Imaging Mass Cytometry Detects the True Dynamic Range of Low-Abundance Biomarker Expression in Human Tumors

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Introduction

Detecting clinically relevant biomarkers in cancer tissues provides key insights into the unique tumor characteristics of patients, allowing for more personalized and effective therapies. Immunohistochemistry (IHC) is the gold-standard technique for biomarker detection and is widely used by pathologists to score low-abundance biomarkers (LABs) in tissues. Limitations related to plexity, quantitation and false signal detection are frequently observed using IHC. Furthermore, day-to-day variability due to multiple steps of signal amplification and pigment mistaken for true signal can misinform pathologists about LAB expression.

Imaging Mass Cytometry™ (IMC™) technology is a multiplexed imaging technique that incorporates quantitative assessment of 40-plus biomarkers simultaneously on the same slide. The Hyperion™ XTi Imaging System, in association with an automated slide loader, permits 24/7 data acquisition and provides biological insights critical for assessment of the tumor microenvironment (TME). We strove to determine whether IMC technology can be used for pathological evaluation of LABs and provide additional key biological insights for clinical evaluation offered through multiplexed analysis.

Methods and materials

We performed a comparison of IHC and IMC technology to detect clinically relevant LABs (PD-1, PD-L1, CTLA-4 and LAG-3) on human tumor tissue microarray and whole tissue samples. For IMC technology, we detected single cells using the Human Immuno-Oncology IMC Panel, 31 Antibodies, which offers cell phenotyping of tumor and immune cell subtypes and their functional states. We stained serial sections of tissues using the same antibody clone and generated IHC and IMC data, which was assessed by a board-certified pathologist. We conducted quantitative image analysis to detect LAB expression on single cells.

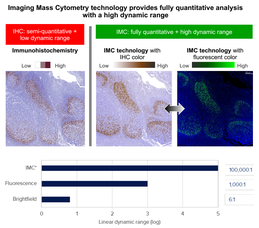

Figure 1. A standard IMC image taken in healthy tonsil sample using a 31-marker panel showing the two markers that are equivalent to IHC – DNA and PD-1 (right). An IMC image equivalent to that used for IHC, in which IMC technology exhibits much clearer delineation of PD-1-expressing regions (middle).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

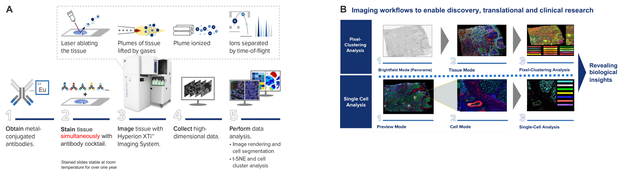

Figure 2. Imaging Mass Cytometry workflows. (A) IMC technology offers a streamlined workflow that simplifies translational and clinical application of multiplexed tissue analysis. The five-step process, which consists of obtaining metal-conjugated antibodies, staining tissues with antibody cocktails, imaging tissues with the Hyperion XTi Imaging System, and the collection and analysis of high-dimensional data, can be accomplished in as little as 72 hours (two slides with two 4 mm^2 ROI each). (B) The novel whole slide imaging (WSI) modes for IMC platforms offer a customized workflow for specific imaging applications. Here we highlight two simple ways for a user to get started. For single-cell analysis, start with Preview Mode, which provides a rapid scan of the whole tissue and highlights all your stained markers. This helps guide ROI placement to capture single cell-resolution image data using Cell Mode. For pixel-clustering analysis of an entire tissue section, users can first identify the placement of tissue using the rapid Brightfield Mode, followed by the novel Tissue Mode, which generates a high-quality scan of the entire tissue section in a matter of hours with higher spot-size ablations enabling entire tissue analysis using pixel-clustering analysis. Combining these new workflows with the newly available slide loader for the Hyperion XTi Imaging System streamlines IMC application and makes it a useful resource for high-throughput clinical and translational studies.

Conclusions

Clinical assessment of tissues using IMC technology offers an advantage over traditional IHC methods by providing true biological context due to multiplexing capabilities. The ability of IMC technology to provide high-dimensional spatially resolved data makes it a powerful tool for clinical and translational applications and shows that it is poised to significantly contribute to biomarker discovery and drug development.

Results

Our results demonstrate that while IMC technology and IHC are similar in detecting LABs, IMC technology can accomplish this without signal amplification, offering an opportunity to evaluate LABs in their true dynamic signal ranges. Qualitative analysis of IHC and IMC data further demonstrated the equivalent performance of both platforms (Figure 3). Multiplexed single-cell analysis using IMC data provided insights about LAB expression on specific immune and tumor cells. While IHC is semi-quantitative and cannot reliably determine the high abundance of a target, IMC technology offers the opportunity of signal quantitation as it displays the complete dynamic range of signal (Figures 4–7).

IMC technology is equivalent to IHC in detecting LABs, and offers the advantage of detecting the linear dynamic signal range along with the power of multiplexity

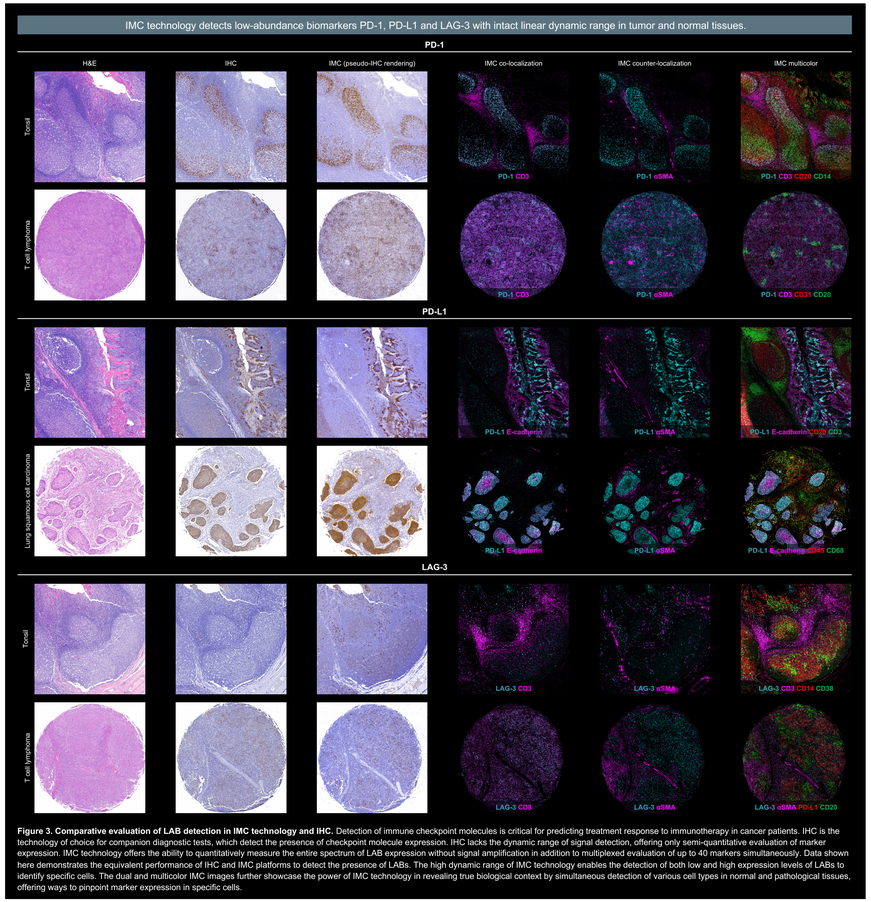

Figure 3. Comparative evaluation of LAB detection in IMC technology and IHC. Detection of immune checkpoint molecules is critical for predicting treatment response to immunotherapy in cancer patients. IHC is the technology of choice for companion diagnostic tests, which detect the presence of checkpoint molecule expression. IHC lacks the dynamic range of signal detection, offering only semi-quantitative evaluation of marker expression. IMC technology offers the ability to quantitatively measure the entire spectrum of LAB expression without signal amplification in addition to multiplexed evaluation of up to 40 markers simultaneously. Data shown here demonstrates the equivalent performance of IHC and IMC platforms to detect the presence of LABs. The high dynamic range of IMC technology enables the detection of both low and high expression levels of LABs to identify specific cells. The dual and multicolor IMC images further showcase the power of IMC technology in revealing true biological context by simultaneous detection of various cell types in normal and pathological tissues, offering ways to pinpoint marker expression in specific cells.

IMC technology detects HER2 ultralow level of expression and reveals the cellular composition of the TME in breast adenocarcinoma tissue

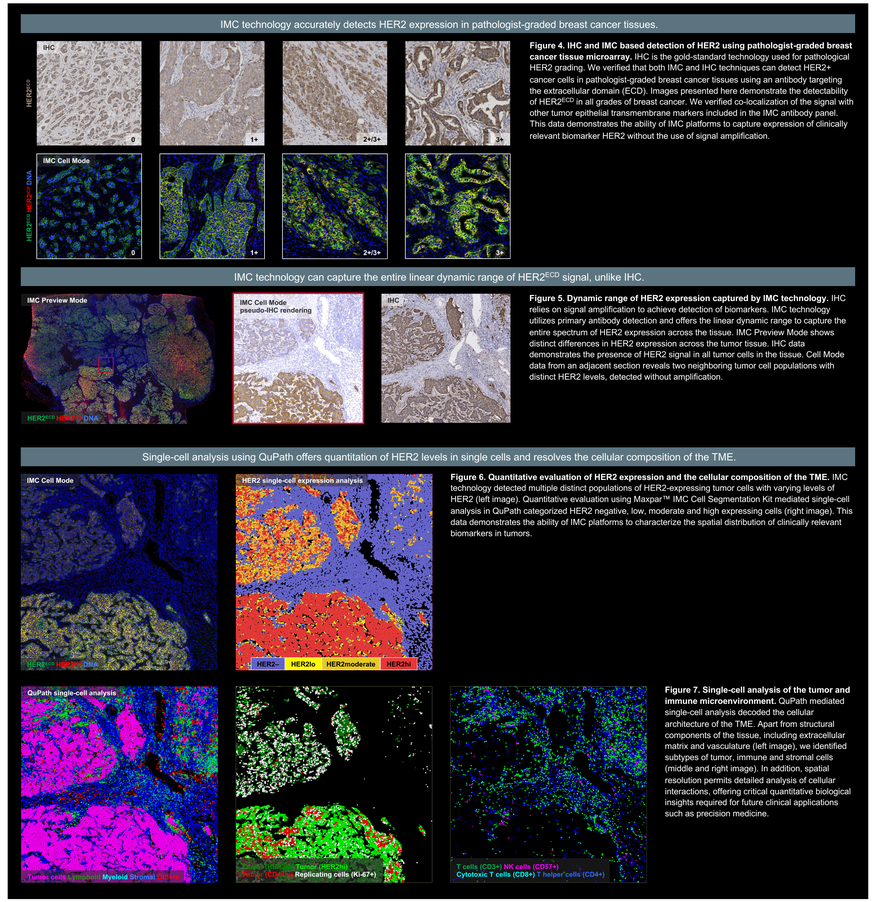

Figure 4. IHC and IMC based detection of HER2 using pathologist-graded breast cancer tissue microarray. IHC is the gold-standard technology used for pathological HER2 grading. We verified that both IMC and IHC techniques can detect HER2+ cancer cells in pathologist-graded breast cancer tissues using an antibody targeting the extracellular domain (ECD). Images presented here demonstrate the detectability of $HER2^{ECD}$ in all grades of breast cancer. We verified co-localization of the signal with other tumor epithelial transmembrane markers included in the IMC antibody panel. This data demonstrates the ability of IMC platforms to capture expression of clinically relevant biomarker HER2 without the use of signal amplification.

Figure 5. Dynamic range of HER2 expression captured by IMC technology. IHC relies on signal amplification to achieve detection of biomarkers. IMC technology utilizes primary antibody detection and offers the linear dynamic range to capture the entire spectrum of HER2 expression across the tissue. IMC Preview Mode shows distinct differences in HER2 expression across the tumor tissue. IHC data demonstrates the presence of HER2 signal in all tumor cells in the tissue. Cell Mode data from an adjacent section reveals two neighboring tumor cell populations with distinct HER2 levels, detected without amplification.

Figure 6. Quantitative evaluation of HER2 expression and the cellular composition of the TME. IMC technology detected multiple distinct populations of HER2-expressing tumor cells with varying levels of HER2 (left image). Quantitative evaluation using Maxpar™ IMC Cell Segmentation Kit mediated single-cell analysis in QuPath categorized HER2 negative, low, moderate and high expressing cells (right image). This data demonstrates the ability of IMC platforms to characterize the spatial distribution of clinically relevant biomarkers in tumors.

Figure 7. Single-cell analysis of the tumor and immune microenvironment. QuPath mediated single-cell analysis decoded the cellular architecture of the TME. Apart from structural components of the tissue, including extracellular matrix and vasculature (left image), we identified subtypes of tumor, immune and stromal cells (middle and right image). In addition, spatial resolution permits detailed analysis of cellular interactions, offering critical quantitative biological insights required for future clinical applications such as precision medicine.

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