WHITE PAPER

From Immunohistochemistry to Mass Spectrometry: Expanding Possibilities in Clinical Protein Analysis

Bridging the protein analysis gap
from **observation** to **interpretation**

SAPIENT

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Introduction

Accurate protein detection and characterization is essential to drug development and deployment, with protein biomarkers used to stratify patients, predict therapeutic response, and guide treatment decisions. Immunohistochemistry (IHC) has long been the go-to clinical method to assess protein expression in tissue biopsy samples, particularly for oncology and immune-related diseases. However, as the use of protein biomarkers in drug development and clinical medicine continues to evolve, with modern clinical practice now demanding more precise, quantitative, multiplexed, and scalable measures for protein detection, the limitations of IHC are becoming increasingly clear – as is the need to look to technologies that can expand upon what IHC provides.

To answer more advanced clinical questions, protein assays must go beyond analysis of protein **presence and localization** in tissue to enable **quantification and functional interpretation** of protein signals in the sample. High-specificity measure of protein abundance, including for post-translational modifications (PTMs) and protein isoforms, in correlation to prognosis or therapeutic response is key to driving better clinical decision-making. While IHC offers spatial context, it is a semi-quantitative, low-plex method that cannot readily detect PTMs and protein variants.

Mass spectrometry, on the other hand, is built for this level of molecular precision. It can not only quantify protein abundance with high accuracy via direct peptide sequencing, but it also measures protein isoforms – including post-translationally modified, variant, or mutant forms – which are increasingly recognized to play important roles in biological processes and drug response, particularly in the oncology space.[1] Though traditionally seen as a research-focused tool too complex for routine diagnostics, mass spectrometry has undergone significant technological advancements in sample handling, protein extraction, hardware, and software that have transformed it into a robust, scalable, and highly quantitative platform for clinical applications.

In this paper, we will delve into the innovations that are driving mass spectrometry-based proteomics methods into the clinic as an accessible platform to deliver specificity and a depth of molecular insights that IHC simply cannot match.

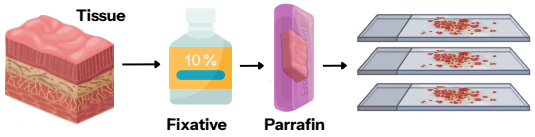
Immunohistochemistry: The Clinical Mainstay for Tissue Diagnosis

IHC has become deeply embedded in clinical workflows because of its simplicity, familiarity, and intuitive visual outputs. The method works by using labeled antibodies, typically linked with an enzyme or fluorophore, to detect a target protein in the tissue sample via light microscopy.

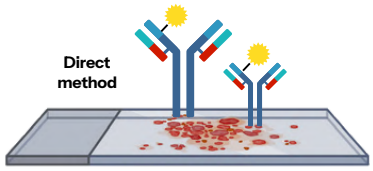
Sample preparation is key to the process, as the tissue must be rapidly preserved to prevent the degradation of cellular protein structures and ensure effective staining that accurately visualizes the target protein's location within the tissue. Formalin-fixed, paraffin-embedded (FFPE) tissue is by far the most widely used format for IHC analysis, using a formaldehyde solution to essentially 'lock' proteins within the tissue in place. The fixed tissue is then embedded in paraffin wax to allow for long-term storage as well as to enable the tissue to be thinly sectioned for analysis, which is crucial to ensure antibody accessibility to the target protein.

The thin tissue sections are mounted onto glass slides for de-paraffinization prior to staining. Because formalin fixation can cause protein cross-linking, which can mask protein and/or epitope accessibility or inhibit binding, additional steps for antigen retrieval are typically performed. Slides are then incubated, first with a blocking solution to reduce non-specific binding, and then with the primary target-specific antibody. If the primary antibody is not directly labeled with an enzyme or fluorophore, a secondary detection antibody is added to bind to the primary antibody.

IHC Method in FFPE Tissue

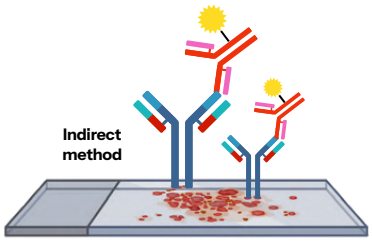


The tissue is first fixed with formalin to halt cellular degradation and then embedded in paraffin wax so it can be thinly sectioned for analysis.

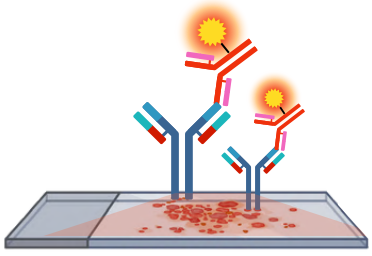


Following de-paraffinization and blocking, the slide-mounted tissue is incubated with the primary antibody.

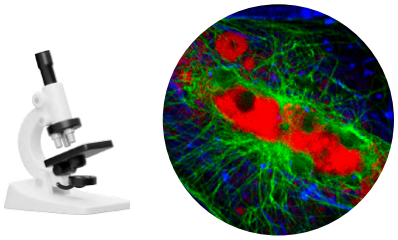
*In a **direct** IHC method, the primary antibody is labeled with the reporter enzyme or fluorophore.*



*In an **indirect** IHC workflow, a secondary detection antibody is added to bind to the primary antibody.*



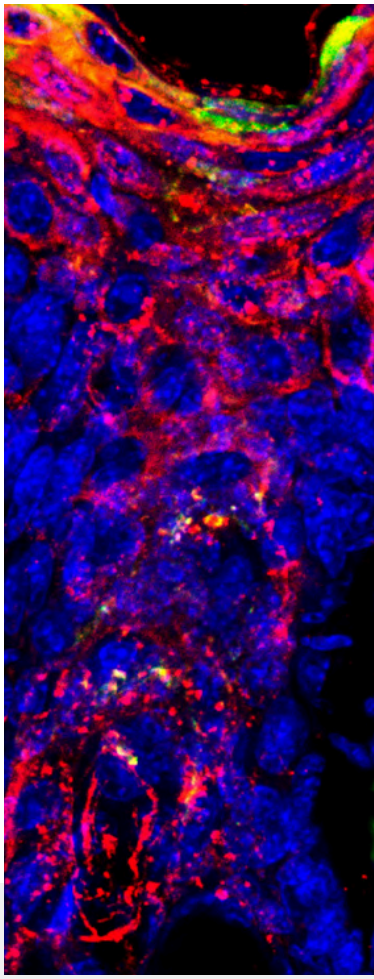
Signal is generated upon addition of a chromogenic substrate or by excitation of the fluorophore.



The signal is visualized under microscope to assess the presence and localization of the target protein.

A chromogenic substrate is added to the sample to cause a reaction with the enzyme (or fluorescence detection is used in the case of fluorophores) to visualize the presence and location of the target protein under a microscope. Protein expression is assessed based on staining intensity, distribution, and percentage of positive cells, and typically confirmed by a board-certified pathologist.

Providing Spatial Context



IHC workflows use standardized, semi-quantitative scoring systems for clinical interpretation of the colorimetric readouts. This makes IHC particularly valuable for evaluating the presence of known biomarkers in a spatial context, given its ability to visualize protein expression in the tissue within the overall structure and cellular organization of said tissue.

In oncology, for example, IHC can be used to distinguish between benign and malignant tumors, assess the tumor grade and stage, and evaluate the presence of key oncogenic mutations including HER2/neu, estrogen receptor (ER), or prostate-specific antigen (PSA). IHC's role in clinical oncology has grown considerably with the rise of targeted therapies and immunotherapies, as measure of tissue biomarkers has become critical for guiding drug development, clinical trial enrollment, and subsequently treatment decisions.

With more therapies relying on biomarker-driven patient selection, IHC will continue to play an important role. However, the growing complexity of clinical research and increasing interest in exploratory approaches is also creating demand for more advanced bioanalytical techniques.

Recognizing the Limitations of IHC

IHC is well-suited for targeted analyses but offers only a narrow view of the complex molecular networks that drive disease. It typically can only measure a limited number of biomarkers at a time, making it difficult to capture the broader biological context needed for more sophisticated clinical questions. Results are also semi-quantitative at best, subject to variance from tissue matrix and preparation, and are reliant on visual interpretation for scoring, limiting precision and scalability. The reliability of IHC data is also fundamentally tied to the quality and specificity of the antibodies used. Even under ideal conditions, variability in antibody performance can affect the accuracy of results. Detecting PTMs such as phosphorylation or glycosylation places even greater demands on antibody specificity. In these cases, antibodies must not only recognize the target protein but also distinguish its variants and/or precise modified state, which is an exceptionally high level of specificity that is difficult to achieve. Such highly validated antibodies are not always available, and even when they are, the performance can vary across sample types and conditions.

Tissue processing and storage further complicate detection by altering or masking these fragile molecular features. As a result, IHC offers a limited and often incomplete view of the dynamic protein modifications that play critical roles in disease mechanisms and therapeutic responses.

IHC: A Snapshot of Trade-offs

Low throughput	Most IHC assays are designed to detect only single or a few protein biomarkers at a time.
Semi-quantitative	While IHC can be used to assess relative protein abundance, it is not designed for highly accurate quantification. Results are based on visual scoring of the staining intensity and staining area, which can be influenced by subjective interpretation as well as antibody quality.
Antibody-dependent	The quality and accuracy of IHC results are heavily reliant on antibody specificity, sensitivity, and batch consistency. Antibody cross-reactivity and non-specific binding are challenges that can arise from subpar antibody performance and/or problems in the sample preparation steps.
Time-consuming assay development	In cases where a validated antibody is not yet available for the target protein of interest, a new antibody must be produced and subsequently optimized and validated to confirm its specificity, sensitivity, and reliability – requiring significant time investment.
Protein isoforms are problematic	PTMs and protein variants often require highly specific antibodies that may not exist or that may not perform reliably across different tissue types or fixation protocols.
Largely limited to FFPE	While IHC can be performed on frozen tissue, frozen samples tend to have less well-preserved tissue morphology which can affect staining quality and the ability to visualize fine cellular details under histological examination.

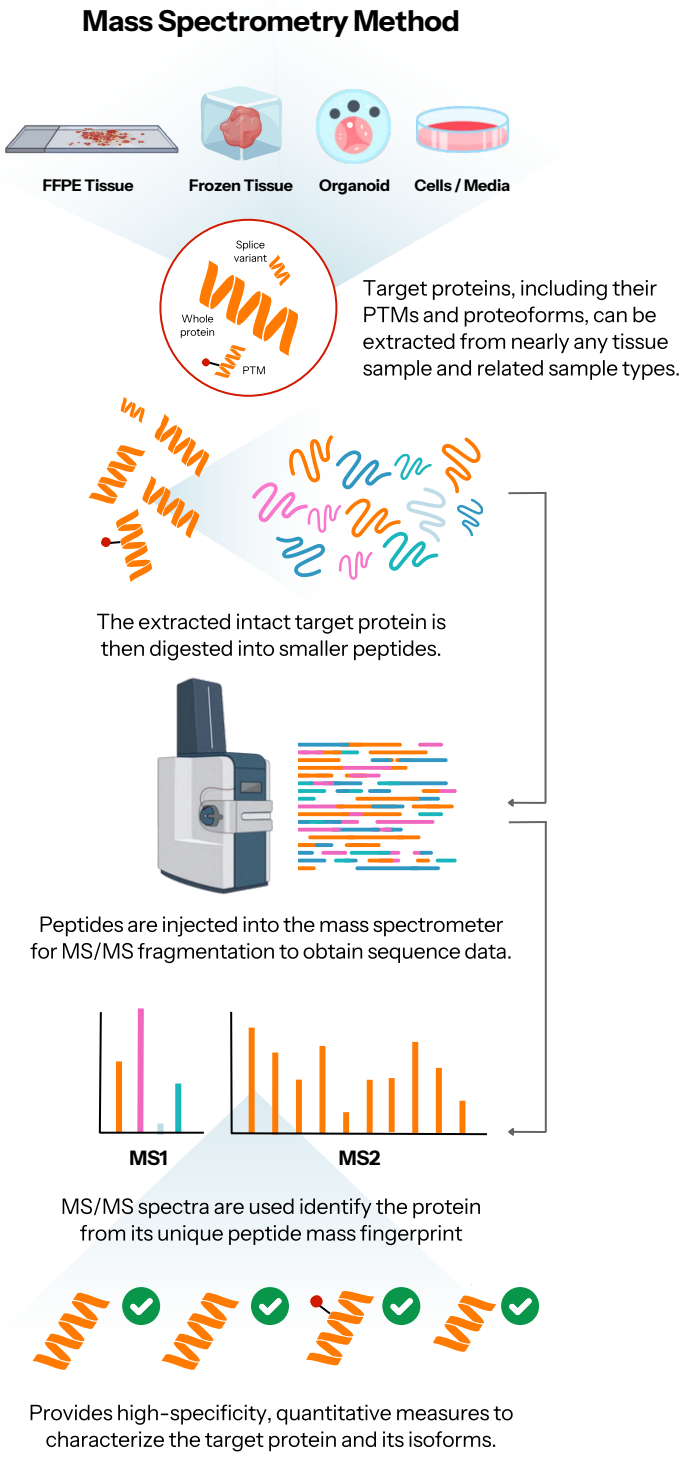
These constraints reduce the utility of IHC for understanding dynamic disease biology, particularly when precise quantification or the detection of subtle protein modifications are needed.

Next-Generation Mass Spectrometry:
A New Era in Clinical Protein Analysis

While mass spectrometry has long been valued for its analytical power, its practical use in clinical settings was historically limited by complex workflows, manual sample preparation, and the need for highly specialized expertise. Significant progress in instrumentation and workflow automation has since removed those barriers, making the new generation of mass spectrometry systems fully equipped for modern clinical demands.

Advances in high-resolution mass analyzers, for example, have dramatically increased sensitivity and throughput while reducing run times. Automated sample preparation platforms minimize human error and accelerate sample processing, while integrated software solutions streamline complex data analysis, making results more accessible to laboratory personnel without specialized training. These changes, paired with the long-renowned analytical capabilities of mass spectrometry, allow researchers to build upon IHC insights with quantitative measures and functional understanding of their proteins of interest.

Unlike IHC, which relies on antibodies to bind and visualize specific proteins, mass spectrometry identifies proteins by direct sequencing of their peptides. This allows for definitive identification of proteins and their isoforms and negates challenges of non-specific binding and cross-reactivity that can occur in antibody-based methods like IHC, thus

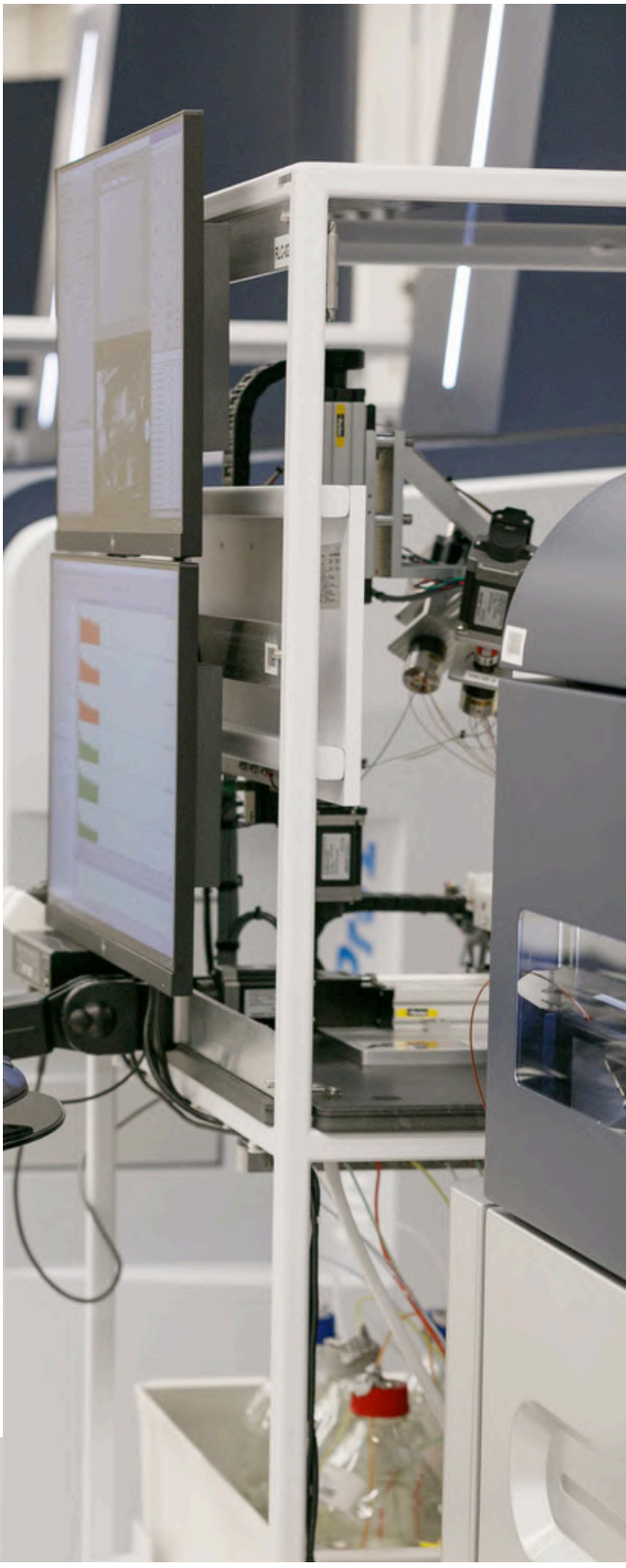


reducing the chance of false positive or false negatives. In comparison to the semi-quantitative nature of IHC, mass spectrometry delivers precise, highly quantitative measurements, enabling researchers and clinicians to make more informed diagnostic and treatment decisions.

Delivering Deeper, Rapid Insight

Next-generation mass spectrometry is amenable to high-throughput targeted workflows, able to perform quantitative analyses of single proteins (or small panels) on the order of thousands of samples per day. It can also be used to quantify hundreds to thousands of proteins in a single experiment, with the same high throughput, to deliver a far more comprehensive view of the molecular landscape underlying disease and drug response. It does not depend on prior knowledge of the proteins present, enabling an unbiased view of the proteome. While advanced multiplex IHC techniques are available, they remain limited in scale compared to mass spectrometry and require antibodies optimized to bind each target protein in the study.

Importantly, mass spectrometry's sensitivity and high resolution reveals layers of molecular insights that are difficult or even impossible to access through IHC. This includes not only the detection of critical protein variants, mutant proteins, and PTMs that can influence disease progression, therapeutic response, and drug resistance, but also the discovery of entirely new biomarkers, including non-canonical proteins in the dark proteome which have recently discovered links to diseases such as cancer.[2] And, because mass spectrometry allows for direct analysis of molecules, it can be reliably applied to nearly any sample type including FFPE as well as frozen tissue, organoids, and even biofluids.



Mass Spectrometry: Benefits that Broaden the Picture

High throughput	Next-generation mass spectrometry can be leveraged in highly multiplexed targeted workflows, across thousands of samples at time.
Quantitative precision	'Bottom-up' mass spectrometry identifies and quantitates proteins based on sequencing of individual peptides, enabling precise detection across a wide range of proteins without the need for antibodies.
Rapid assay development	Developing a new mass spectrometry assay is significantly faster and more resource-efficient as it eliminates the steps required to generate, optimize, and validate a new antibody, as would be required by IHC.
Protein isoforms included	Through direct peptide sequencing, mass spectrometry can measure variant and mutant forms of a protein, as well as PTMs such as phosphorylation and glycosylation which have been shown to be promising drug targets.[1]
Deeper coverage of the tissue proteome	In contrast to IHC which is used to measure one or a few known proteins at a time, next-generation mass spectrometry can assay up to 12,000 proteins in a single tissue sample in 'discovery' mode or hundreds of proteins for targeted, quantitative measure, providing a comprehensive view that can reveal related or additional biomarkers and functional mechanisms which correlate with disease activity or therapeutic response.
Amenable to any tissue sample	As a biochemical technique, mass spectrometry does not rely on tissue morphology or visualization for analysis. If the protein can be extracted and digested from the sample – whether FFPE, frozen tissue, cells, organoids, or even biofluids – it can be assayed via mass spectrometry.

Mass spectrometry enables clinical researchers to ask more than whether a protein is present in their sample of choice, but how much, in what form, and its functional relevance.

Mass Spectrometry and IHC: Complementary, Not Competitive, Methods for Modern Clinical Analysis

The clinical proteomics landscape is rapidly evolving. IHC remains a foundational tool in clinical research and diagnostics, especially when spatial localization of known protein biomarkers is critical, but its limits are clear in an era where clinical questions demand quantitative accuracy, isoform-level resolution, and the ability to explore molecular complexity at scale. Mass spectrometry now offers a scalable, highly quantitative, and clinically accessible platform to fill these gaps – **no longer confined to the research realm but ready to drive next-generation clinical decisions.**

IHC provides a well-established clinical method to assess protein expression directly within tissue architecture and continues to be indispensable in areas such as oncology, where the tissue context informs patient stratification and therapy selection. Next-generation mass spectrometry expands upon IHC analysis by significantly improving quantitative fidelity and throughput while offering a means to readily measure PTMs or protein variants that may underlie disease heterogeneity or treatment resistance.

Now, clinical protein analysis can extend beyond presence and localization, to accurately quantify protein levels, measure proteoforms that were previously undetectable, and make decisions based on molecular precision rather than subjective assessment. Together these methods can form a complementary framework: one that leverages IHC for important spatial information and mass spectrometry to contextualize and correlate protein profiles with specific histological features identified, enabling deeper analysis for functional insights. This empowers researchers and clinicians to move from observation to interpretation – and from detection to better drug development and clinical decision-making.

References

1. Capturing the diversity of protein modifications on presented tumor antigens. *Nat Biotechnol* 41, 195–196 (2023). https://doi.org/10.1038/s41587-022-01465-1

2. Posner, Z. et al. Shining a light on the dark proteome: Non-canonical open reading frames and their encoded miniproteins as a new frontier in cancer biology. *Protein Sci.* (2023) https://doi.org/ 10.1002/pro.4708