



Imaging Mass Cytometry (IMC) Technology Uniquely Reveals Clinical Impact of HER2 Expression Levels

Why the high dynamic range of IMC systems matters in spatial proteomics to capture biomarker insights from high and low protein expression

Introduction

Deciphering both therapeutic and immune responses is a difficult undertaking but is critical to accurately determine cancer progression and the safety and efficacy of a potential therapy.

In this context, detection and monitoring of impacted proteins can be challenging depending on their abundance within the microenvironment.

Key to analytic success is the ability to measure the distinct expression levels of these proteins because the degree of underexpression or overexpression of a specific protein could uncover the difference in predicted response. For example, overexpression of certain antigens, such as HER2 in breast cancer, directly influences the aggressiveness of the tumor and helps guide treatment strategies.

While immunofluorescence methods are limited by low dynamic range leading to early saturation of signals, Imaging Mass Cytometry™ (IMC™) technology is uniquely positioned for assessing high and low expression levels due to its high dynamic range (Figure 1).

In this application note, we outline how:

- The high dynamic range of IMC technology empowers detection of high- and low-expressing markers
- Quantification of HER2 expression levels with IMC technology enables more precise tumor grading
- IMC technology enables association of tumor and immune cell phenotypes with quantitative spatial biomarker expression

IMC technology reveals the full range of protein expression, enabling immune insights unable to be captured with cyclic fluorescence

A high dynamic range better matches the already high biological dynamic range of the proteome, providing the capability to capture high- and low-expressing proteins. This enables the assessment of expression level without saturation of signals rather than relying on presence or absence, providing the opportunity to:

- Acquire accurate relative quantification of biomarkers without the need to compensate for autofluorescence
- Allow cell identification
- Characterize cells based on specific expression levels

For example, HER2 is a critical biomarker for subtyping breast cancers and guiding treatment strategies. It is assessed by immunohistochemistry (IHC) using a three-tier scoring system and reflex in situ hybridization (ISH) for an IHC score of 2+. HER2 grading is used to

determine HER2-directed therapies and is performed by measuring the level of HER2 expression in patient samples.

The ability to distinguish between HER2 grades, with levels of 0, 1+, 2+ and 3+, is critical for determining whether a sample qualifies for these therapies. Using this method, patients with HER2+ breast cancer, i.e., IHC score 3+ or ISH-positive IHC score 2+, are eligible for anti-HER2 targeted therapy. Recently, the DESTINY-Breast04 clinical trial demonstrated that targeting HER2 also provides significant benefits for patients with metastatic breast cancer showing low levels of HER2 expression, i.e., IHC score 1+ or ISH-negative IHC score 2+.

As more is learned about the dynamics of HER2 expression and how it impacts cancer progression, additional parameters must be accounted for, including distinguishing and treatment of low and ultralow HER2 breast cancer. The field is challenged by the need for precise, quantitative measurement of HER2 expression, whose dynamic range remains linear in both low-expression and high-expression environments.

IMC technology scales with the highest dynamic range and multiplex abilities

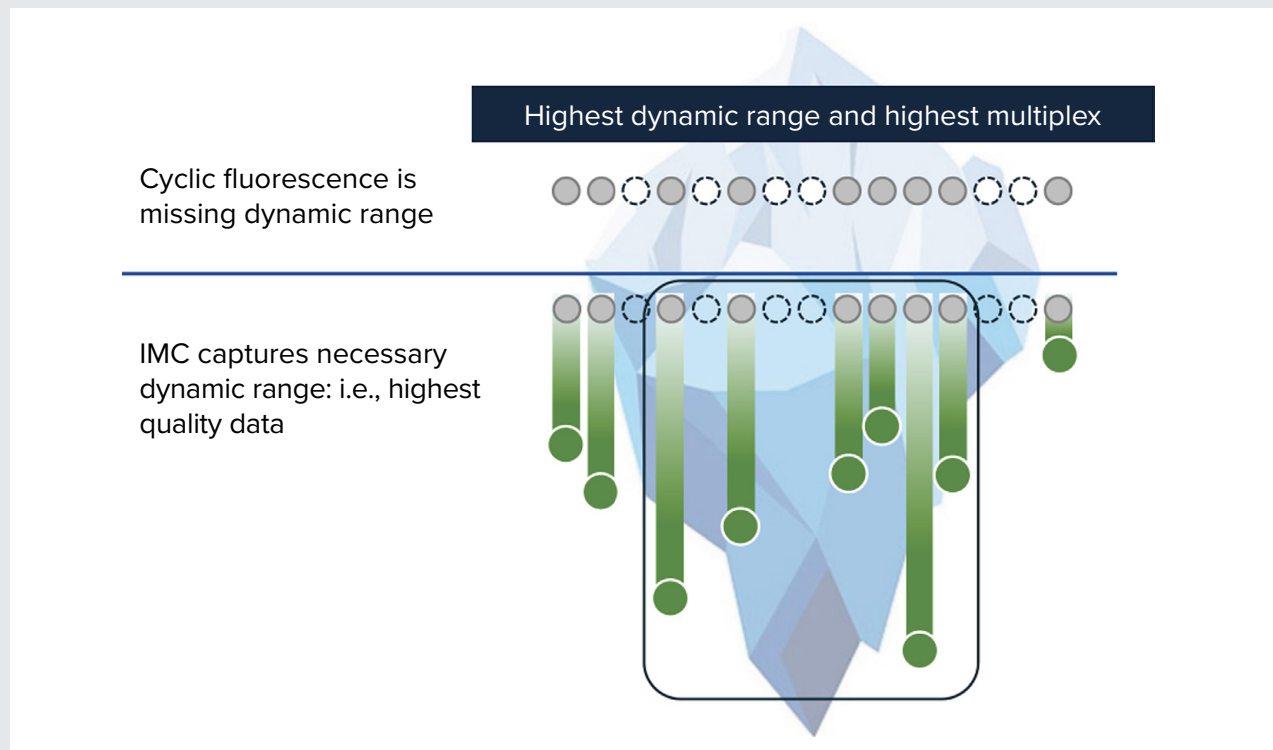


Figure 1. IMC technology enables unique patient stratification via its ability to capture the dynamic range of spatial proteomics. Cyclic fluorescence is unable to capture the dynamic range of spatial biomarkers.

IMC uniquely quantifies low and high abundance biomarkers

Compared to IMC, immuno-histochemistry is subjective and thus does not meet the need for fully automated, quantitative evaluation of HER2 signal in breast cancer tissues (Figure 2).

Clear gradation of HER2 signal is observed by IMC technology across graded breast cancer tissues **without** amplification.

IMC accurately determines the expanded spectrum of HER2 expression in breast cancer tissue due to the dynamic range of signal detection (Figure 3).

IMC technology provides the largest linear dynamic range among technologies

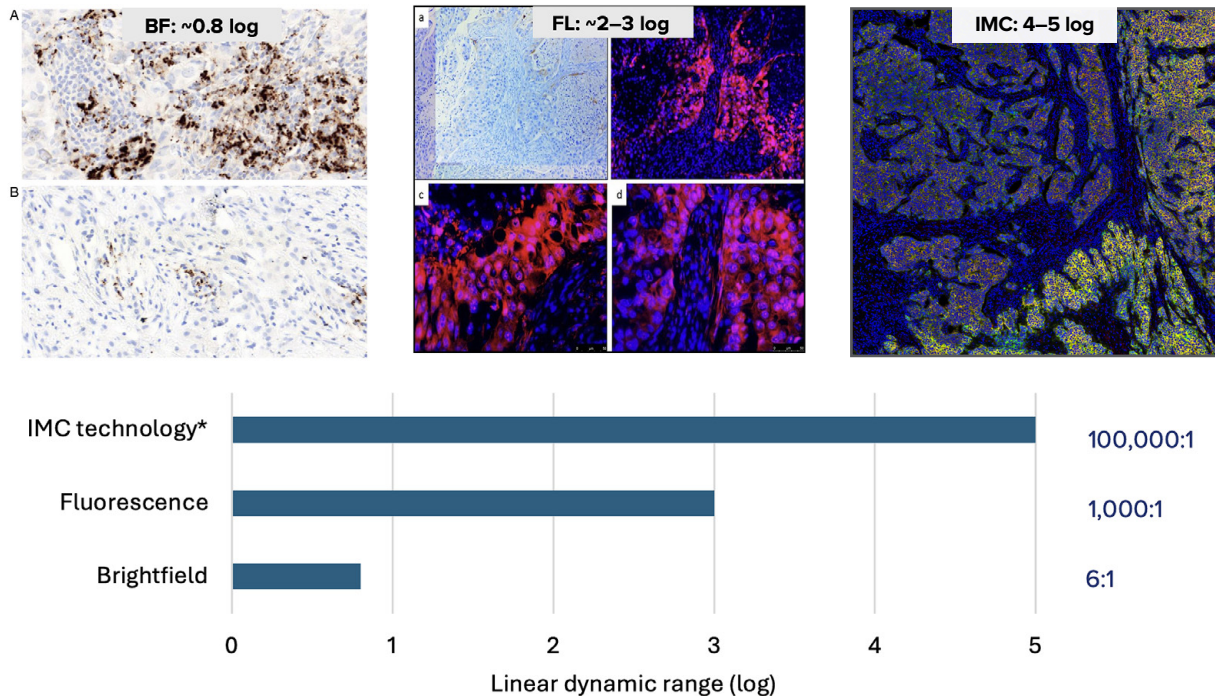


Figure 2. IMC technology has the unmatched ability to quantify data, sensitively detecting varying expression levels across a 4–5 log range where other technologies cannot capture these critical differences.

* Bollhagen, A. et al. "Highly multiplexed tissue imaging in precision oncology and translational cancer research." *Cancer Discovery* 14 (2024): 2,071–2,088.

As demonstrated in Figure 3, IMC technology clearly detects HER2 expression in the upper tumor locations, where IHC shows no expression differences across the tumor locations.

This type of clear gradation is a significant step toward enhanced patient stratification, selecting those who could benefit from targeted treatments and who might have otherwise been overlooked.

IMC technology captures the entire dynamic range of expression for biomarkers at single-cell resolution

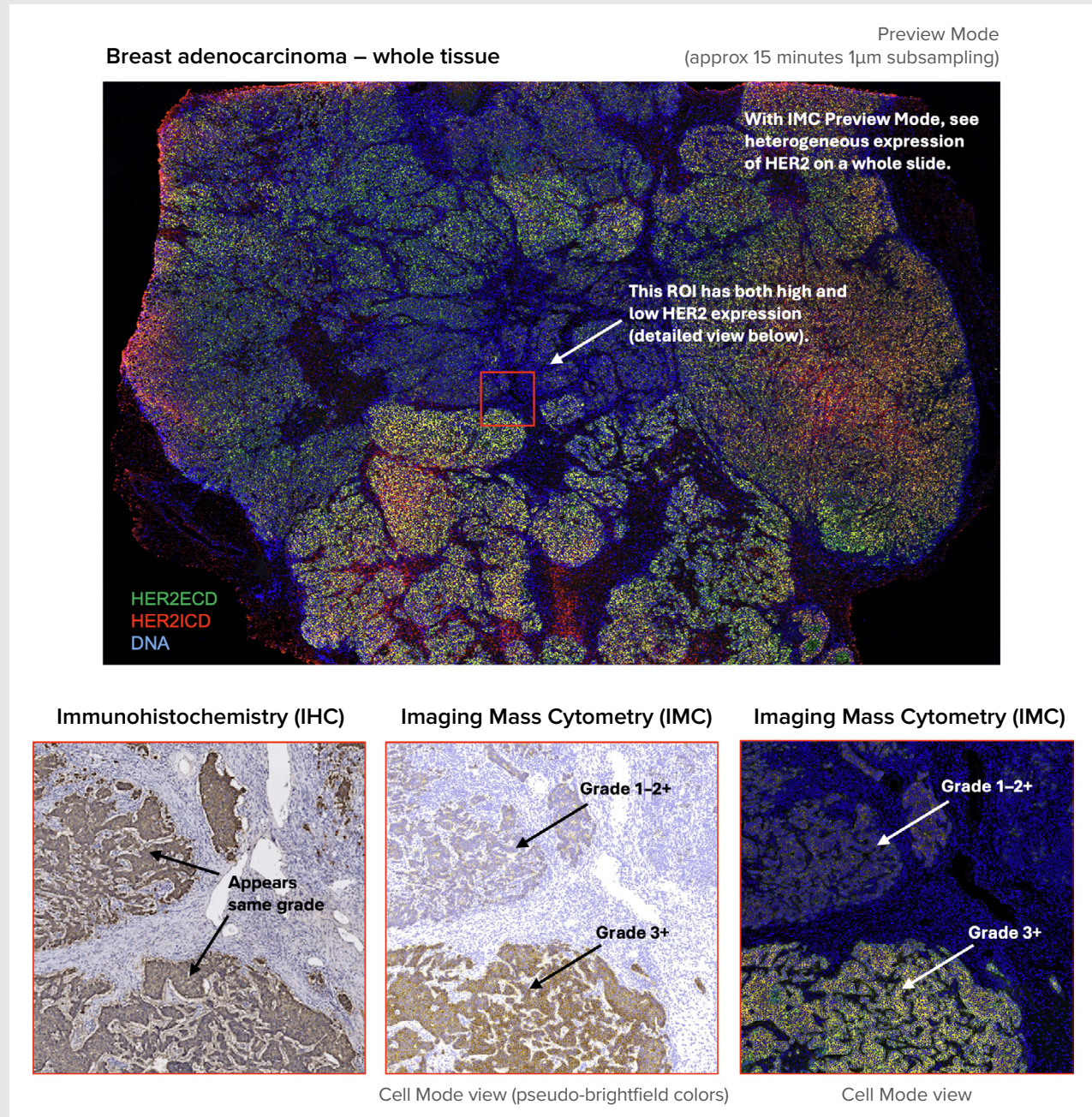


Figure 3. A distinct difference in HER2 expression, which cannot be seen with IHC, can be observed in the IMC images across the tumor locations. With Preview Mode (top), a preview of panel expression across the entire tissue was generated in minutes. In the IHC image (left), variations in HER2 expression are not captured by nonlinear signal detection, resulting in a binary signal (present/not present) that makes it difficult to properly measure low-expressing markers. With Cell Mode (right), the entire dynamic range of expression for biomarkers is captured at high resolution. A rapid preview of whole slide can be generated in minutes, followed by high resolution cell mode imaging.

Further quantitative analysis using IMC technology (Figure 4) identifies how these differences in expression can affect grading outcome and establish effective biomarkers for disease progression and treatment response.

These grading differences are not easily observed using the equivalent IHC image.

IMC offers the capability to quantify spatial biomarker expression at the single-cell level

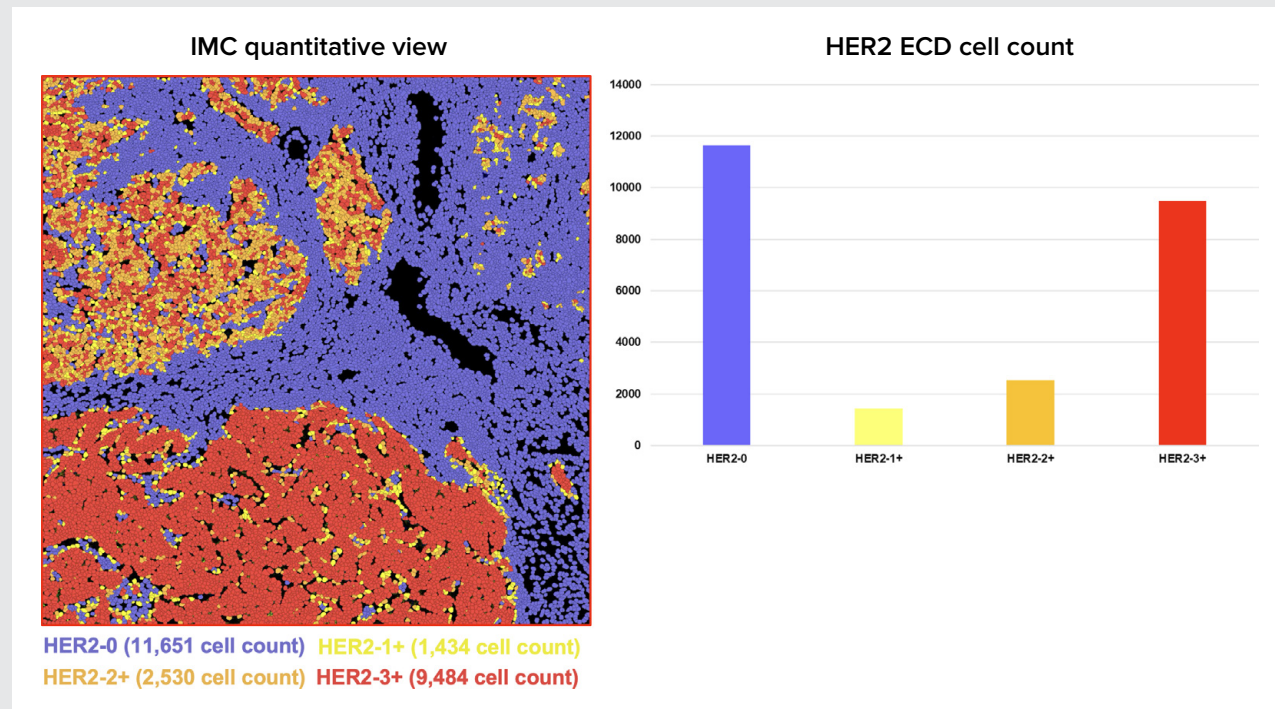


Figure 4. The same IMC Cell Mode image using quantitative analysis. Single-cell detection and quantification of positive HER2 cells with numbers of expressing single cells associated with biomarker expression levels across a tissue. Image is a quantitative view of the ROI highlighted in Figure 3. The graph shows the proportion of HER2-expressing cell types.

IMC technology enables association of tumor and immune cell phenotypes with quantitative spatial biomarker expression and co-localization

IMC systems provide the highest dynamic range available, along with the ability to detect 40-plus markers at once, compared with one marker using IHC. This unique feature of IMC technology enables easy phenotyping of the entire tumor microenvironment (TME), as well as a deeper dive into tumor cell phenotypes, precisely identifying those cells with varying expression of HER2, replicating tumor cells and tumor cells with a stem cell-like phenotype (Figure 5).

Specifically identified by IMC technology, cancer stem cells (CSCs) are a small subpopulation of cells within tumors that possess characteristics similar to normal stem cells. These cells can self-renew, differentiate and initiate tumor formation. CSCs are responsible for tumor initiation, growth, recurrence and resistance to chemotherapy and radiation therapy.

The 3 IMC images in Figure 5 provide detailed information showing:

- Quantitative evaluation of cellular phenotype and neighborhoods across the tissue with single-cell analysis (image A)
- Diverse HER2+, immune, myeloid and structural single-cell phenotype clusters (image A)
- The heterogeneity of tumor cells expressing combinations of biomarkers (image B)
- Stem cell-like tumor cells (panCK+, CD44+) observed within high HER2-expressing cells (image B)
- T cell populations that are more concentrated around a HER2_{low} tumor population (image C)

These insights can help direct whether HER2_{low}-expressing cells associated with replicating tumor cells respond better to a specific treatment. The ability to see the difference in administering a HER2_{low} targeted therapeutic vs. a HER2_{high} targeted therapeutic provides significant value in cancer treatment strategies.

IMC data analysis identifies spatial phenotypes of tumor and immune cells with quantifiable biomarker expression levels

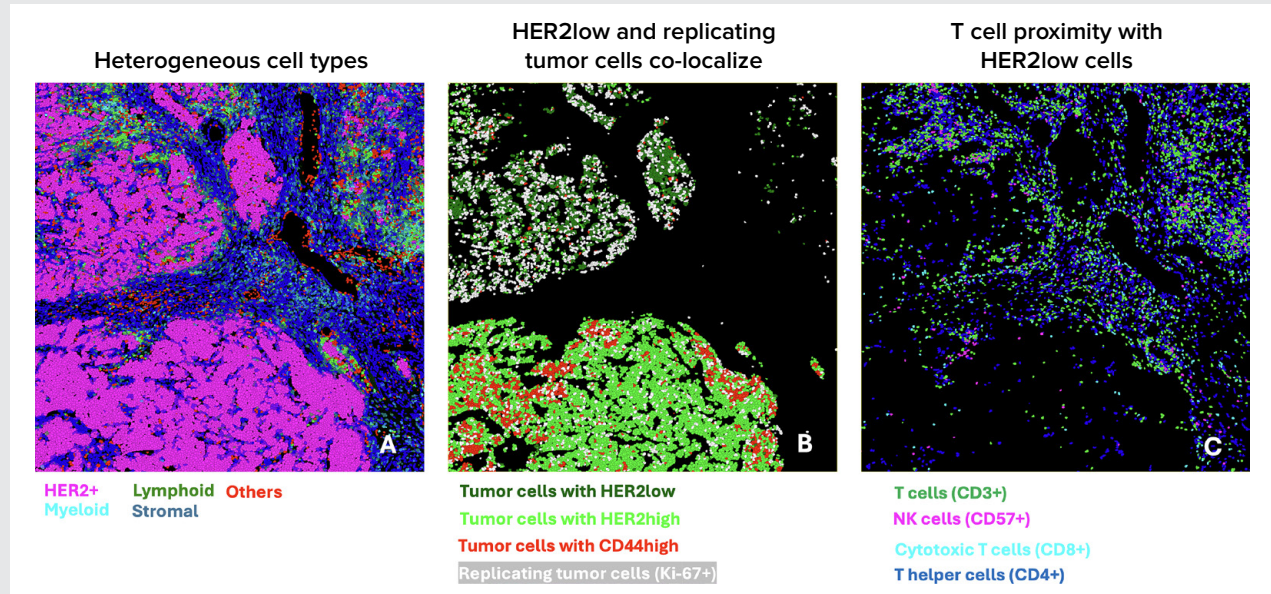


Figure 5. In addition to examining protein expression levels using the high dynamic range of IMC technology, the ability to detect 40-plus markers at once allows this expression data to be associated with cell type and behavior. Comprehensive analysis provides insights into the mechanisms important to targeting the right cells with the right treatments.

IMC technology captures the dynamic range of spatial biomarkers to enable predictive patient stratification

In a Phase 3 clinical trial of trastuzumab for breast cancer in 180 patients, IMC technology enabled predictive patient response stratification by capturing the dynamic range of HER2 (high/low expression) and spatial proximity of the HER2 extracellular domain (ECD) and CD8+ T cells.

The ability to objectively measure HER2 domains, signaling targets and targeted immune cells using IMC technology delivers information with the potential to help identify patients who could benefit from targeted treatment or immunotherapy. For this study, the measurement of HER2 ECD and intracellular domain (ICD) in the same tissue and their association with better outcome was uniquely made possible by IMC technology.

Due to its high dynamic range, IMC technology identified the mechanism of action by which high expression of HER2 ECD attracts CD8+ cytotoxic T cells to the tumor site, facilitating the immune system's ability to target and eliminate cancer cells.

IMC technology provided a broader dynamic range vs. quantitative immunofluorescence, allowing for more precise stratification of HER2 levels in clinical samples, as stated in the paper: “[quantitative immunofluorescence] has less dynamic range than IMC and appears to be saturated.”

Study at a glance:

- A Phase 3 clinical trial evaluated whether breast cancer tumors that do not express the ECD of the HER2 protein are less likely to benefit from checkpoint therapy in 180 patients
- Due to high content coverage and high dynamic range capabilities provided by IMC technology, the study was able to determine outcomes only IMC technology could identify to successfully stratify patients
- IMC technology identified that high expression of HER2 and proximity to CD8+ T cells in the TME was indicative of response

High and low HER2 expression detected by IMC reveals predictive patient response stratification

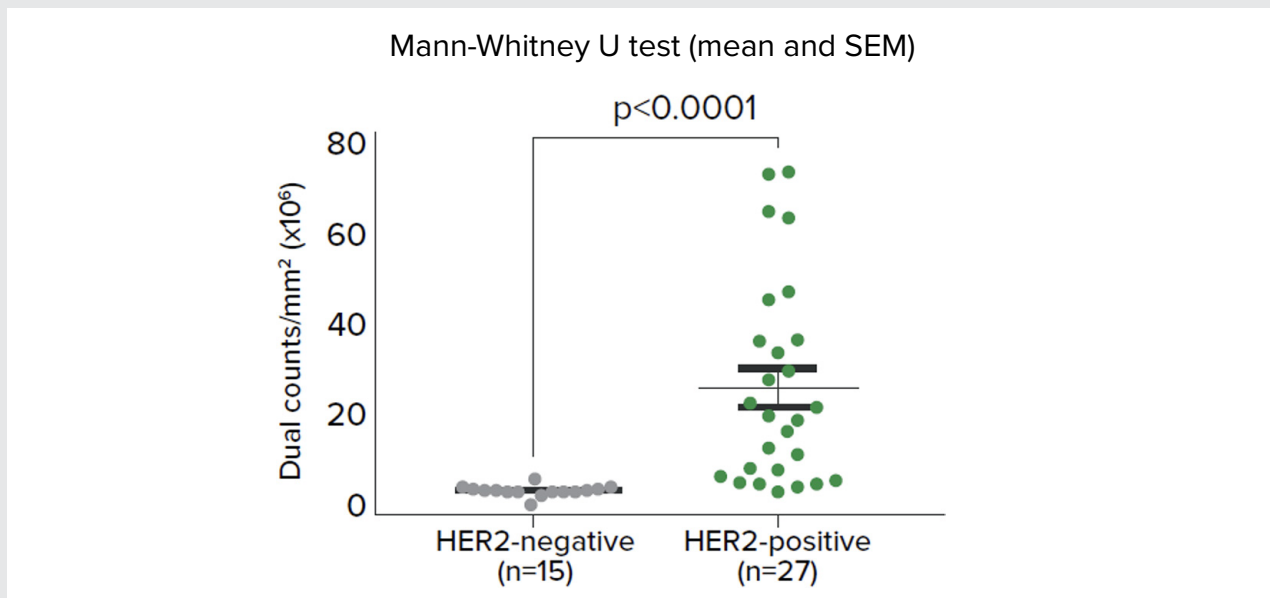


Figure 6. Dynamic range of HER2 expression captured by IMC technology is predictive of response. The study analyzed samples from HER2+ breast cancer patients who were part of the HeCOG 10/05 clinical trial, captured in pixels on a standard tissue microarray (YTMA263).

Conclusion

Imaging Mass Cytometry technology on the Hyperion™ XTi Imaging System has the potential to provide significant value to drug development initiatives, patient stratification and informing treatment decision-making strategies.

This application note has demonstrated that:

- IMC technology has the largest linear dynamic range – essential to accurately and quantitatively measure levels of low-expressing biomarkers, a significant advancement over IHC
- IMC technology has large plexity (40-plus markers) – to generate more data and obtain more insights out of each sample than is possible with IHC

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IMC Technology Uniquely Reveals Clinical Impact of HER2 App Note

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