




# Capture the immune markers that matter

Identify novel **disease drivers** and design **better therapies**

Uncover diverse and rare immune cell subsets and their function with CyTOF™ XT PRO  
– capturing critical biomarkers for improved immunotherapies.



Proof of mechanism  
Patient stratification  
Biomarker translation

**CyTOF™ XT PRO**

# CyTOF single-cell proteomics

## Every marker, all at once

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CyTOF XT PRO is a powerful cytometry platform with proven sensitivity, dynamic range and resolution to capture 50-plus targets simultaneously, including cell surface markers, signaling proteins and cytokines.

Powered by mass cytometry, the use of metal-conjugated antibodies overcomes spectral spillover limitations associated with fluorescence flow cytometry – enabling the precise detection of phenotypic and functional variation in single cells.

### Widest coverage and highest multiplex

**5-10x**

more functional proteins

**2x**

more markers

Designed with clinical researchers in mind, CyTOF XT PRO takes mass cytometry to the next level. With the enhanced throughput mode, integrated compliance-enabling software, increased sample multiplexing and improved walk-away capabilities, CyTOF XT PRO meets the throughput and regulatory needs of clinical and translational research.

### **XTra coverage**

Reveal differentiating markers of immune health

### **XTra reliability**

Stable signals and reproducible results for any study design

### **XTra speed**

Up to 4x enhanced throughput to expedite insights

### **XTra compliance**

21 CFR Part 11 available to meet clinical research requirements



## Widest immune coverage

### Comprehensive profiling for clinical and translational research

Both cell type and function are key in determining mechanisms behind immune potency and persistence, immune-related adverse events and immunosuppression. For this reason, CyTOF has been adopted for use in hundreds of clinical trials worldwide for immune monitoring and biomarker discovery studies across broad areas including immuno-oncology, infectious disease, autoimmune disease and neuroscience.

### The top cancer immunotherapies<sup>‡</sup> have leveraged unique biomarkers – found by CyTOF technology

Conventional panel in spectral fluorescence

25 surface markers\*

Additional simultaneous coverage on CyTOF system

+25 intracellular/functional markers\*



*Biomarker for identification of drug targets in multiple myeloma trials*

**BMS<sup>†</sup> | nivolumab**  
*Predictive biomarker of response in combination nivo Phase 2 trial*

**MERCK<sup>†</sup> | pembrolizumab**  
*Predictive biomarker of survival in pembro Phase 1 trial*

**NOVARTIS<sup>†</sup> | CAR T**  
*Predictive biomarker of response in CAR T*

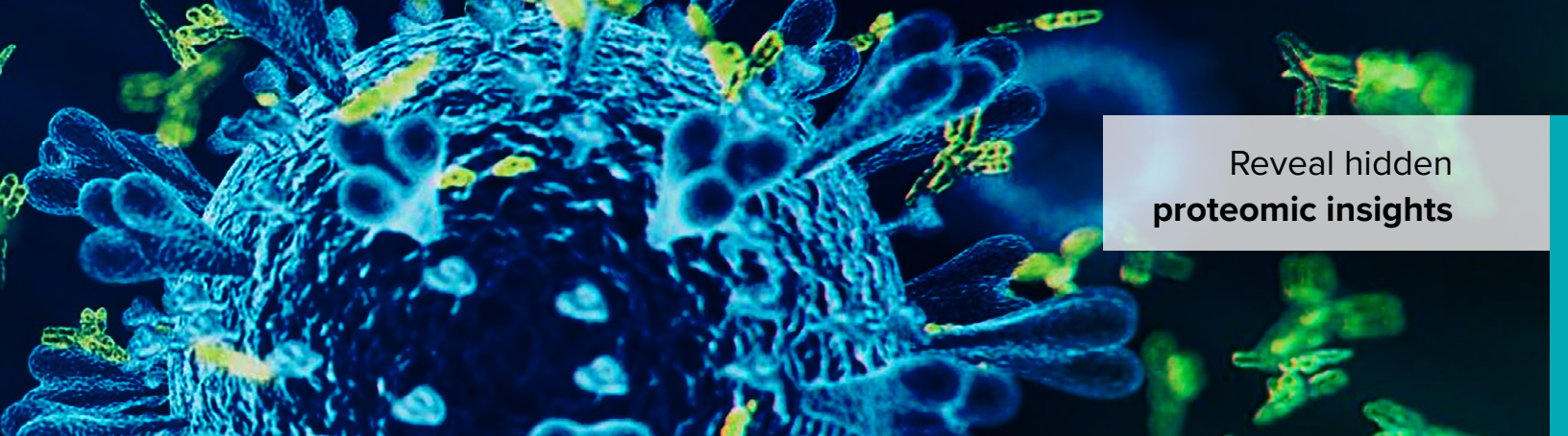
Unique predictive biomarker

**CyTOF simultaneously profiles 50-plus surface and functional markers – in a single panel**

\* Competitor 25-color immunoprofiling assay.

<sup>†</sup> Pharma companies listed are the firms that manufacture and/or market the therapies in the published studies.

<sup>‡</sup> Top cancer immunotherapies by revenue in 2023.



Reveal hidden  
proteomic insights

## Identify unique biomarkers with early predictive insights

Understanding phenotypic and functional biological variation can generate actionable insights into molecular mechanisms and disease pathogenesis that refine targeted therapies and advance precision medicine approaches. For this reason, clinical researchers are including CyTOF in their clinical trials to better understand disease activity, drug response or outcome prediction.

## CASE STUDY

In a Phase 1 clinical trial<sup>1</sup>, CyTOF technology reveals **predictive biomarkers of response and survival** for checkpoint therapy in advanced non-small-cell lung carcinoma.

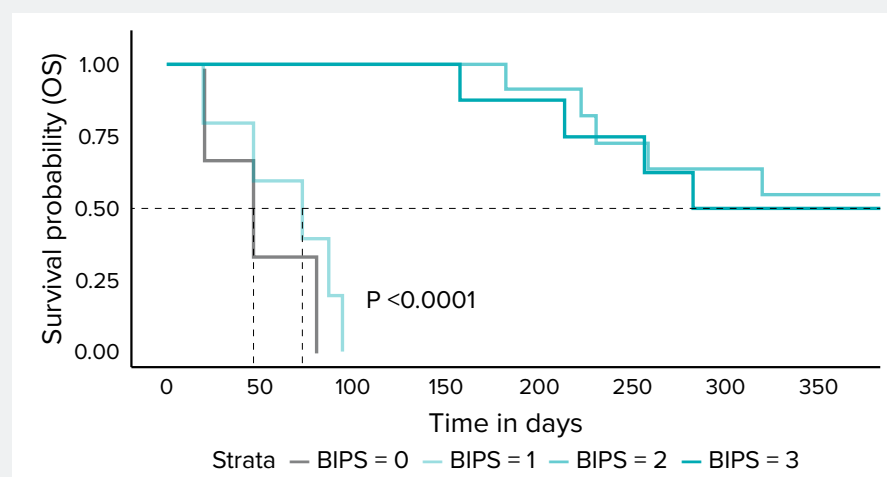


Figure 1. CyTOF technology demonstrates that blood baseline frequencies of classical monocytes, natural killer cells and ICOS<sup>+</sup> CD4<sup>+</sup> T cells are significantly associated with improved objective response rates, progression-free survival and overall survival.

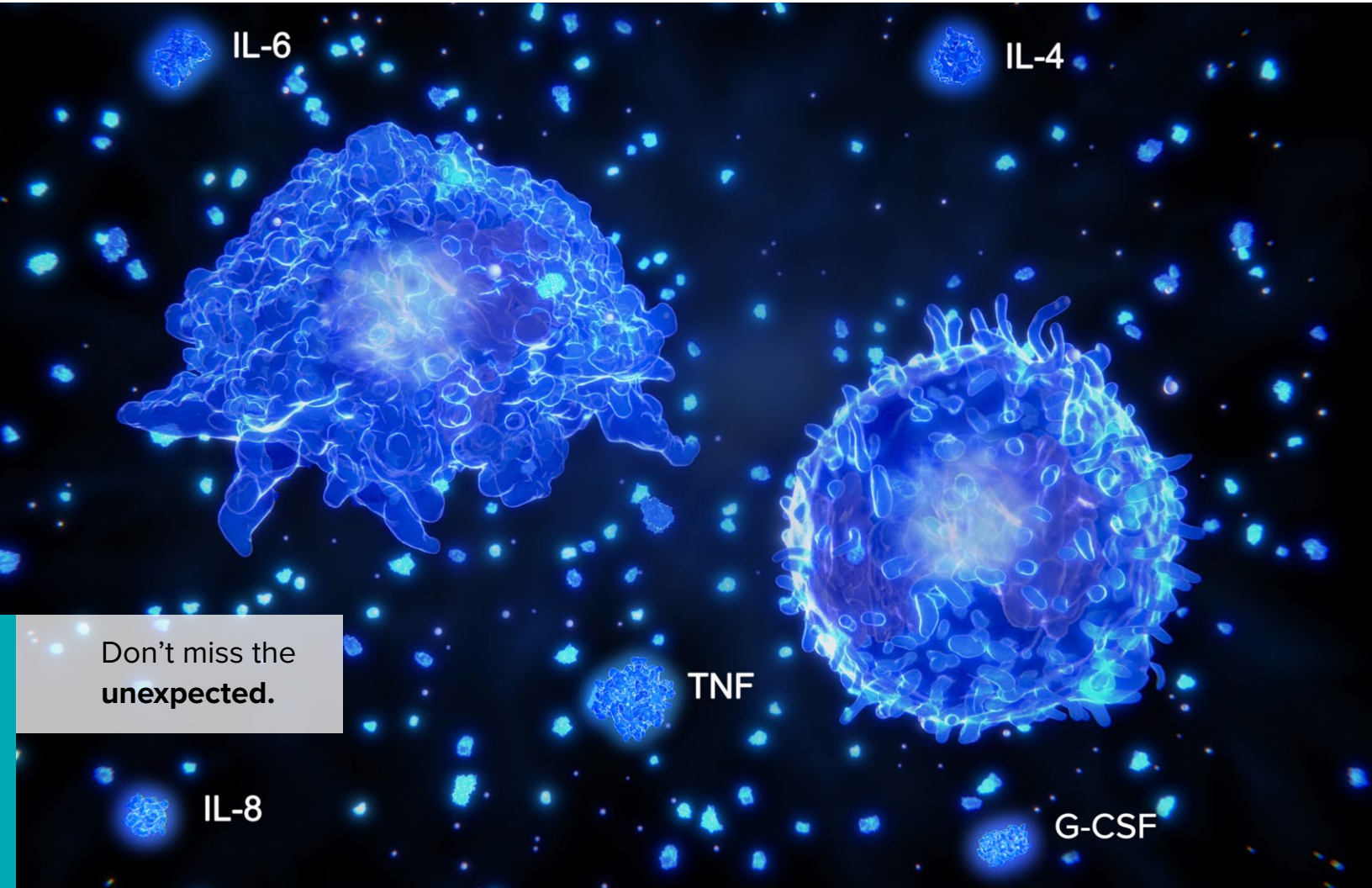
Rochigneux, P. et al. *Clinical Cancer Research* (2022)

# Superior intracellular resolution

CyTOF XT PRO detects more functionally diverse subsets — with accuracy.

Cytokines represent a large percentage of published immune potency biomarkers; however, they are notoriously difficult to capture by fluorescence flow cytometry due to autofluorescence and spillover challenges. CyTOF XT PRO provides superior signal resolution for a wide range of intracellular markers, many of which cannot be clearly detected with spectral flow cytometry<sup>2</sup>. Characterizing the complex crosstalk of cytokines and their signaling pathways simultaneously with surface markers enables a clearer understanding of distinct immune signatures.

Capturing both cell type and function is essential in understanding mechanisms behind immune potency and persistence, immune-related adverse events and immunosuppression.

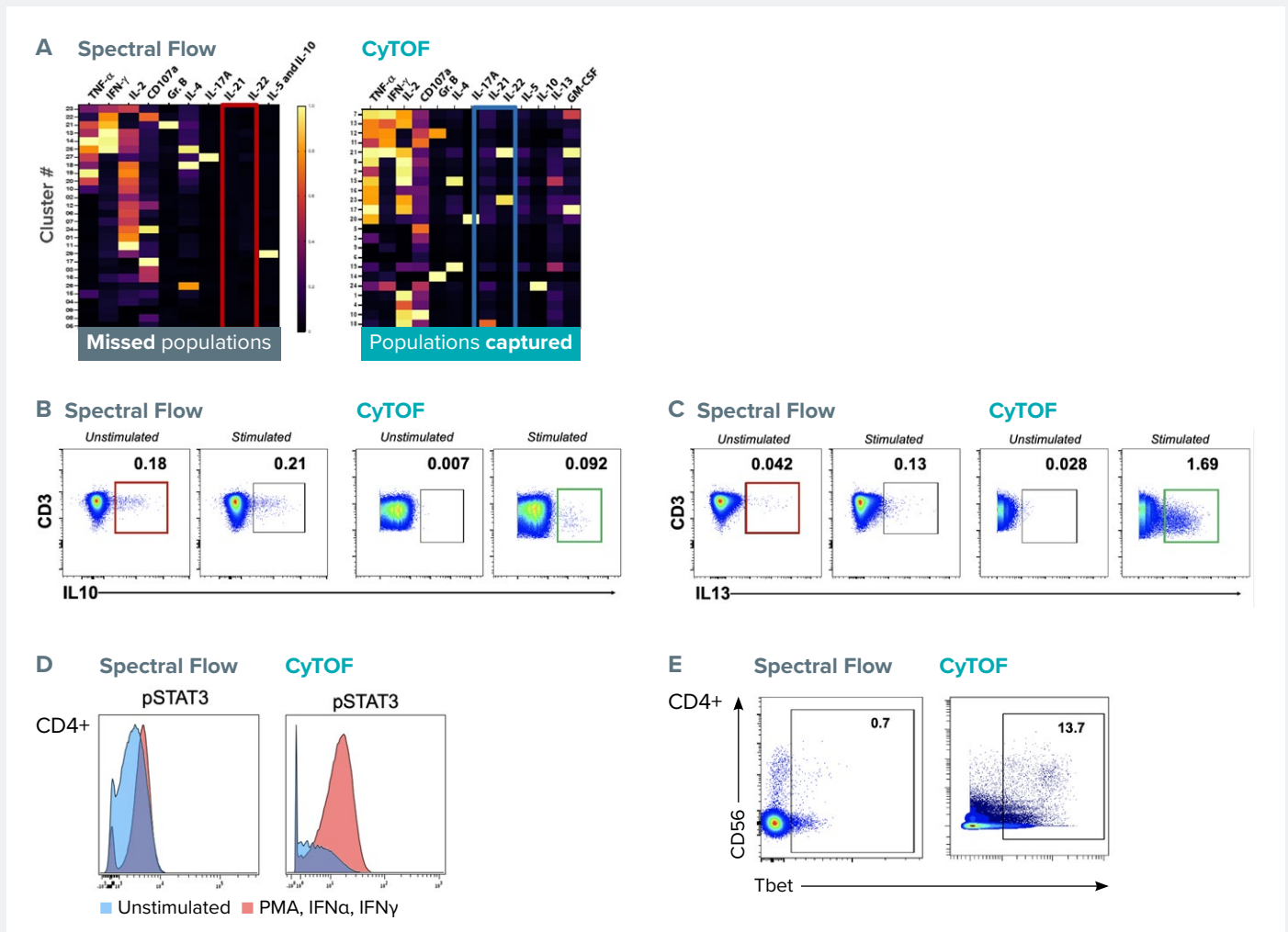


Don't miss the unexpected.

## CASE STUDY

### CyTOF identifies critical functional markers undetectable with spectral flow cytometry

In a comparison study<sup>2</sup>, Boston University found that the CyTOF XT system consistently demonstrates superior signal:noise resolution of intracellular markers compared with spectral flow cytometry. CyTOF was strikingly superior in the detection of all intracellular categories, including cytokines, phosphorylated proteins and transcription factors. In addition, they concluded that CyTOF XT is the only suspension cytometry instrument able to capture IL-5, IL-10 and IL-13 with ease and accuracy.



**Figure 2. Distinct differences in intracellular cytokine staining by spectral flow and mass cytometry.** A) Right: spectral flow staining heatmap, blue box highlights no IL-21 and IL-22 signal. Left: CyTOF staining heatmap, the red box highlights IL-21 and IL-22 detection. B) Left: Spectral flow cytometry gating of IL-10 expression, boxes highlight population with positive expression; Right: CyTOF gates of IL-10 expression, boxes highlight population with positive cytokine expression. C) Same as B) for IL-13 expression comparison. D) Comparison of pSTAT3 expression within the CD4+ population of stimulated and unstimulated samples. E) Comparison of Tbet expression within the CD4+ population.



#### Watch the seminar

Exceptional Resolution of Anti-Inflammatory Cytokines Using CyTOF.

# Rapid and scalable immune profiling

## CyTOF facilitates standardized and large-scale immunophenotyping initiatives

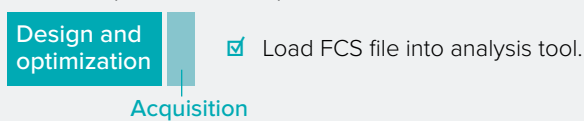
Mass cytometry analyzes immune responses at a scale not possible with other methods. The unique CyTOF advantage of discrete mass signals without overlap, and subsequent large number of available antibodies, simplifies panel design and eliminates the need for complicated spectral unmixing algorithms. Further, this wider range of available channels provides the unmatched capability for multiplexed sample barcoding, a powerful method for harmonizing large clinical trial datasets. By staining and acquiring large numbers of samples in a single tube, sample barcoding significantly reduces workflow burdens, acquisition times and technical variability, while conserving precious clinical material.

CyTOF XT PRO expedites the end-to-end workflow and provides greater data consistency for large immune profiling studies.

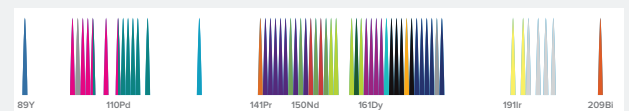
## WORKFLOW COMPARISON

### CyTOF

Time required for 30-plus markers is **less than 1–2 weeks**.



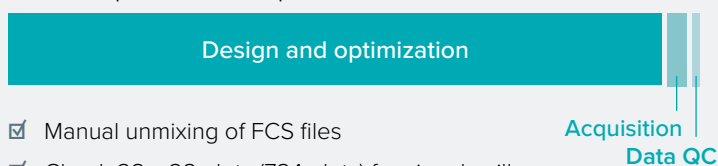
In **CyTOF**, studies, sample preparation is simple. There are no complicated algorithms to apply to deconvolute or compensate for spectral overlap.



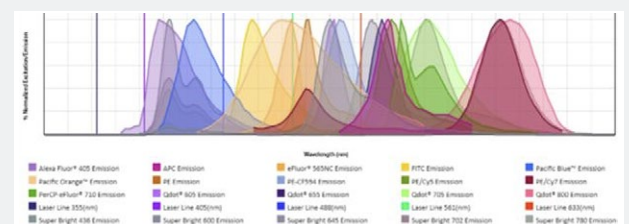
## VS

### Spectral flow

Time required for 30-plus markers is **8–12 weeks or more**.



In **spectral flow**, there are strict requirements for fluorophore combinations that need rigorous testing and optimization, single-stain controls, and additional sample processing and algorithms<sup>7,8,9</sup>.





Quality data,  
faster.

## Reduce time of study with pre-optimized panels and analysis templates

Several clinical trials<sup>10,11</sup> have used the **Maxpar™ Direct Immune Profiling Assay (MDIPA)** for its comprehensive and convenient immunophenotyping screening in a single assay. MDIPA is a lyophilized and validated 30-marker antibody panel, that can be easily combined with expansion panels and an automated five-minute analysis solution to quickly identify cell types and cell differentiation states.

## CASE STUDY

### Scalable immune profiling in clinical trials with CyTOF reagents

In one Phase I/II multiple myeloma trial<sup>10</sup>, this assay was customized with six additional markers to find correlates of response and resistance following TIGIT–LAG3 blockade.

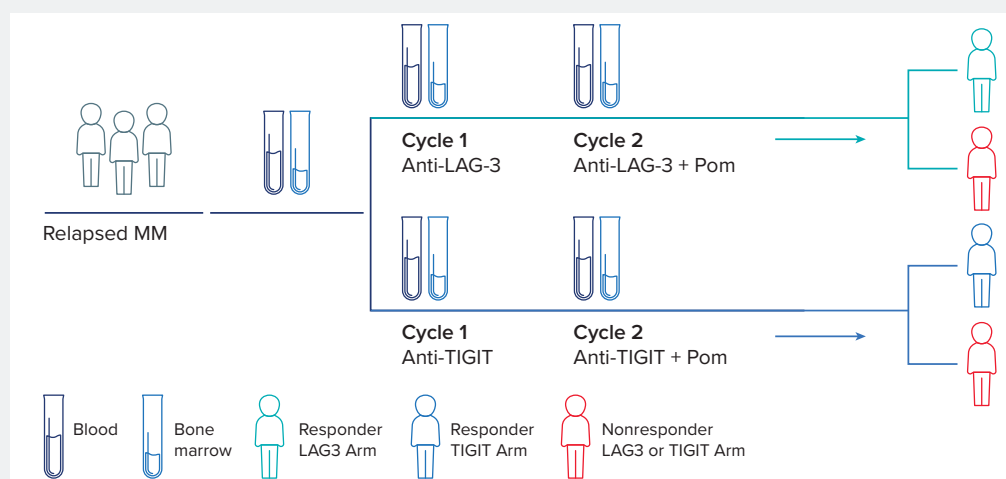


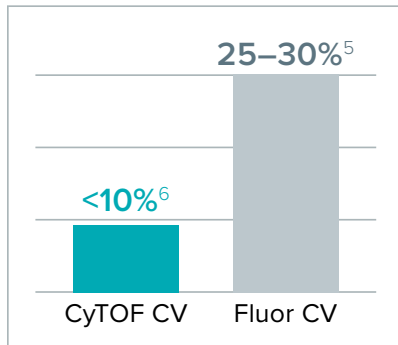
Figure 4. Rapid and comprehensive immunophenotyping with MDIPA for two study arms and at different time points generated important insights into the distinct clinical response pathways associated with TIGIT or LAG3 blockade resistance.

# Highest reproducibility for any workflow

Analysis of clinical samples requires rigorous attention to data quality, comparability and reproducibility. CyTOF technology has exceptionally high reproducibility, as metal-tagged antibodies can withstand the harsh elements of time and temperature. Unlike fluorescent tags, which are light-sensitive, CyTOF reagents are extremely stable when storing, freezing or shipping samples. This alleviates time-sensitive restrictions when processing samples, especially required for large-scale clinical trials.

## Ideal for:

- Multi-site and longitudinal studies
- Biobanked or stored samples
- Stain-freeze-ship workflow



## Consistency between sites and instruments

In a multi-site study, CyTOF technology demonstrated a high degree of reproducibility, with all population frequencies >5% having a coefficient of variation (%CV) of less than 10% in whole blood<sup>6</sup>.

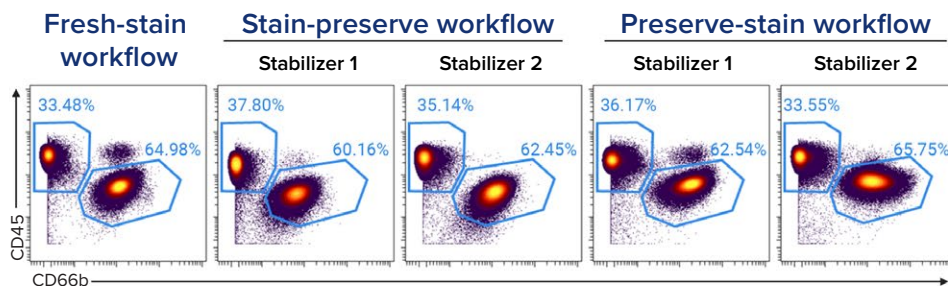
Flexible protocols,  
**same robust quality.**



## Consistency across different sample collection methods<sup>4,5</sup>

In a comparison study with different experimental workflows, CyTOF demonstrates reproducible data quality<sup>4</sup>. For all 32 immune cell populations  $\geq 5\%$  in frequency, the %CV within each donor across the different workflows was  $< 9.2\%$ . In addition, frozen antibody cocktails are stable over time and yield comparable signal intensities over time.

- Fresh-stain
- Stain-preserve
- Preserve-stain
- Frozen antibody cocktails



[Read the application note](#)



Figure 5. Representative dot plots show the compatibility of the Human Broad Immune Profiling CyTOF Panel, 20 Antibodies with stabilization reagents and sample multiplexing in three different workflows<sup>3</sup>.

## CASE STUDY

### Improving immunophenotyping strategies for longitudinal clinical trials

Researchers at the University of Sydney<sup>12</sup> developed a robust CyTOF workflow to overcome the limitations in remote sample collection quality, aiming to better represent more diverse populations in clinical trials.

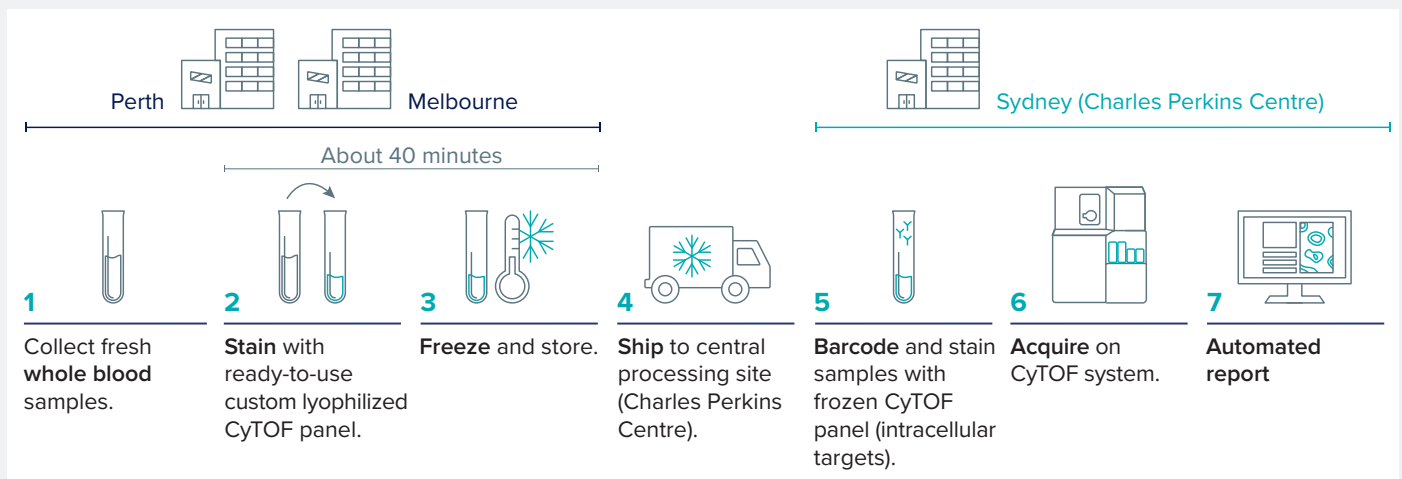


Figure 6. This graphic highlights a highly reproducible and robust stain-freeze-ship CyTOF workflow with a 50+ functional and phenotypic panel to capture pre- and post-treatment responses, across multiple sites. Further, they employed sample barcoding, a unique CyTOF advantage, which provides even greater data consistency for large studies.

# Taking CyTOF XT to new heights

## Widest immune coverage – at faster speeds

CyTOF XT PRO is the latest advancement in mass cytometry. Designed specifically for clinical research needs, CyTOF XT PRO is up to 4x times faster and complies with 21CFR Part 11 requirements. In addition, the enhanced software of CyTOF XT PRO improves sample multiplexing capabilities for large-scale clinical trials, by streamlining data analysis for the de-barcoding of custom isotope combinations.

With the CyTOF XT family of systems, researchers will benefit from simplified sample processing and data acquisition that offers the ability to **load samples and walk away**. The chilled sample carousel, which holds up to 13 tubes, and real-time signal optimization stabilize the detection system for uninterrupted 23-hour acquisition. In addition, the simplified front-end assembly, easy daily setup, and automated tuning and sample dilution streamline experimental throughput, allowing you to do more with your time – while seeing more from your samples.



The next evolution of  
mass cytometry is here –  
**CyTOF™ XT PRO.**

**Do more** with your time.  
**See more** from your samples.



### Enhanced throughput.

Combining faster acquisition with automated sample handling

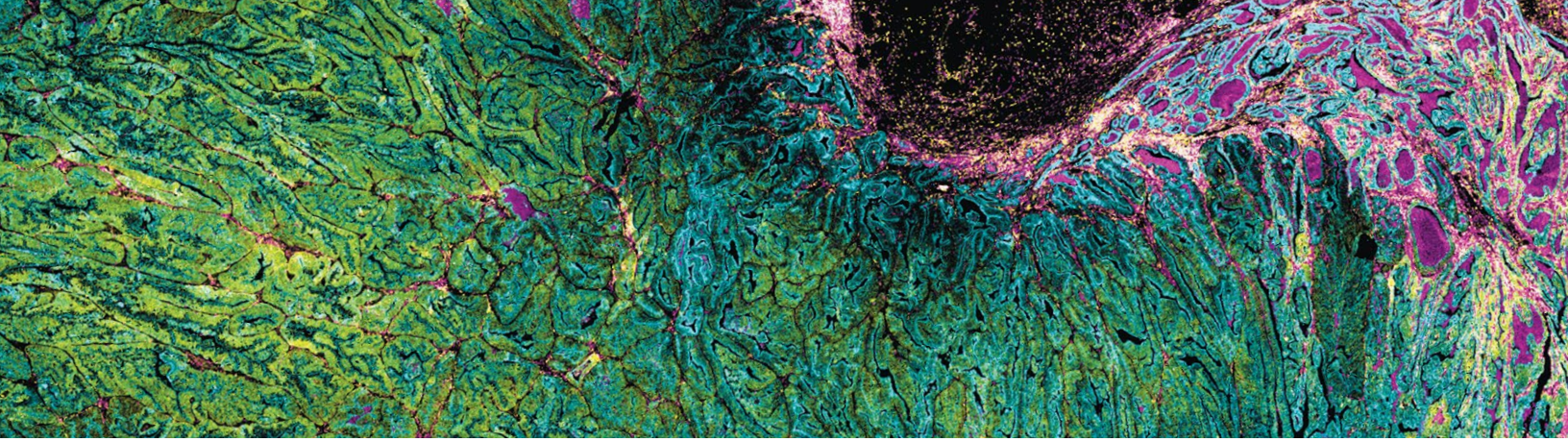
### Compliant software.

Meets requirements for authenticity, integrity and confidentiality of clinical trial data with 21 CFR Part 11 compliance.

## A solution for any phase of research

Whether you're doing discovery, translational or clinical research, we have a solution to meet your laboratory requirements.

	CyTOF XT PRO	CyTOF XT
<b>21 CFR Part 11 compliance</b>	✓	
<b>Enhanced throughput mode</b> Up to 4x faster than CyTOF XT.	✓	
<b>Autosampler Module</b> Automated sample handling with up to 13 tubes that can be loaded into the carousel for unattended acquisition. Additional samples can be added on the fly as space is freed up.	✓	✓
<b>CyTOF Software v9.2</b> Includes major feature upgrades that improve system automation and data analysis. Key enhancements include support for custom debarcoding to advance downstream analysis of sample multiplexing and automated sample dilution to improve hands-free operation.	✓	✓
<b>Tissue imager-compatible</b> Can be purchased as a single system or retrofitted later.	✓	✓



## A multimodal instrument

### Maximize your investment with add-on spatial proteomics capabilities

Add the tissue imager module to CyTOF XT or CyTOF XT PRO for powerful spatial proteomics insights. Imaging Mass Cytometry™ (IMC™) has become a widely adopted method due to the mounting evidence demonstrating the important role that spatial context has in understanding disease progression, identifying biomarkers, developing therapeutics and monitoring therapeutic response. Since the tissue microenvironment is dynamic and highly heterogeneous, characterizing cell phenotypes, correlated locations and cell-to-cell interactions can provide predictive insights derived from the quantitation of spatial relationships and context.

### One instrument, dual functions

Leverage the unique integrated dual modality of single-cell and spatial proteomic capabilities with the **Hyperion™** XTi Imaging System.



**Hyperion XTi**  
Imaging Mass Cytometry

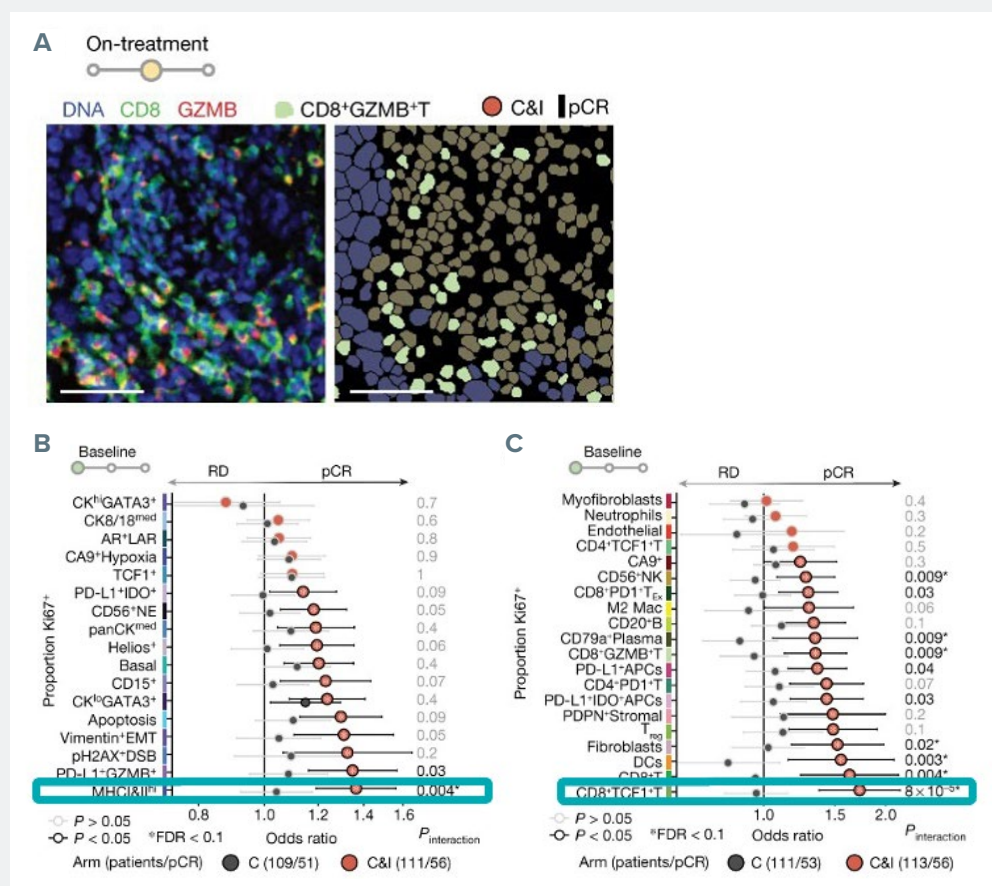
**CyTOF XT PRO**  
Mass Cytometry

Interpret tissue heterogeneity.

## CASE STUDY

### Predictive biomarker of response in checkpoint blockade combination therapy

IMC technology identified a unique biomarker of response in this Phase 3 clinical trial of atezolizumab (anti-PD-L1) combination therapy for 280 patients with early high-risk triple-negative breast cancer. Both cellular composition and spatial organization within the tumor microenvironment were predictive of response in patients both pre- and on-treatment<sup>13</sup>.



**Figure 7. Proliferating cancer and tumor microenvironment cell phenotypes predict response to immunotherapy.** A) Highlights CD8+GZMB+T cell density for immunotherapy on-treatment responder with image (left) and cell phenotype masks (right). White scale bar, 50  $\mu$ m. B, C) For subjects treated with immunotherapy, 12 epithelial and 16 cell phenotypes predicted response. The blue outline represents the strongest predictors of immunotherapy response, with the proliferative fraction of MHCII<sup>hi</sup>I<sup>hi</sup>H cells strongest among epithelial (cancer) cells and CD8+TCF1+T cells the strongest response predictor overall. pCR: Pathological Complete Response; RD: residual disease.



## Unleashing tools to accelerate breakthroughs in human health™

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