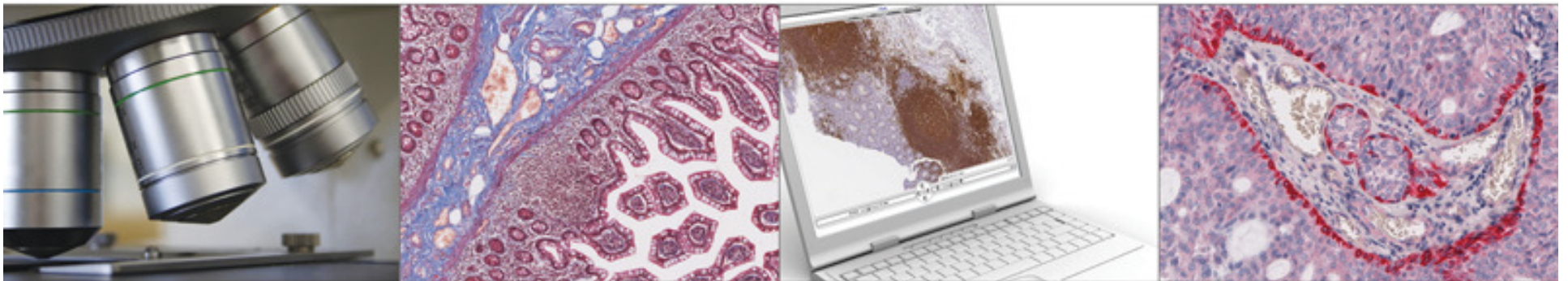
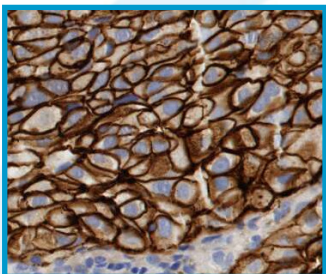
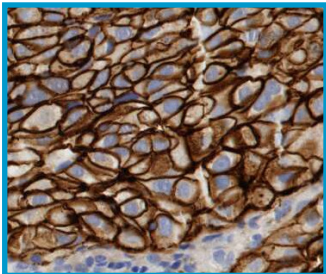
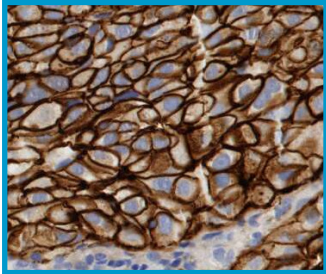




Application of image analysis as an adjunct to manual evaluation of HER-2 status: a diagnostic tool to standardise interpretation.





Personalised Medicine- Introduction

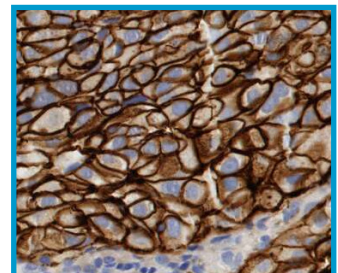
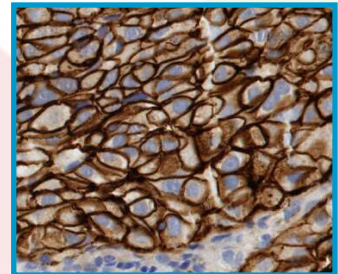
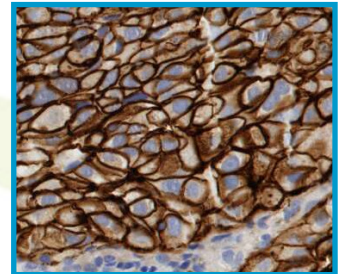
- Targeted therapeutics or personalised medicine regimes are driving a new era of integrated diagnostics and therapeutics, particularly in the oncology domain.
- Many anti-cancer drugs have been approved in association with companion tests for biomarker expression to identify the most response patients.
- Ensures that accurate evaluation of biomarker status has become particularly acute in the clinical laboratory.

FDA approved targeted therapies and biomarkers

Drug	Disease	Biomarker	Companion Test
Cetuximab	Metastatic colorectal cancer	EGFR	IHC, FISH
Imatinib Mesylate (Gleevec)	Gastro-intestinal stromal tumour	Bcr/abl, c-kit	IHC
Bevacizumab (Avastin)	Colorectal cancer	VEGF	IHC
Retuximab	Non-hodgkin lymphoma	CD20	Immunophenotyping, IHC
Gemtuzumab- ozogamicin	AM Leukemia	CD33	Immunophenotyping, IHC
Alemtuzumab (Campath)	BCL Leukemia	CD52	Immunophenotyping, IHC
BMS 354825	GIST	Kit	IHC
Trastuzumab (Herceptin)	Metastatic breast cancer	HER-2	IHC, FISH

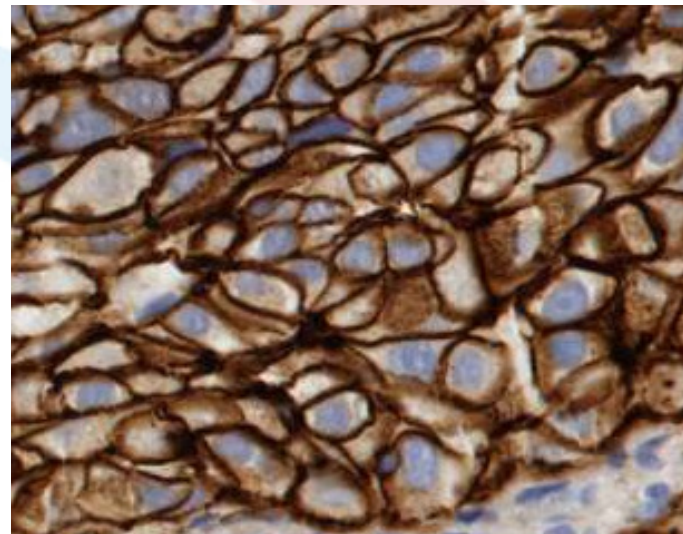
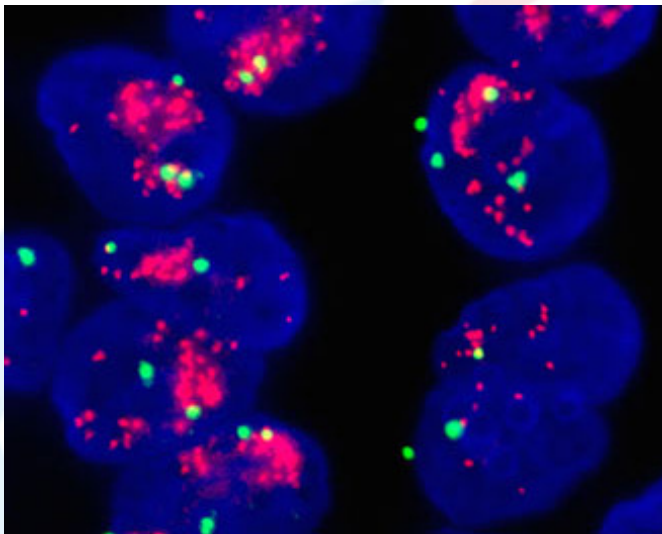
Clinical significance of HER-2

- Over-expressed in approx 25% cases
- Biomarker associated with disease progression and poor prognosis
- Herceptin developed by Roche/Genentech that targets HER-2 receptor
- Significantly improve prognosis of HER-2 positive breast cancer patients reducing risk of relapse by 50%
- Use restricted
 - expense of treatment: £20,000 per year per patient
 - risk of cardio-toxicity
- Assessment of HER-2 status key focus of BMS and pathologists to identify patients for whom drug would be of most benefit



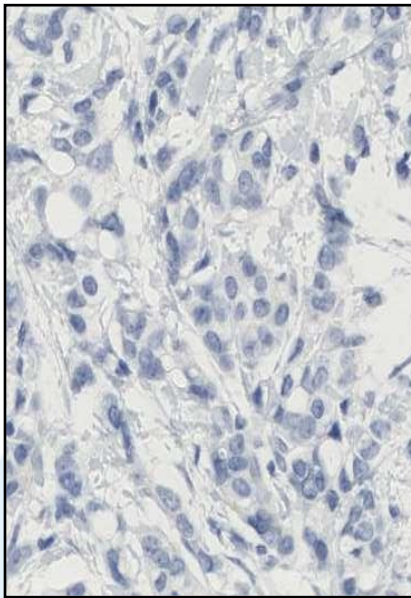
Evaluation of HER-2 status

- FISH (gene amplification)
 - requires specialised equipment
 - fading fluorochromes
 - poor long term stability
- IHC (protein expression)
 - readily available
 - easily performed
 - advantages speed/ economics/ automation



Negative

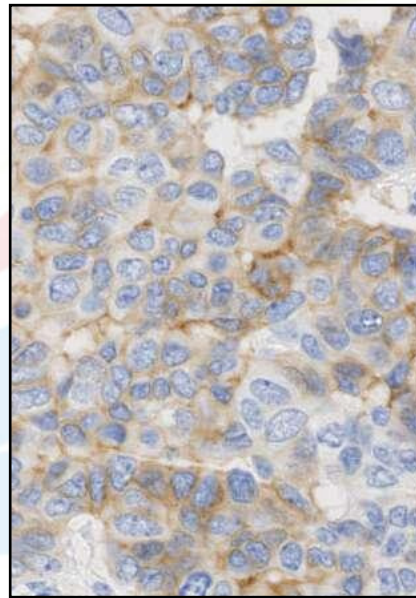
0/1+



- Faint staining in <10% tumour cells
- Cells only stained in part of membrane

Equivocal

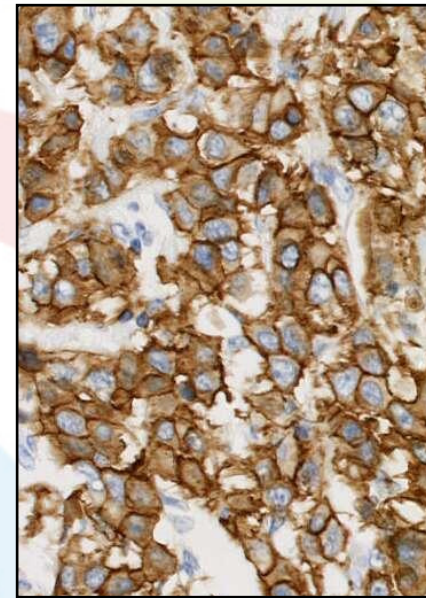
2+



- Weak/moderate complete membrane staining in >10% tumour cells

Positive

3+



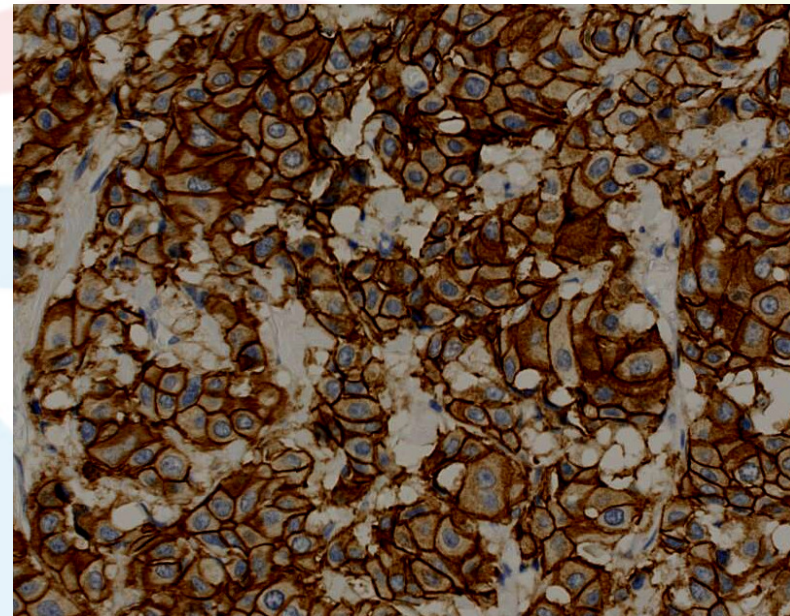
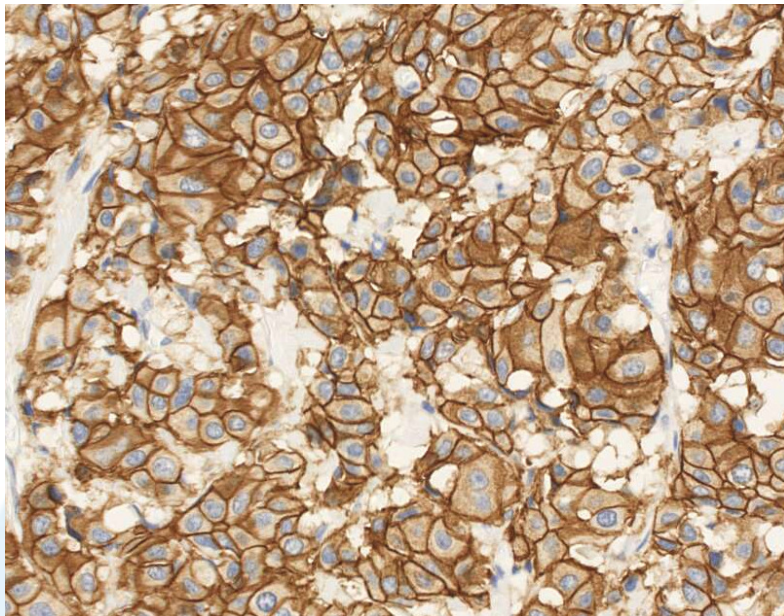
- Strong complete membrane staining in >30% tumour cells

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Difficulties associated with IHC interpretation

- Inherently subjective, due to external factors, human limitations
- Over use of intermediate category



Best practice

HER2 testing in the UK: further update to recommendations

R A Walker,¹ J M S Bartlett,² M Dowsett,³ I O Ellis,⁴ A M Hanby,⁵ B Jasani,⁶ K Miller,⁷
S E Pinder⁸

“Currently no QA scheme to assess proficiency in interpretation...virtual systems are being explored”

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JOURNAL OF CLINICAL ONCOLOGY

ASCO SPECIAL ARTICLE

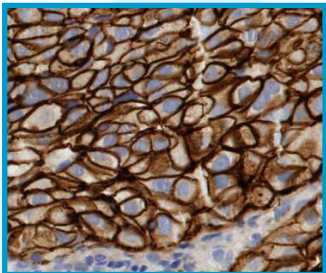
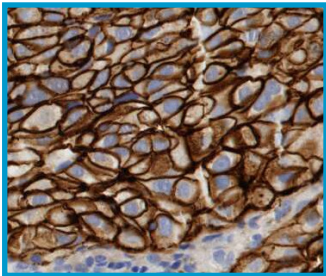
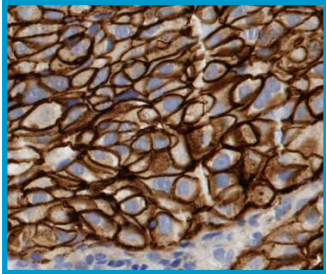
American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer

Antonio C. Wolff, M. Elizabeth H. Hammond, Jared N. Schwartz, Karen L. Hagerly, D. Craig Allred, Richard J. Cote, Mitchell Dowsett, Patrick L. Fitzgibbons, Wedad M. Hanna, Amy Langer, Lisa M. McShane, Soonmyung Paik, Mark D. Pegram, Edith A. Perez, Michael F. Press, Anthony Rhodes, Catharine Sturgeon, Sheila E. Taube, Raymond Tubbs, Gail H. Vance, Marc van de Vijver, Thomas M. Wheeler, and Daniel F. Hayes

“Image analysis could be an effective tool for achieving consistent interpretation”

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Requirement for Image Analysis

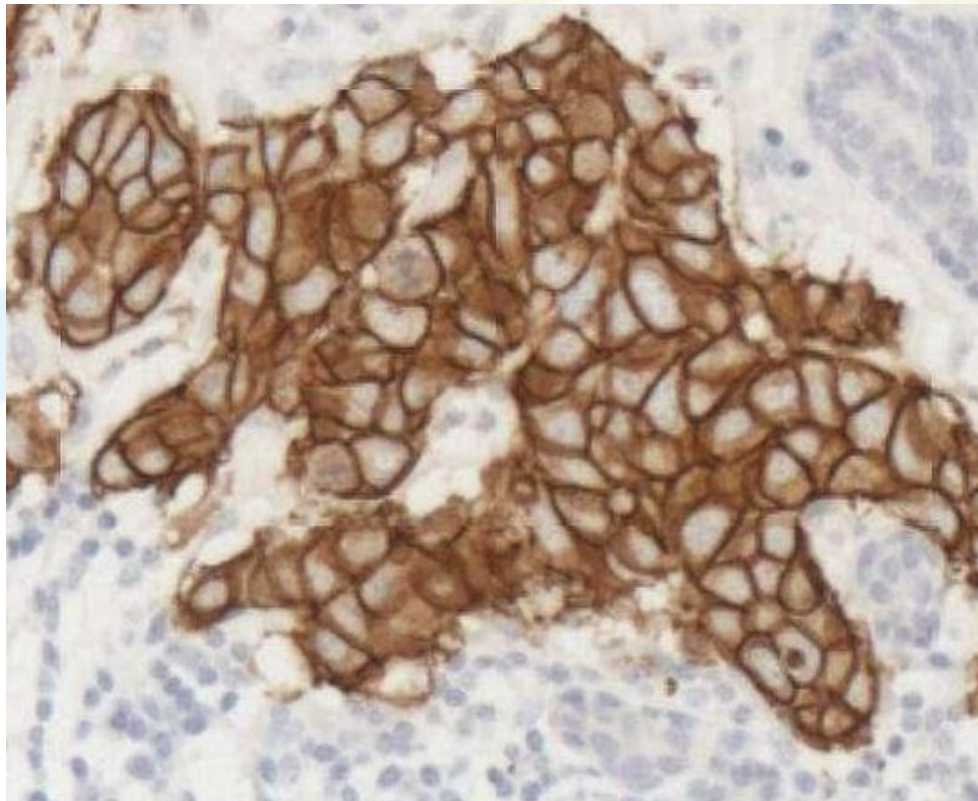
Key advantages of Image Analysis

- **Quantification** - image analysis can provide enumeration and intensity data that's difficult if not impossible to obtain using conventional analysis.
- **Consistency** - computers return the same result for the same specimen on each review.

Algorithm Development

Development of HER-2 Algorithm

- First we take in an image, a colourcube file and some input parameters

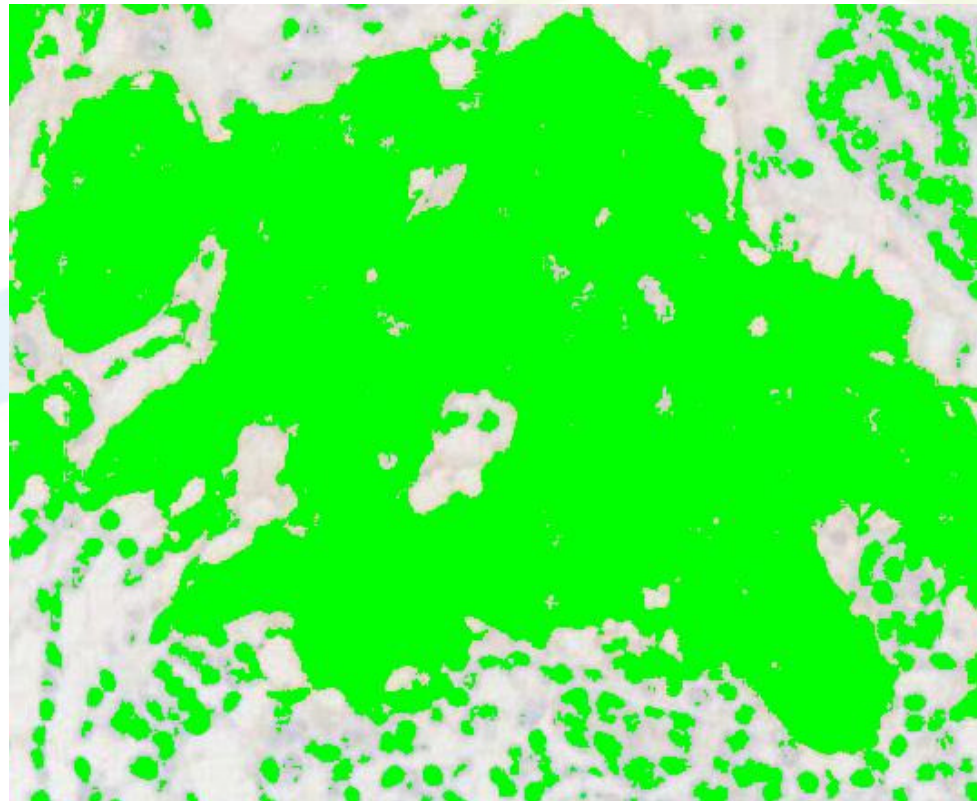
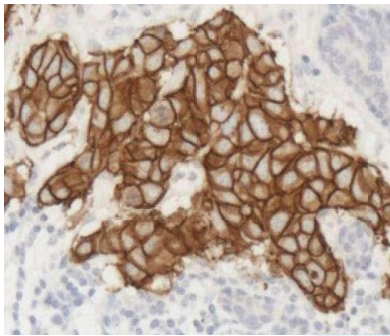


Input Image

Algorithm Development

Development of HER-2 Algorithm

- We then use an input parameter for background colour to determine the tissue

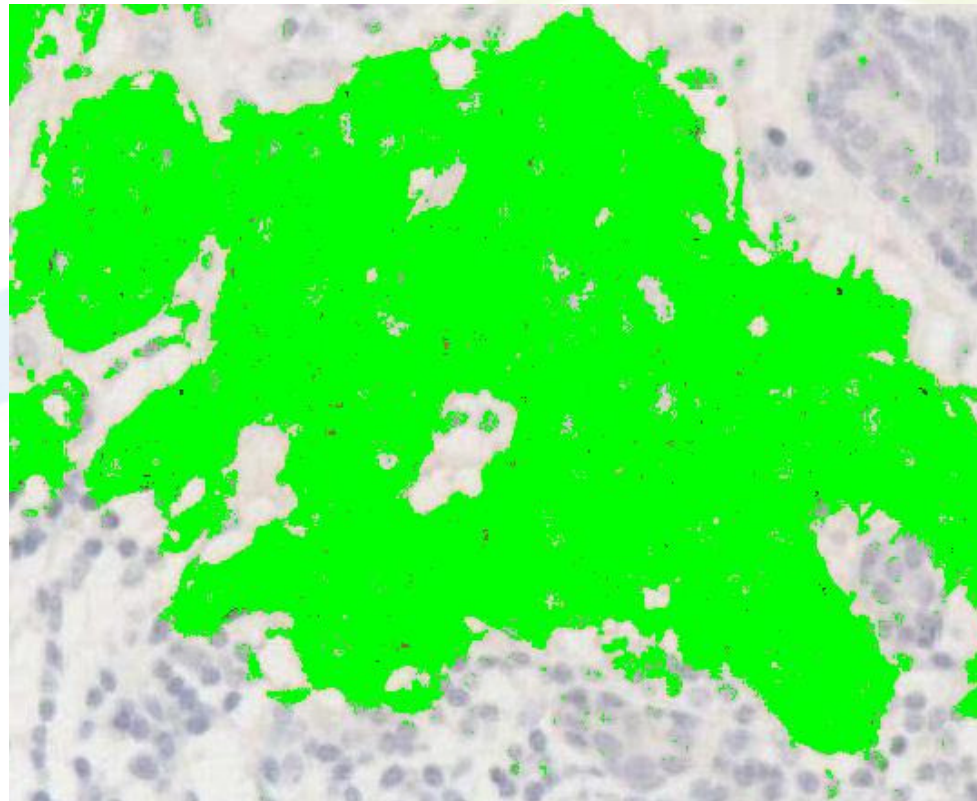
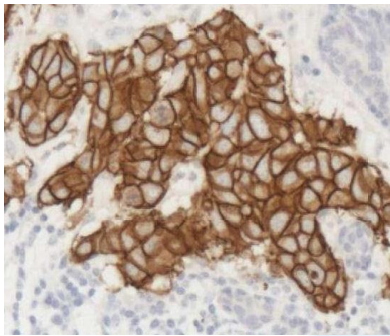


Tissue Image

Algorithm Development

Development of HER-2 Algorithm

- Applying the colourcube to the tissue image isolates the stained tissue

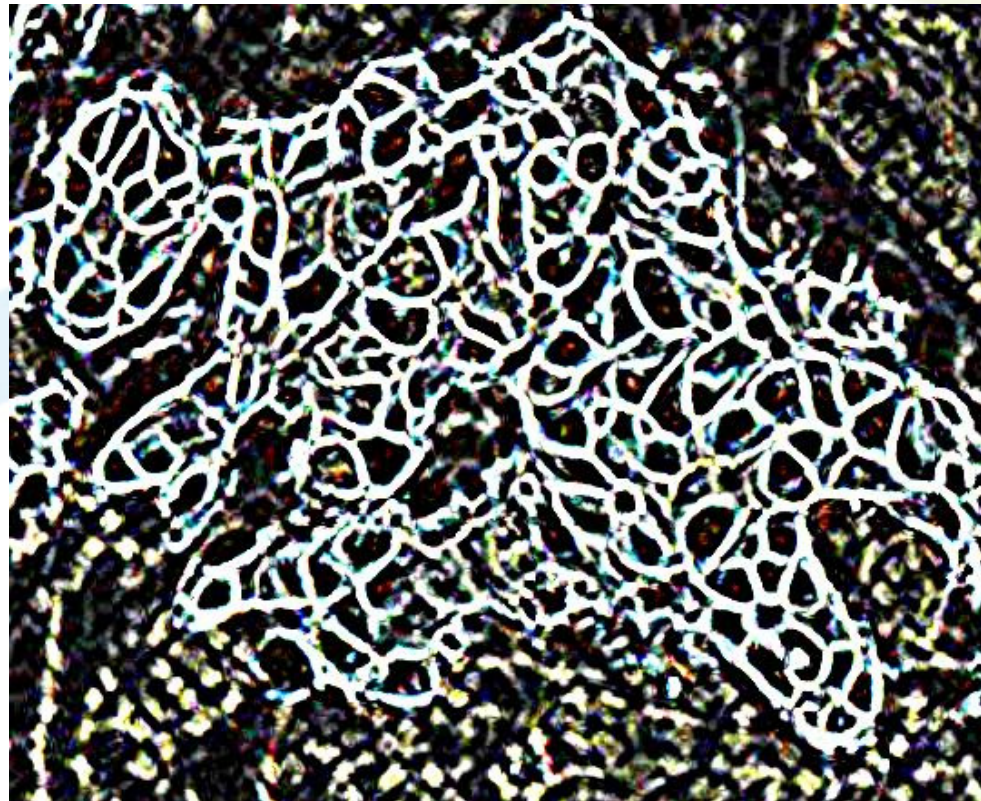
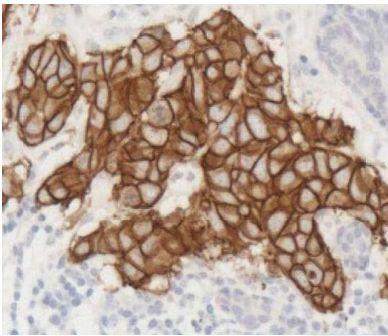


Staining Image

Algorithm Development

Development of HER-2 Algorithm

- Running edge detection is used to find potential membrane

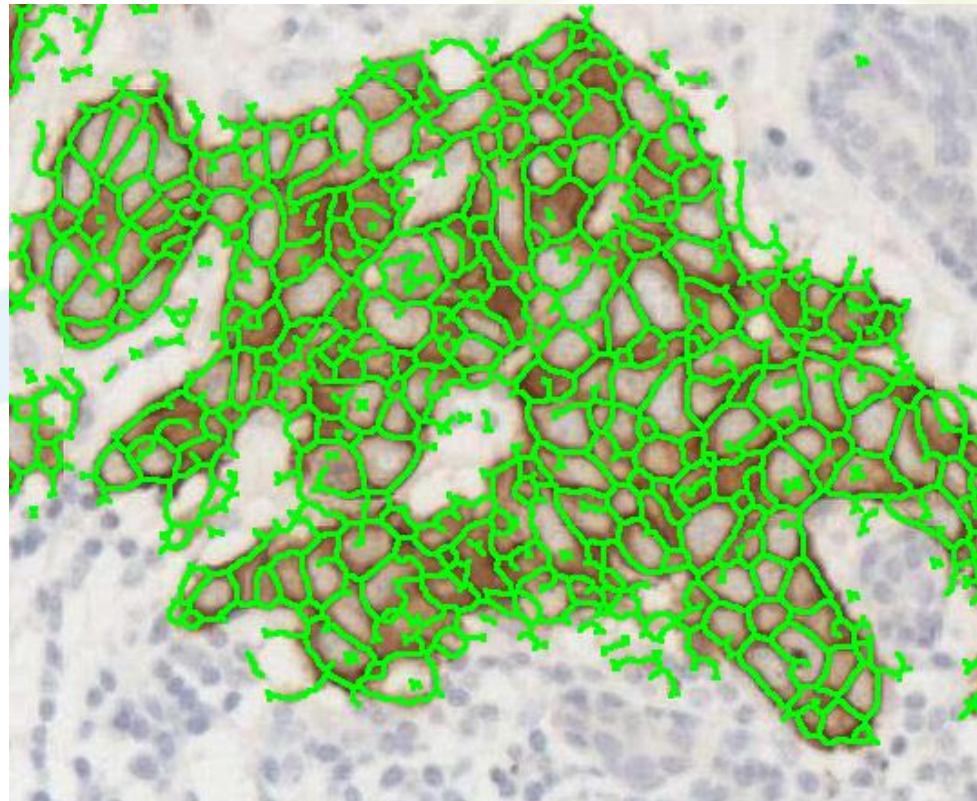
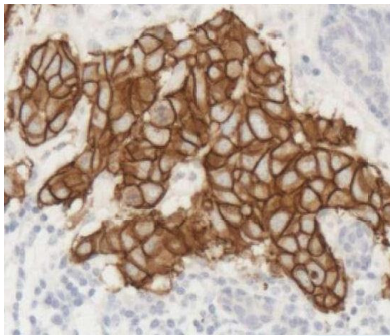


Edges Image

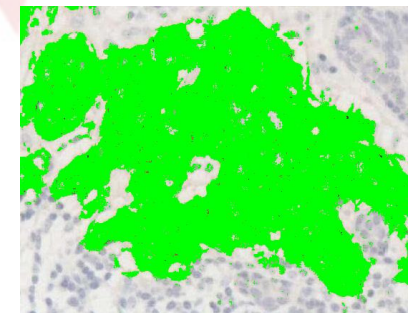
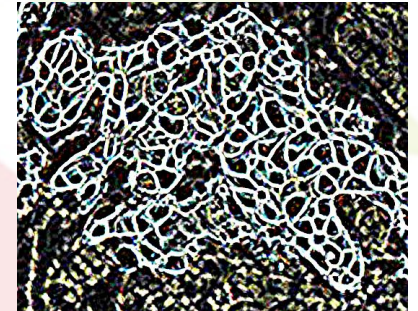
Algorithm Development

Development of HER-2 Algorithm

- Determine membrane image from edge and stain images



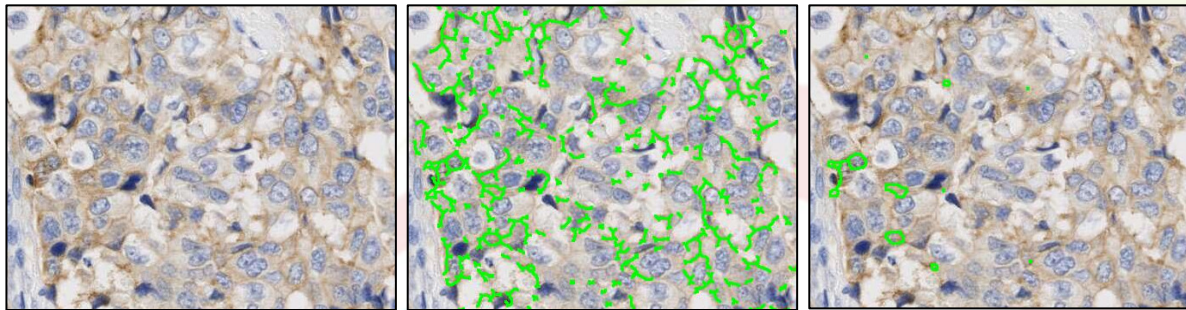
Membrane Image



Development- Stage 2

- Intensity alone insufficient, evaluation of membrane continuity

A: 0/1+
in-house
control
tissue



B: 2+
in-house
control
tissue

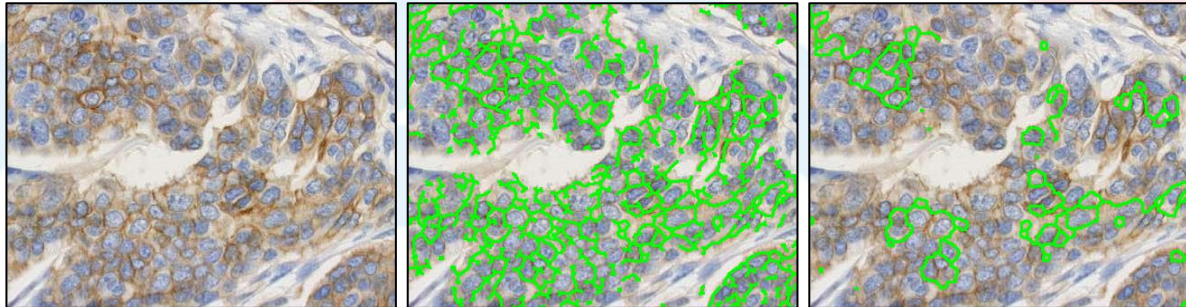
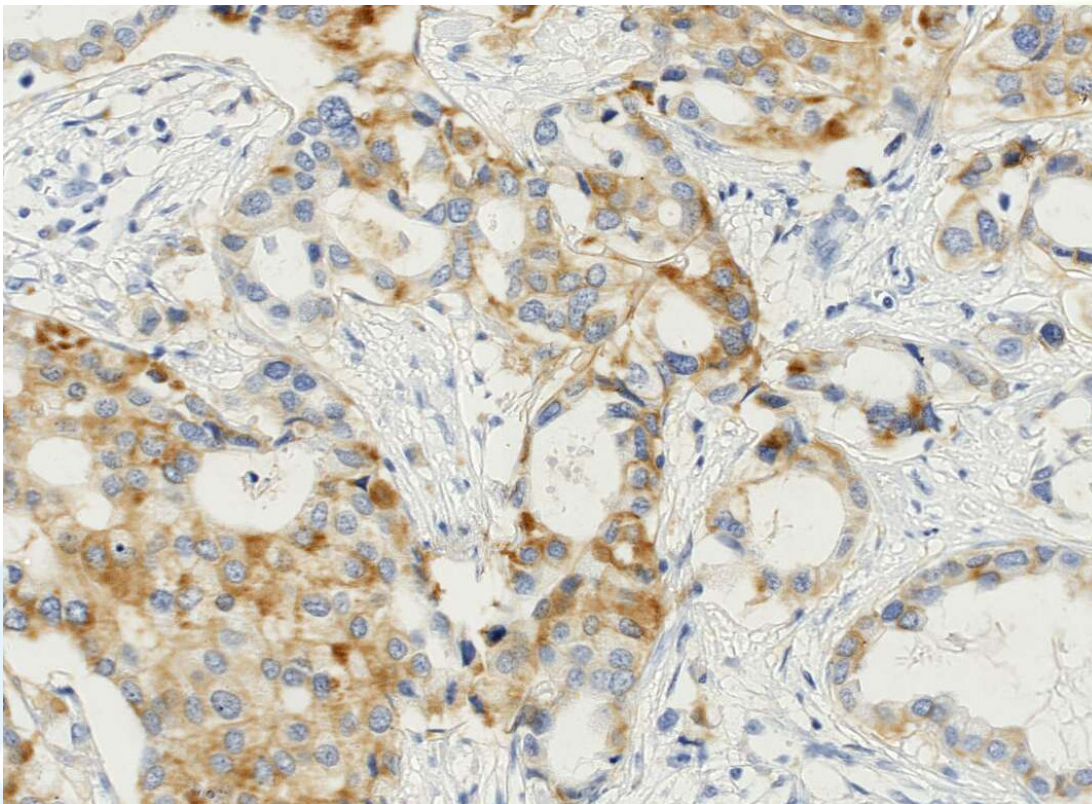


Figure 5: Membrane continuity enables differentiation of 0/1+ and equivocal (2+) cases by image analysis. **A:** 0/1+ in-house control tissue; **B:** 2+ in-house control tissue stained with Leica Oracle HER-2.

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Case 1: HercepTest, Manual Classification 2+, FISH Unamplified



Predicted HER-2 Score

- 2

Confidence in HER-2 0/1 Score

- 49

Confidence in HER-2 2 plus Score

- 51

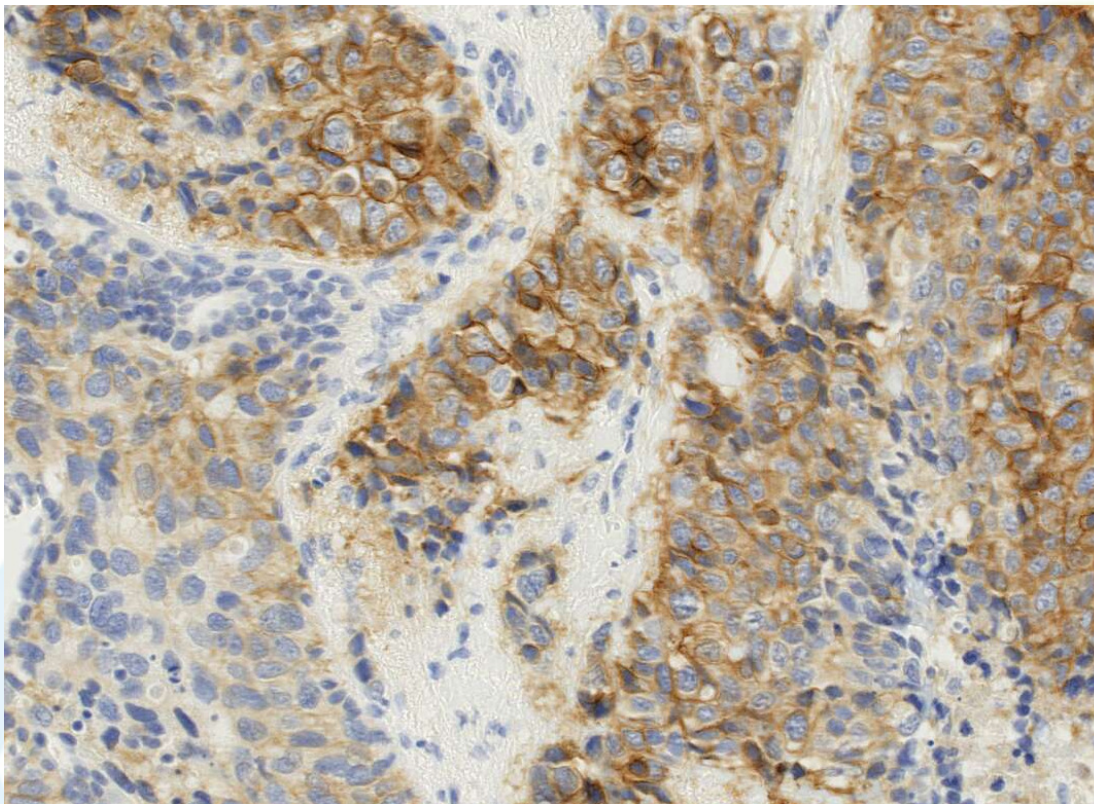
Confidence in HER-2 3 plus Score

- 0

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Case 2: Oracle, Manual Classification 2+, FISH Equivocal



Predicted HER-2 Score

- 2

Confidence in HER-2 0/1 Score

- 0

Confidence in HER-2 2 plus Score

- 61

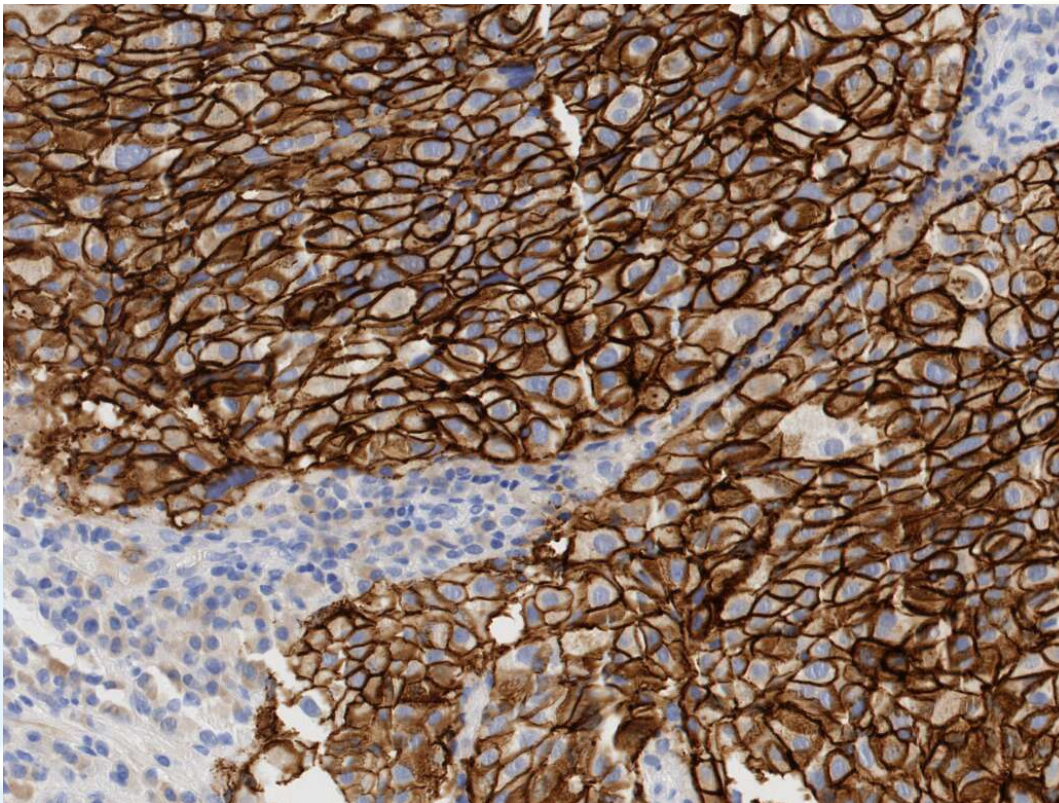
Confidence in HER-2 3 plus Score

- 39

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Case 3: Ventana Pathway, Manual Classification 3+, FISH Amplified



Predicted HER-2 Score

- 3

Confidence in HER-2 0/1 Score

- 0

Confidence in HER-2 2 plus Score

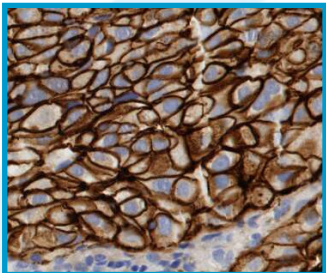
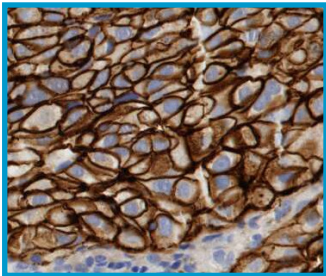
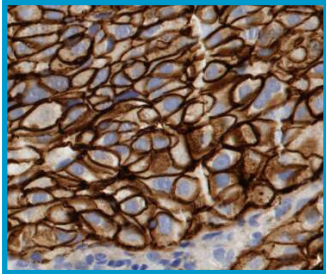
- 0

Confidence in HER-2 3 plus Score

- 100

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Conclusions

- Image Analysis as an Adjunct to Manual Review.
- Image Analysis increases throughput, reproducibility, accuracy and provides quantitative analysis of immunohistochemical staining.
- SlidePath developed a HER-2 algorithm which obtains 91% agreement with manual scoring, higher than competitors.
- Algorithm reduces the number of 2+ cases.
- Image Analysis can only be as good as the raw materials and the scientist training the system.



European Head Office

SlidePath,
Invent DCU,
Dublin 9,
Ireland.

Tel: +353 1 7007576
Fax: +353 1 7007555

Australasian Head Office

SlidePath,
133 Alexander St, Crows Nest,
Sydney, 2065 NSW.
Australia.

Tel:+61 9431 5368
Fax:+61 9439 2738

www.slidepath.com • info@slidepath.com