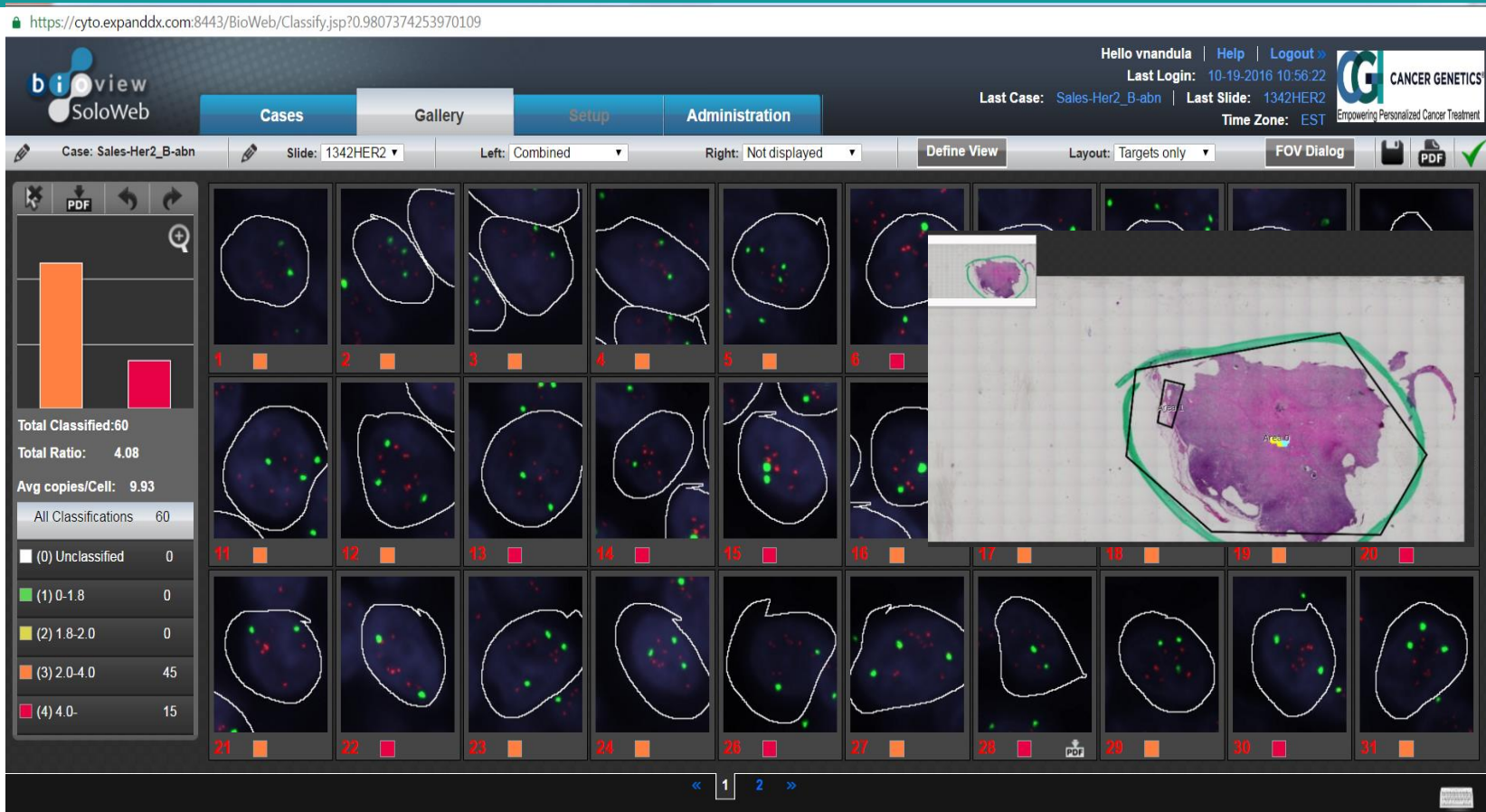
Solutions for Technical Challenges using Bioview

H&E and DAPI Scan (Tissue Matching)



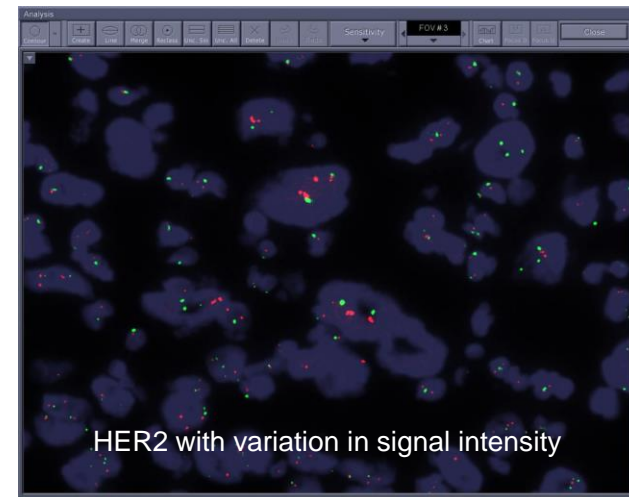
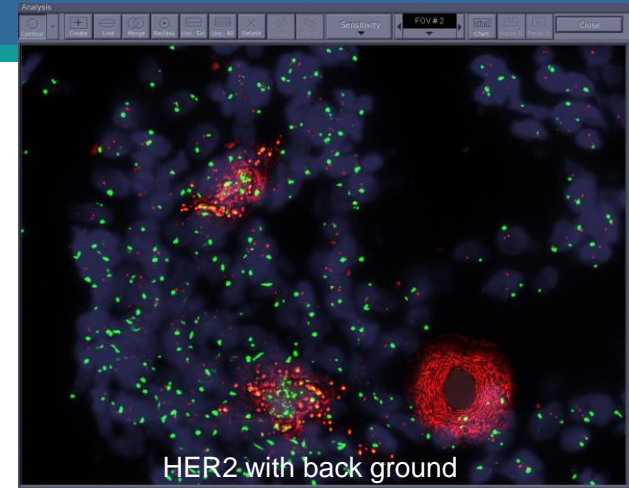
Solutions for Technical Challenges using Bioview

Review of FISH Images



New Technical Challenges with the Use of Bioview and Their Solutions

- Background signals vs actual signals
 - Ability to view and scroll each fluorophores under different planes
- Variation in the signal intensities
- Choosing cells from the representative regions
 - Possibility to review the cell distribution with signal patterns



Clinical Laboratory, a Challenging Environment

Scientific Challenges

- Equivocal cases in HER2 FISH
 - True Equivocal
 - Equivocal due to cherry picking cells
 - Possibility to review multiple FOVs
- Genetic heterogeneity in HER2
 - Possibility to review H&E again during FISH analysis
 - Matching H&E FOVs with the FISH FOVs

Scientific Challenges Solutions Using Web Based FISH Capture and Analysis System

- **Equivocal cases**

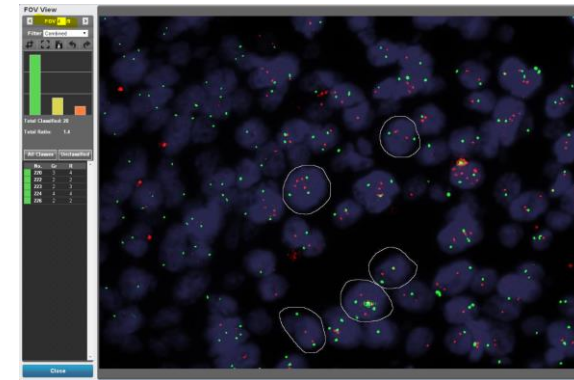
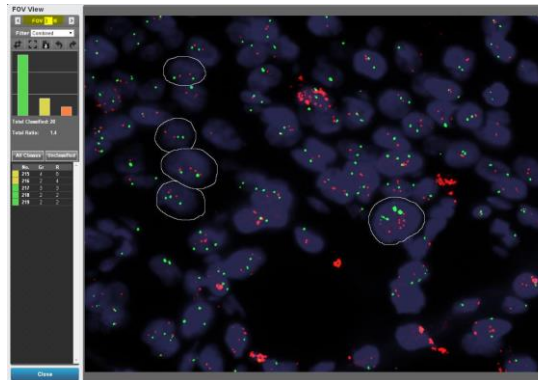
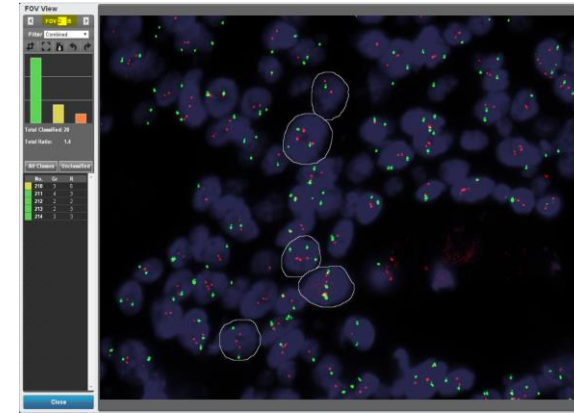
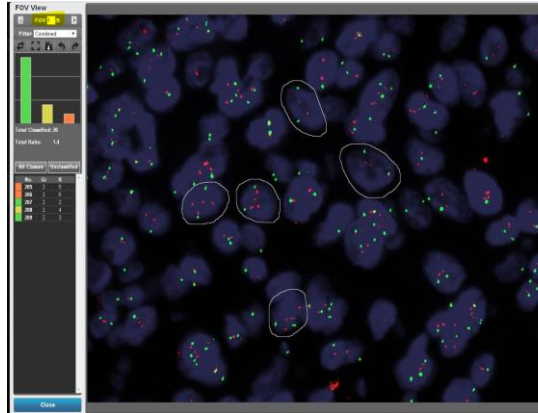
- True Equivocal vs false positives
 - Possibility to review multiple FOVs
 - Choosing the representative cells

- **Genetic heterogeneity**

- Possibility to review H&E again after FISH analysis
- Matching H&E FOVs with the FISH FOVs
- Web-based H&E review with identification of the area of interest
- Case study

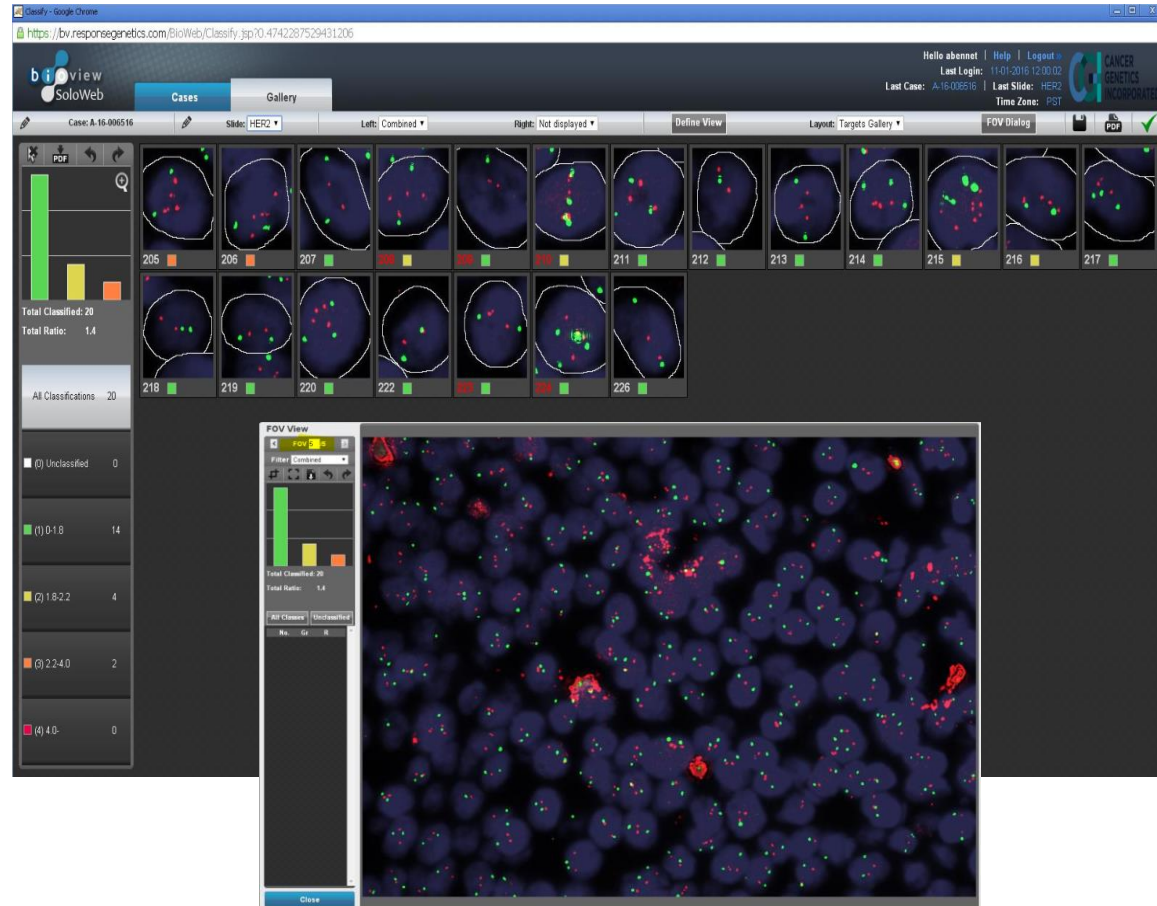
Case Study 3

- Case is IHC 2+ equivocal
- FISH results are non-amplified (1.4 ratio and 3.5 avg HER2 copy number)
- The 20 cells circled are taken from FOV 1-4
- FOV 5 is also from within the inscribed area on the slide (and many other FOVs), but all cells here are 2G2R and don't exemplify the IHC 2+ results



Case Study 3

- FOV 5 is also from within the inscribed area on the slide (and many other FOVs), but all cells here are 2G2R and don't exemplify the IHC 2+ results
- FOV5 vs the Case Analysis Based on FOV1-4



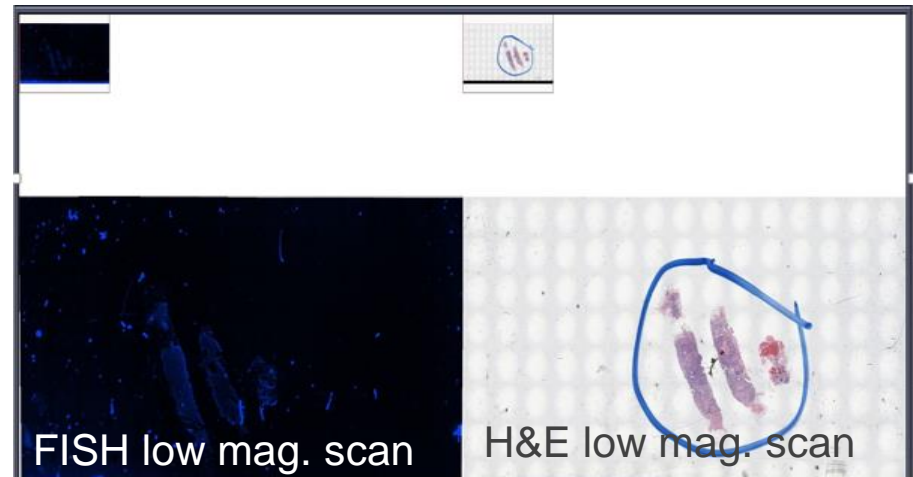
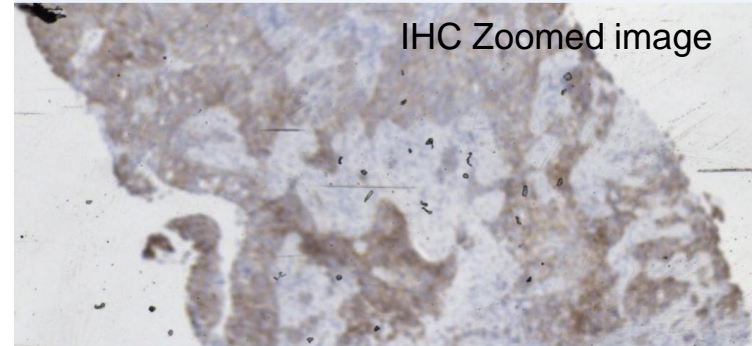
Scientific Challenges

Genetic Heterogeneity for HER2

- Intra tumoral heterogeneity
- Sub clonal diversity
- HER2 – 5% - 30%
- Increases subjectivity in HER2 interpretation by pathologist

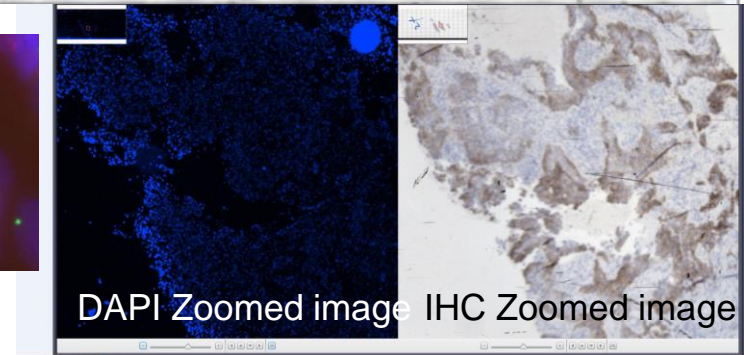
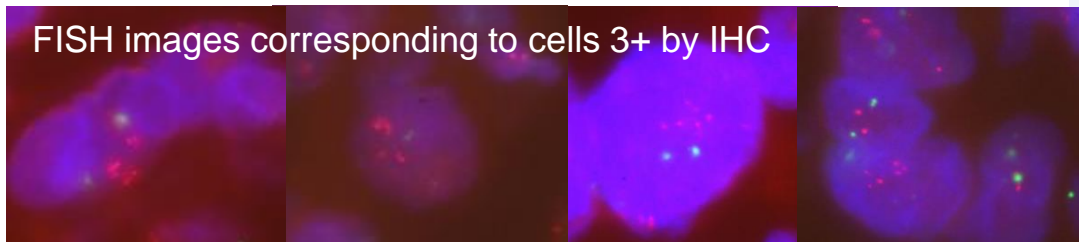
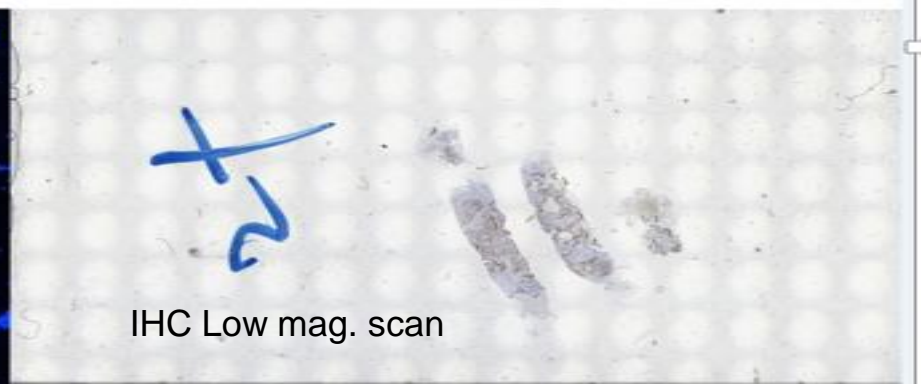
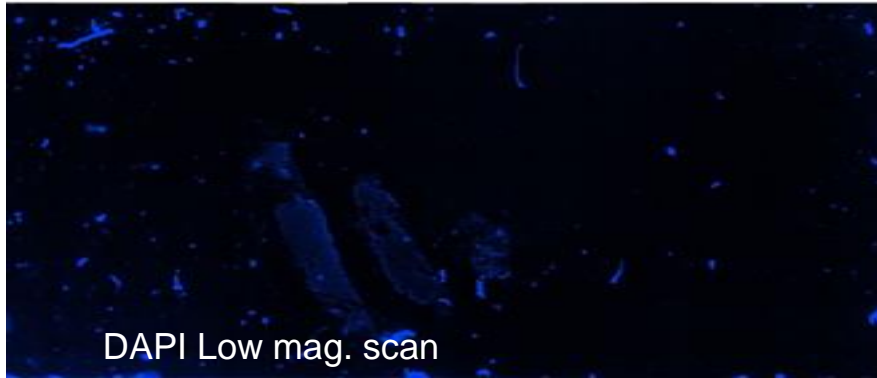
Case Study 4

- IHC for her2neu called as 2+ and FISH
- It showed scattered positive cells (3+)
- Tumor region marked
- Review of the cells corresponding to IHC or H&E slide
- Cells positive for HER2 by IHC – amplified by FISH



Case Study 4

IHC, H&E and DAPI Image – Corresponding Cells with FISH Signals



Addressing GH Using Web Based System

- Whole tumor region scanned
- Scanning performed under 20X
- Quick review of the low magnification scan
- Identifying the representative region in case of GH
- Pathologist can re-review the scan in case of discrepancy with IHC or in challenging cases

In Summary Bioview's Web Based FISH Review and Analysis system brings solutions to Scientific Challenges

- Tissue matching between H&E and DAPI image is highly beneficial in equivocal cases and cases with genetic heterogeneity
- Reduction in review time
- Ease to view multiple loci in a single view
- Ease to eliminate Clusters/overlapping cells from analysis

Telepathology

Web based FISH

- Patient identification - Slide/Image ID
- Access to clinical information during analysis of the case
- Telepathology training
- Patient confidentiality
- Result documentation
- Quality management program


Addressing Telepathology Requirements

- **Patient identification - Slide/Image ID**
 - Bar coding
- **Access to clinical information during analysis of the case**
 - All the specimen information is present in the clinical history for reference
- **Telepathology training**
 - The technologists are trained and documents details of training is provided by Bioview who is assigned as champion
 - The champion trains all the pathologists and other techs who perform sign out and analysis respectively

Addressing Telepathology Requirements Contd.

- **Patient confidentiality**

- System and user authentication is achieved by password protected user names for each remote pathologist
- System provides the activity logs
- Access restrictions is provided based on the user level
- The images reside at the host server and users are given access via a password protected user name



Login

User Name:

Password:

Login

[Forgot Password >>](#) [Change Password >>](#)

Case Notes:				Edit Case Details
Slide Name	Date	Assigned to:	Status	
1342HER2	28 Aug 2015	pdossantos	Ready For Archive	30

Activity Logs			
1.8-2.0	0	(0%)	
2.0-4.0	45	(75%)	
4.0-	15	(25%)	
Total Targets	60		
Total Ratio	4.08		
Avg copies/Cell	9.93		

3	10-19-2016, 11:26:37 Assigned By:vrandula Assigned To:pdossantos	Status:From Ready for path review Notes:Review completed	To Ready For Archive
2	10-19-2016, 11:24:51 Assigned By:pdossantos Assigned To:vrandula	Status:From Ready for path review Notes:	To Ready for path review
1	10-26-2016, 13:43:30 Assigned By:N/A Assigned To:salesdemo	Status:From N/A Notes:	To Ready for path review

Show all

Addressing Telepathology Requirements Contd.

- **Result documentation**

- Diagnostics and specimen adequacy results are documented in the system
- Report is generated consisting of the results of the review of the images/slides

- **Quality management program**

- The laboratory is required to include QM plan
 - The system provides the statistics of TAT, inadequate, normal and abnormal cases
 - Comparison to onsite analysis can be performed by making copies of the case.

bio view

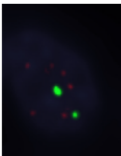
Case: Sales-Her2_B-abn

ERBB2 (HER2/neu) FISH REPORT			
Patient Name:	S.M.	Accession #:	Sales-Her2_B-abn
Sex:	Female	Date:	DR. Nandula
DOB:	28-Aug-2015	Physician:	
Specimen:	Nt tissue	Block ID #:	SSG-0008
Received Date:	28-Aug-2015		
Reported Date:	19-Oct-2016		
Clinical Use:	Invasive ductal Breast/Met A C		

Summary: POSITIVE FOR HER2/neu GENE AMPLIFICATION

Interpretation: AMPLIFIED: The fluorescence in situ hybridization (FISH) analysis showed AMPLIFIED signal patterns for HER2/neu both by CEP17/CEP17 ratio and HER2/SIGNAL chromia.

Number of targets	60
Total CEP17	146
Total Her2/neu	596
Focal Ratio	4.08
Her2/NeuCell	9.93
Number of Randoms	1



CEP17 (green)(HER2/neu red)

Tumor area was analyzed by pathologist on H&E and identified appropriate area(s) for scoring

Updated HER2/CEP17 Guidelines of ASCO/CAP 2013:

- An AMPLIFIED result should have HER2 gene copy/Nucleus of 5 or more or HER2/CEP17 ratio of 2 or more.
- An EQUIVOCAL result should have HER2 gene copy/Nucleus of 4 or more and less than 6 AND HER2/CEP17 ratio of less than 2.
- A NORMAL result should have HER2 gene copy/Nucleus of greater than 4 AND HER2/CEP17 ratio less than 2.

FISH testing for HER2/neu (amplified cancer) (Abbott and Vysis), ALU Break Apart test for Non-Small Cell Lung Cancer (NCCN), DOB for microsatellite instability, TP53/MLH1 & D13S1851/MSI2/CDKN2A/p16INK4a were performed at Cytochrome Laboratories as approved by Cancer Genetics Inc. as required by CLIA regulations. This test has been cleared or approved by the specific state for the use and performance of this laboratory. The CLIA certificate number for this laboratory is: 04D0000001. This test is not used for clinical diagnosis. Genetic Counseling Inc., 222 Maple Street, Waltham, MA 02451, Phone Number: 888-339-4888, Email: info@geneticcounseling.com, Fax: 781-932-1100

Image (click on for reference only)

Intended Use and Assay Description:

The human epidermal growth factor receptor 2 gene ERBB2 (commonly referred to as HER2/neu) is amplified in approximately 15% to 20% of breast cancers. HER2 positivity is associated with worse prognosis (higher rate of recurrence and mortality) in patients with newly diagnosed breast cancer who do not receive any adjuvant systemic therapy. The purpose of HER2 testing is stratification of patients who could benefit from effective HER2-targeted therapies.

The HER2 human gene FISH Probe Kit is designed to detect labeled DNA of the HER2-neu gene via fluorescence in situ hybridization (FISH) in formalin-fixed, paraffin-embedded (FFPE) tissues, in primary, recurrent & metastatic human breast cancer tumors. The HER2 DNA probe kit consists of two labelled DNA probes. The LSI Her2 labeled in spectrum orange scans the whole of Her2 gene. The control probe, CEP17, is located at centromere 17 (17p11.1-q11.1). Clinical HER2 FISH testing has, more recently, been employed in gastric adenocarcinoma including metastatic gastric or gastric esophageal junction adenocarcinoma, and less commonly in lung cancer NSCLC.

Pre-analytically, specimen used for HER2 testing (cytologic specimens, needle biopsies, or resection specimens) should begin the fixation process quickly (time to fixative within 1 hour) and is fixed in 10% neutral buffered formalin for 6 to 72 hours.

References:

- Watt, A. et al. JCO November 1, 2013 vol. 31, no. 35: 4013 Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer. American Society of Clinical Oncology College of American Pathologists Clinical Practice Guideline Update.
- Zhang MC et al (2012) "Genetic heterogeneity" in HER2/neu testing by fluorescence in situ hybridization: A study of 2,522 cases. Mol Pathol 20(3):343-346
- Shah SS, et al. 2008. Effect of high copy number of HER2 associated with polyomy 17 on HER2 protein expression in invasive breast carcinoma. Diagn Mol Pathol 19:346-350
- Rochon P, et al. Mod Pathol 2012 May;25(5):657-658 HER2 testing in gastric cancer: a practical approach.

Electronically signed on: Wednesday, 19-Oct-2016, 11:27:09 By: Subhadra NV.

FISH testing for HER2/neu (amplified cancer) (Abbott and Vysis), ALU Break Apart test for Non-Small Cell Lung Cancer (NCCN), DOB for microsatellite instability, TP53/MLH1 & D13S1851/MSI2/CDKN2A/p16INK4a were performed at Cytochrome Laboratories as approved by Cancer Genetics Inc. as required by CLIA regulations. This test has been cleared or approved by the specific state for the use and performance of this laboratory. The CLIA certificate number for this laboratory is: 04D0000001. This test is not used for clinical diagnosis. Genetic Counseling Inc., 222 Maple Street, Waltham, MA 02451, Phone Number: 888-339-4888, Email: info@geneticcounseling.com, Fax: 781-932-1100

Page 1 of 2

Page 2 of 2

Innovative Web Based FISH Solution

Implementing Bioview in our lab

- Requirements and comparison with other systems
- High resolution images under low magnification
- Ability to handle large image files
- Provided by Bioview
 - Automated Microscope with 50 slide loader with a shock absorbance Equipment
 - In-house high capacity server
 - Review stations

Validation of the System

Validation of System for Internal Use

System validated against manual analysis

- Training the techs on usage of the system
- The number of cells to be captured
- The default signal patterns to be analyzed
- The review of all the signal patterns by the Tech
- Reporting of the case

Compliance

CAP requirements

- Slide /Image ID
- Clinical information access
- Telepathology training
- Telepathology and confidentiality (HIPAA)
- Telepathology result documentation
- Remote analysis and remote reporting validation

Conclusion

- Bioview brings solutions for some of the challenges in the Molecular cytogenetic laboratory
- CAP compliance, HIPAA requirements, 21 CFR Part II (Electronic Signature)
- Resources and staffing
- Scientific Challenges
- Market outreach

For further information, please contact us at contact@cgix.com



Thank you

Nasdaq
(CGIX)

RUTHERFORD, NJ

CGI Headquarters
201 Route 17 North
Rutherford, NJ 07070

Phone: +1 201-528-9200

RALEIGH, NC

Research Triangle Park
133 Southcenter Court
Morrisville, NC 27569

Phone: +1 919-465-0100

LOS ANGELES, CA

1640 Marengo Street
Fourth Floor
Los Angeles, CA 90033

Phone: +1 323-224-3900

HYDERABAD, INDIA

#3-1-135/1A CNR Complex
Mallapur Main Road, R.R. Dst.
Hyderabad – 500 076, Telangana

Toll-free: +91 040-2717-8178

SHANGHAI, CHINA

781 Cai Lun Road, Room 803
Shanghai 201203
P.R. China

Toll-free: +91 040-2717-8178