



Imaging Mass Cytometry (IMC) Technology Uniquely Detects Low-Abundance Biomarkers

How the high dynamic range of IMC systems reveals variation in protein expression for better assessment of important markers such as PD-1, PD-L1 and CTLA-4

Introduction

The success of therapeutic development relies on the thorough detailing of mechanisms of action and biological response. Measuring the levels of relevant proteins provides needed insight for these applications because the degree of underexpression or overexpression of a specific protein could uncover the difference in predicted response.

With a greater dynamic range than fluorescence imaging technologies, Imaging Mass Cytometry™ (IMC™) technology enables the detection and quantification of distinct expression levels to identify critical proteins.

In this technical note, we outline:

- Why the high dynamic range of IMC technology is critical for protein quantification, enabling the assessment of expression level without saturation of signals
- The significance of detecting low-level marker expression, highlighting PD-1, PD-L1 and CTLA-4
- How IMC technology allows for clear analysis of low-abundance markers
- Examples of PD-1, PD-L1 and CTLA-4 marker expression analysis using IMC systems

Linear dynamic range is extremely important for protein quantitation

The high dynamic range of IMC technology 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, immune checkpoint inhibitor (ICI) therapies targeting immune evasion genes, such as programmed death 1 (PD-1), programmed death ligand 1 (PD-L1) and cytotoxic T lymphocyte antigen 4 (CTLA-4), have been developed based on knowledge of how tumor and immune cells interact. In order to effectively use PD-1, PD-L1 and CTLA-4 as predictive biomarkers for these therapies, their varying expression levels must be accurately measured.

The IMC platform provides the largest linear dynamic range among technologies

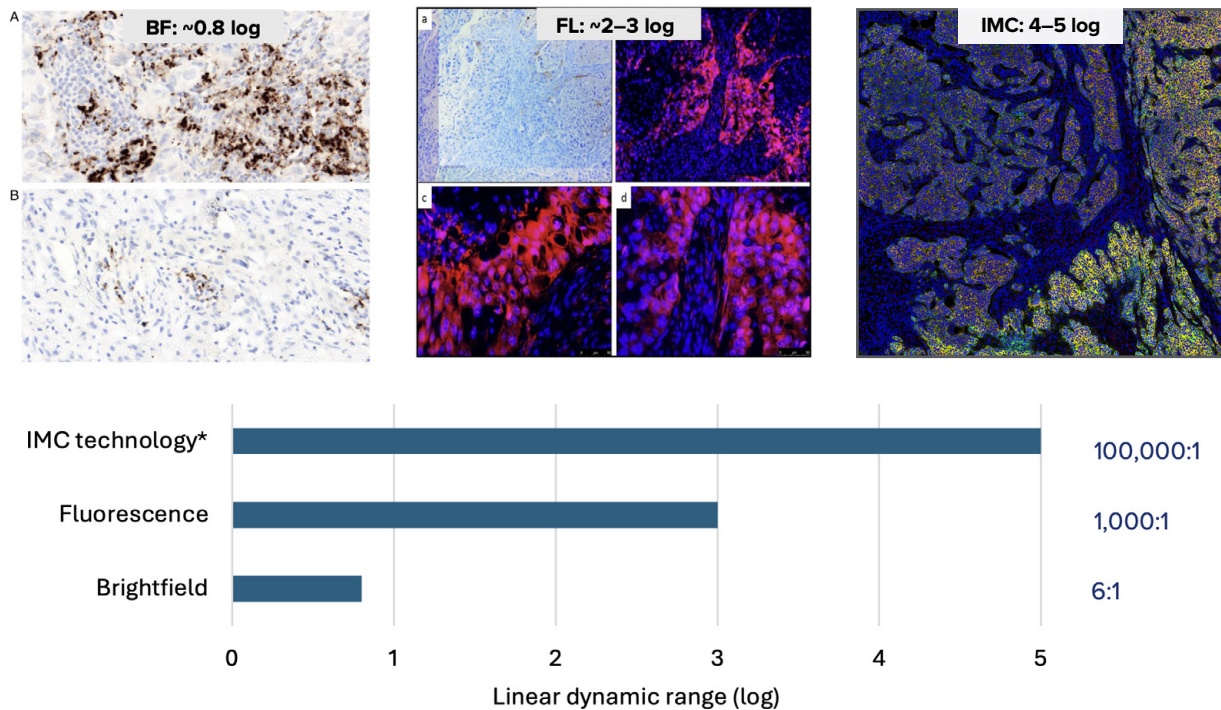


Figure 1. IMC technology has the unmatched ability to quantify data, sensitively detecting varying expression levels across a 4–5 log range where other technologies cannot capture these critical differences.

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

Quantitation of protein expression with IMC enables clear analysis of low-abundance markers

The high dynamic range of IMC technology allows the thorough quantification of antibody concentration across all markers on a slide, revealing relevant differences in low expression levels that could inform therapeutic decision-making.

With the flexibility in applying biomarker color formats, IMC data can be visualized in accordance with field standards – for example, images can be represented in a format similar to fluorescence imaging or to immunohistochemistry (IHC)/brightfield imaging.

This data consistency with conventional methods is enhanced by the ability of IMC systems to detect 40-plus markers targeting a wide array of phenotypes. Spanning immune cell function and activation, vascularization, tissue architecture and stromal population, it is possible to fully explore the complete complex phenotypes exhibited in any sample.

High-plex imaging also enables co-localization, key to verifying data within the same image – a benefit not possible with IHC due to the use of a single parameter.

IMC technology provides fully quantitative analysis with a high dynamic range

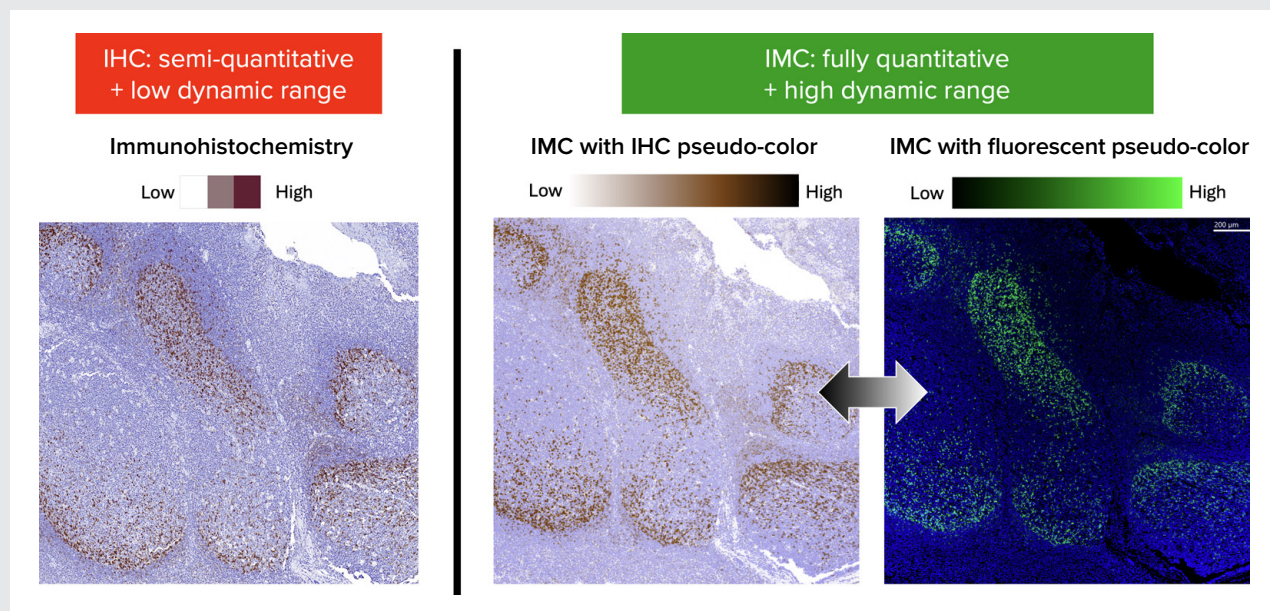


Figure 2. An IHC image of a serial section from a healthy tonsil sample showing DNA and PD-1 (left). An IMC image equivalent to that used for IHC, in which IMC technology exhibits much clearer delineation of PD-1-expressing regions (middle). A standard IMC image of the same sample using a 31-marker panel showing the two markers that are equivalent to IHC – DNA and PD-1 (right). Equivalent signal distribution and intensity is observed across images.

Detecting PD-1 expression in tonsil with IMC technology

PD-1 is an immune checkpoint protein and can inhibit T cell activity when bound by PD-L1. Anti-PD-1-based immunotherapies block the PD-1/PD-L1 interaction so T cells can reactivate their antitumor response. Given its diverse roles and expression on different immune cell

types, it can be important to gather as much context as possible for this marker.

Since PD-1 expression on CD3+ T cells can indicate activation or differentiation of the cells, quantitative detection could suggest progression of immune response and provide spatial context for varying expression levels not captured by other technologies.

Co-localization of PD-1 and CD3 expression in tonsil with IMC technology

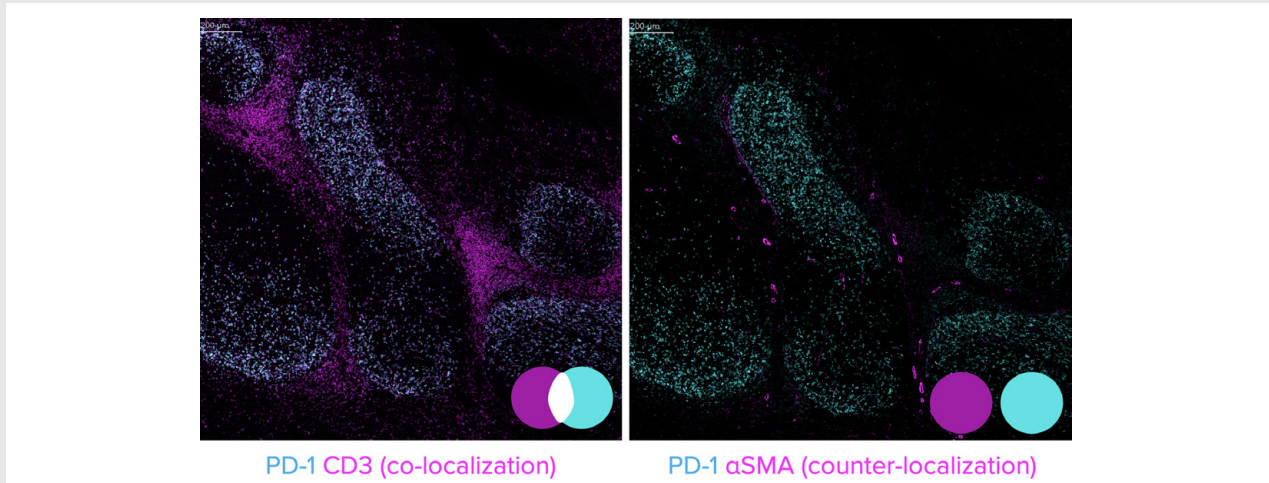


Figure 3. CD3 + PD-1 co-localization confirms PD-1-expressing T cells in the germinal center.

Equivalent PD-1 expression in tonsil with IMC technology, with the benefit of 31 total markers on the same slide for context of spatial behavior and interactions

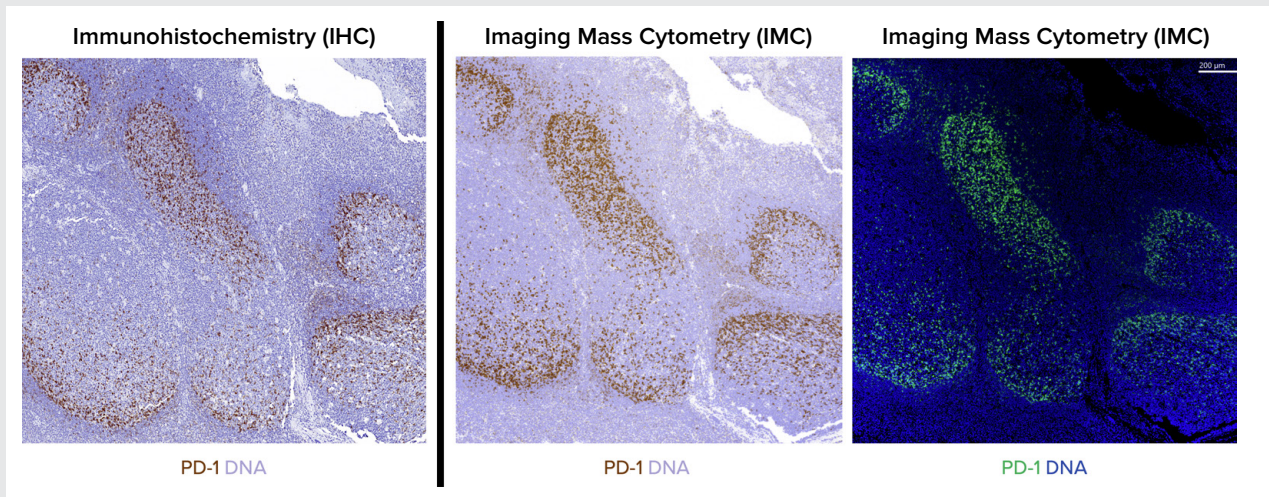


Figure 4. An IHC image (left) of a serial section from a healthy tonsil sample. A standard IMC image (right) taken in the same sample using a 31-color panel, here showing the two markers that are equivalent to IHC: DNA and PD-1. Equivalent signal distribution and intensity is observed across images.

Detecting PD-L1 expression in lung squamous cell carcinoma with IMC technology

PD-L1 is used as a predictive biomarker to help stratify patients who could benefit from ICI therapies. But this dynamic marker can vary both spatially and over time and thus can easily be overlooked in a single sample. Additionally, accounting for PD-L1 expression on immune cells and/or tumor cells can alter results from scoring depending on which method is used.

The linear and high dynamic range of IMC technology has the potential to improve PD-L1 assessment by ensuring its detection across variable expression and quantifying that expression for more accurate grading. Co-localization of PD-L1 with E-cadherin (Figure 5) verifies expressing cells are located within the tumor environment.

PD-L1 co-localization with e-cadherin using IMC verifies spatial context

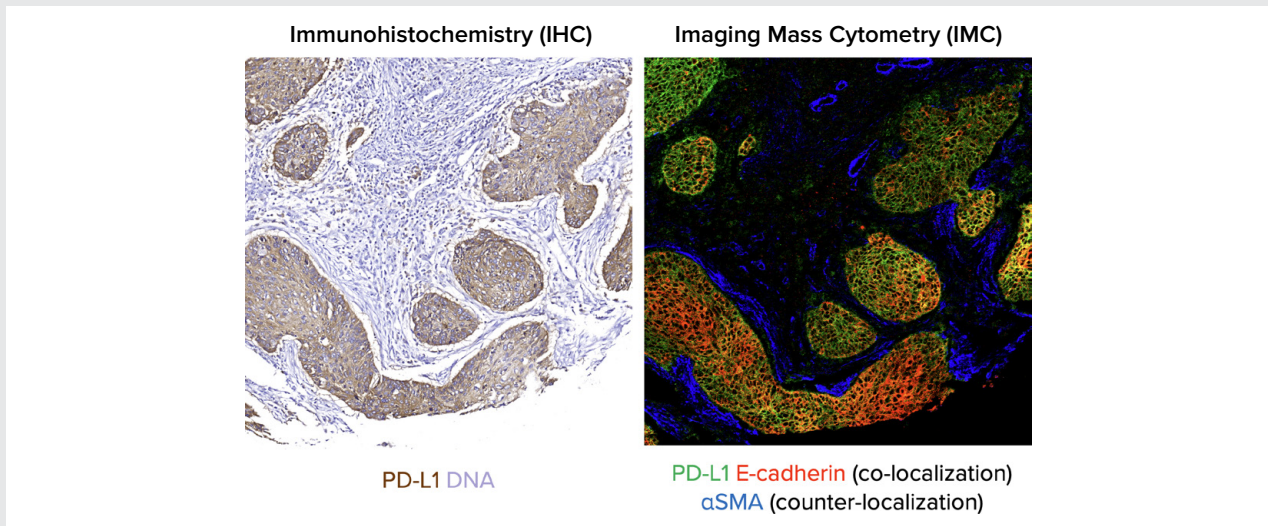


Figure 5. High expression of PD-L1, shown localized to E-cadherin-expressing cells, is associated with effective ICI therapy response. Expression levels cannot be determined using standard IHC staining.

IMC technology detects variations in PD-L1 expression not visible with IHC

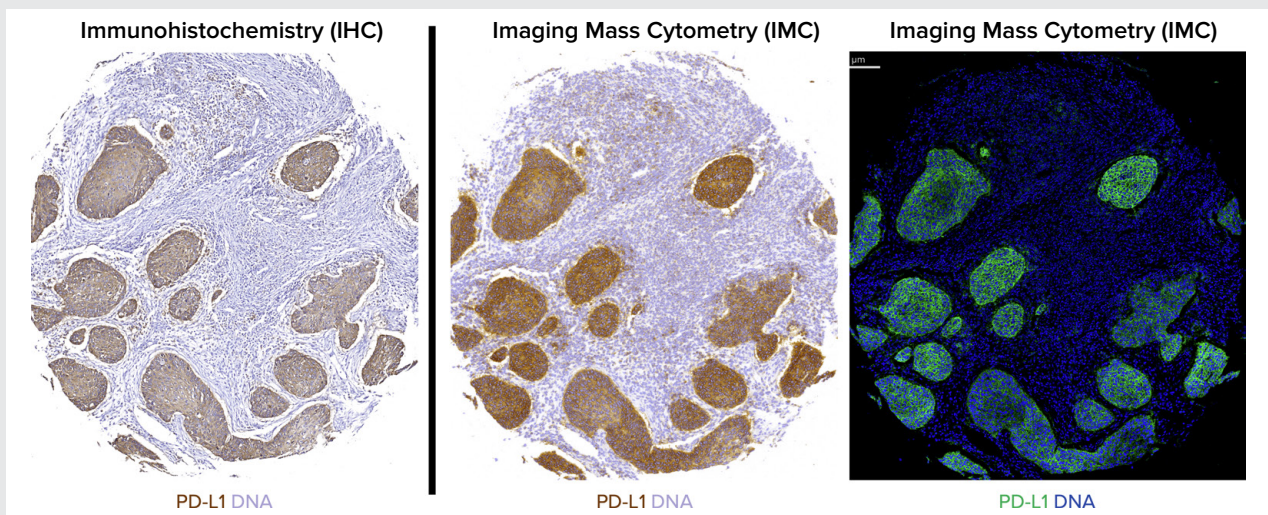


Figure 6. An IHC image (left) of a serial section from a lung squamous cell carcinoma sample. A standard IMC image (right), here showing the two markers that are equivalent to IHC: DNA and PD-L1. The ability to detect high and low levels of PD-L1 expression can be observed with IMC technology (right), including the varying expression levels of PD-L1.

Detecting CTLA-4 expression in malignant melanoma with IMC technology

CTLA-4 plays an inhibitory role in T cell regulation and is a target for ICIs. Because CTLA-4 expression can vary depending on cell type and activation status, the ability of IMC technology to detect all expression levels due to a high dynamic range can help distinguish

current cell status. For example, efficiency of response to treatment is shown to be proportional to CTLA-4 expression levels.

Detecting the co-localization of CTLA-4 and E-cadherin spatially verifies that CTLA-4 is expressed in tumor cells, pinpointing cellular expression. This localization can associate CTLA-4 expression with response to ICIs.

CTLA-4 co-localization with E-cadherin highlights potential ICI response

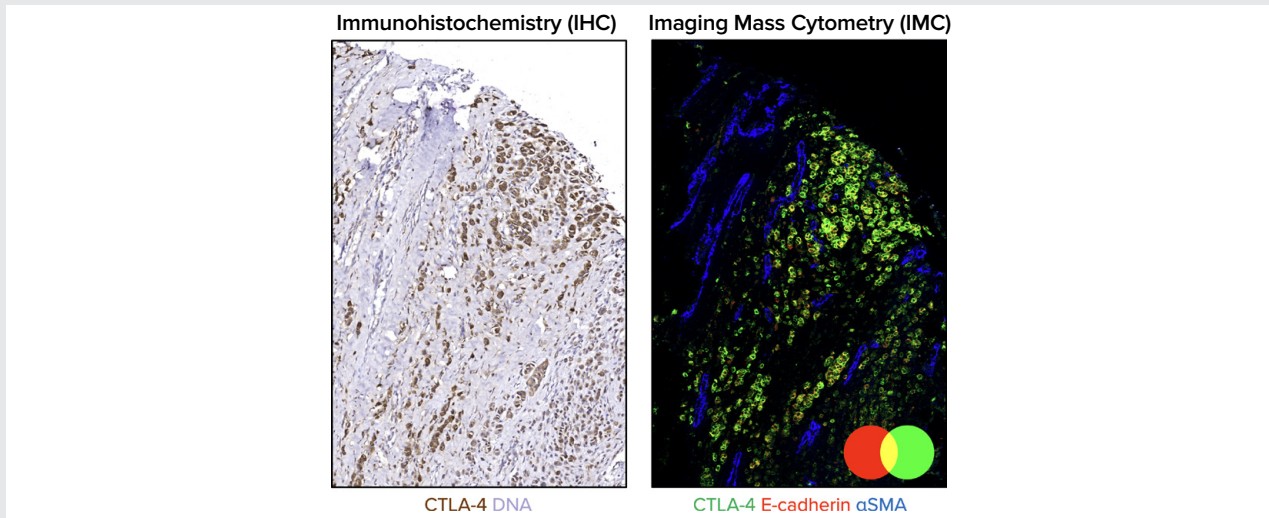


Figure 7. Upregulated CTLA-4 expression on melanoma cells suggests effective ICI response localized to the tumor, shown by co-localization with E-cadherin.

Detecting CTLA-4 expression in malignant melanoma with IMC technology enables quantification of expressing cells

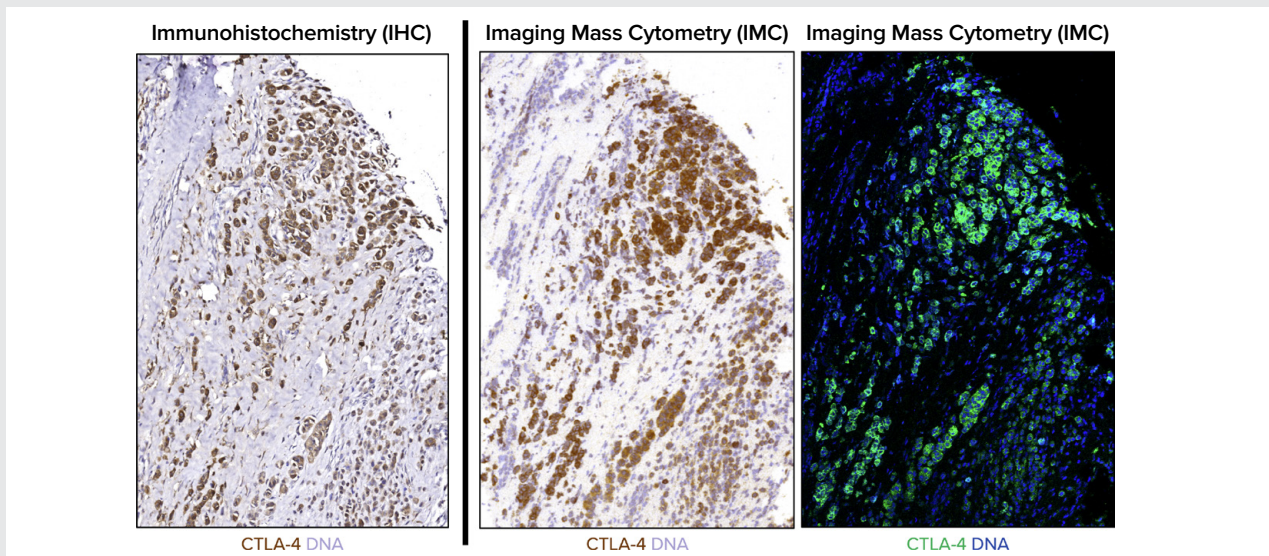


Figure 8. An IHC image (left) of a serial section from a malignant melanoma sample. A standard IMC image (right), here showing the two markers that are equivalent to IHC: DNA and CTLA-4. IMC technology enables the quantification of CTLA-4 expression levels, which is not possible with IHC.

IMC technology enables the detection and quantification of low-level marker expression

Detecting low-abundance biomarker expression is important for generating biological insights that guide decisions about therapeutic intervention – for example:

- Enhanced disease detection – low-abundance biomarkers can enable early detection of diseases before symptoms manifest
- Precision in drug administration – low-abundance biomarker detection enables more tailored therapeutic approaches and paves the way for personalized medicine
- Therapeutic monitoring – low-abundance biomarker detection enables continuous monitoring of disease progression during treatments, allowing for more effective management

IMC technology reveals the full range of protein expression, which includes low-abundance markers, enabling immune insights unable to be captured with cyclic fluorescence.

The Imaging Mass Cytometry platform offers:

- Detection of low-abundance biomarker expression without the use of signal amplification with fully intact dynamic range of signal
- Verification of signal authenticity through co- and counter-localization analysis
- Adaptability to pathologist workflows

The Hyperion™ XTi Imaging System demonstrates exceptional dynamic range to capture multiple insights from a single sample, including from low abundance proteins such as PD-1, PD-L1 and CTLA-4. Through detection of high and low expression, IMC technology can provide critical data for key decision-making strategies on ICI treatment and its effectiveness in different individuals.

Table 1. Therapies targeting PD-1, PD-L1 and CTLA-4 and the associated cancer types.

	FDA-approved molecule	Year of 1st approval	Cancer type
CTLA-4	Ipilimumab	2011	Melanoma, RCC, Metastatic colorectal cancer, HCC, NSCLC, Malignant Pleural Mesothelioma
PD-1	Pembrolizumab	2014	Melanoma, NSCLC, Bladder cancer, Hodgkin's lymphoma, HNSCC, RCC, Merkel cell carcinoma, MSI-high cancer, Gastric cancer, HCC, Cervical cancer, PMBCL, Endometrial Carcinoma, Esophagus Cancer, Colorectal cancer, Cutaneous Squamous-cell carcinoma, Breast cancer, TMB-high cancers
	Nivolumab	2014	Melanoma, NSCLC, RCC, Bladder cancer, Hodgkin's lymphoma, HNSCC, Colorectal Cancer, Hepato-cellular carcinoma, SCLC, Esophagus cancer, Malignant Pleural Mesothelioma
	Cemiplimab	2018	Cutaneous squamous-cell carcinoma, NSCLC, Basal cell carcinoma
PD-L1	Atezolizumab	2016	Melanoma, NSCLC, Bladder cancer, SCLC, Hepatocellular carcinoma, Breast cancer
	Avelumab	2017	Merkel cell carcinoma, Bladder cancer, RCC
	Durvalumab	2017	NSCLC, Bladder cancer, SCLC

Pilard, C. et al. "Cancer immunotherapy: it's time to better predict patients' response." *British Journal of Cancer* 125 (2021): 927–938.

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IMC Technology Uniquely Detects Low-Abundance Biomarkers Technical Note

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