TECHNICAL NOTE

Unparalleled Reproducibility of the Hyperion XTi System for Spatial Proteomics

Assessing technical and biological reproducibility of the Imaging Mass Cytometry (IMC) platform across time and instruments

Introduction

The Standard BioTools™ Imaging Mass Cytometry™ (IMC™) platform enables highly multiplexed spatial proteomics, allowing researchers to visualize and quantify protein expression at subcellular resolution. Ensuring reproducibility across instruments and time is critical for generating reliable biological insights. This technical note examines the Hyperion™ XTi Imaging System's reproducibility by evaluating instrument sensitivity over time and assessing biological data consistency across multiple instruments.

In this technical note, we outline:

- Instrument sensitivity consistency: performance stability over a 60-plus-day period
- Spatial proteomic reproducibility: biological and technical consistency within and across instruments

Instrument sensitivity consistency

To assess the stability of instrument sensitivity over time, a uniform standard slide was measured over 60-plus days while the instrument was continuously acquiring data. Instrument performance remained highly consistent, with a coefficient of variation (CV) of 4.5% over the test period (Figure 1).

The observed trendline slope of 0.15% indicates minimal drift in sensitivity. Routine maintenance and reference energy calibration, as outlined in the user guide, effectively correct this minor drift, ensuring stable performance.

Long-term stability sensitivity of the Hyperion XTi Imaging System

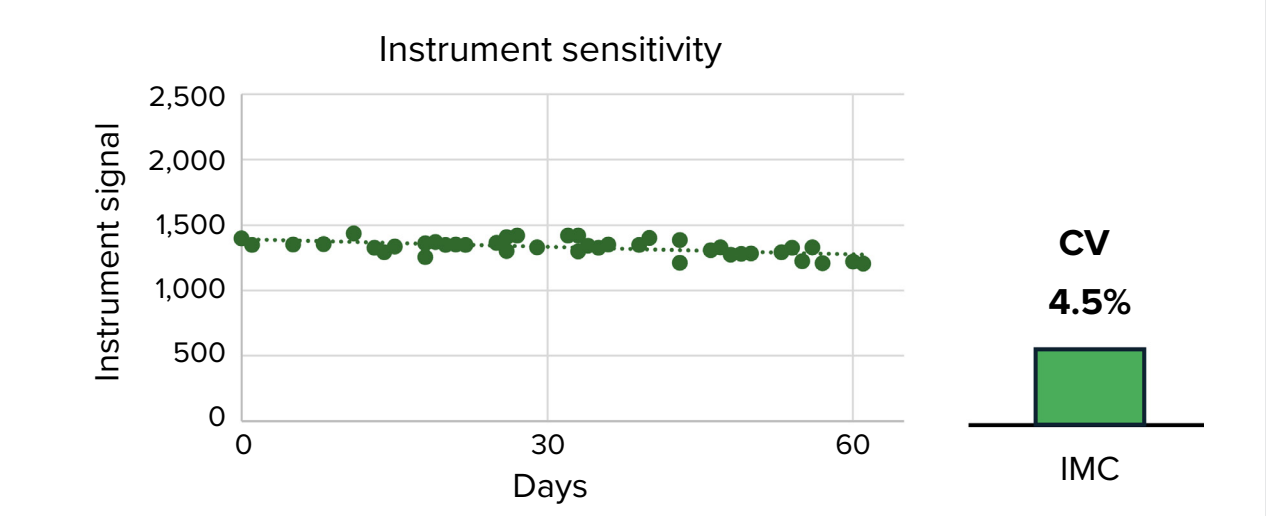


Figure 1. Instrument sensitivity measured on a uniform standard slide over 60-plus days, demonstrating high stability with a CV of 4.5% and trendline slope of 0.15%. Routine maintenance and calibration mitigate minor variations, ensuring reproducible instrument performance.

Imaging Mass Cytometry data reproducibility: Biological and technical consistency of the Hyperion XTi Imaging System

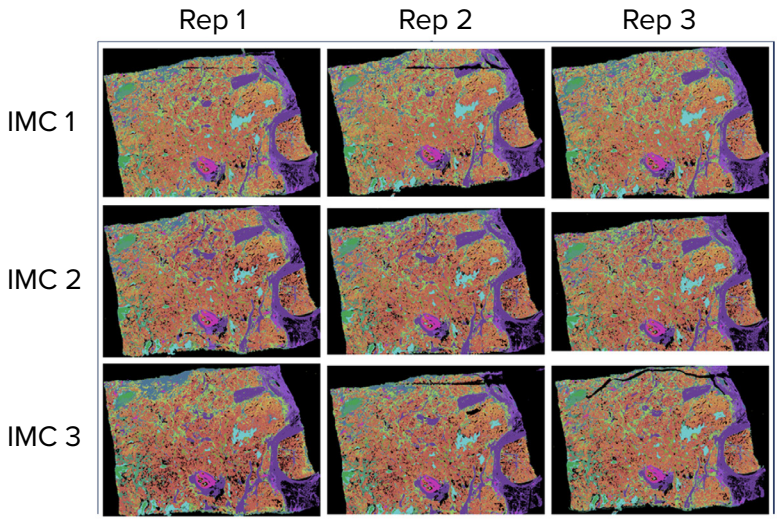
To evaluate biological reproducibility, serial tissue sections of colon adenocarcinoma were imaged on three different Hyperion XTi Imaging Systems, with three sections per instrument. Pixel-based phenotyping was performed, and phenotype clusters were mapped back onto the images to visualize consistency across sections and instruments (Figure 2).

31-marker spatial panel

β-catenin	CD45	HLA-DR
CD3	CD45RA	Ki-67
CD4	CD45RO	PD-L1
CD8	CD57	Pan-cytokeratin
CD11b	CD68	T-bet
CD16	CD163	Vimentin
CD20	Collagen 1	αSMA
CD31	E-cadherin	pERK1-2
CD34	FoxP3	pS6
CD44	Granzyme B	p-tyrosine

Panel contains a variety of commonly used markers to enable visualization of all known cell types in the sample.

IMC biological consistency across instruments



Phenotype clusters (representative colors)

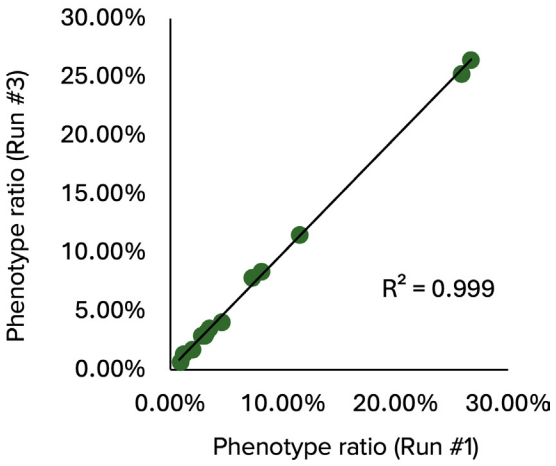
Cytotoxic leukocyte	ECM	ECM (low expression)	Myeloid	Myeloid/ macrophage	Necrotic core
pERK + necrotic core	Replicating tumor	TLS germinal center	Tumor	Tumor/immune	Vascular

Figure 2. Phenotype cluster overlay from multiple instruments, showing consistent pixel-based phenotyping across serial tissue sections (Pearson correlation coefficients of phenotype frequencies shown on following page).

Pearson correlation coefficients were calculated for phenotype frequencies between replicate acquisitions within the same instrument (intra-instrument reproducibility) and between different instruments (inter-instrument reproducibility). Results showed exceptionally high correlation, confirming the system's reproducibility (Figure 3).

IMC technical reproducibility across instruments and runs

a) Intra-instrument reproducibility



b) Intra-instrument correlation (n=36)

	Median	Mean
	0.988	0.988

c) Inter-instrument correlation (n=3)

	Median	Mean
IMC 1	0.988	0.988
IMC 2	0.999	0.999
IMC 3	0.984	0.988

Figure 3. High correlation values confirm excellent reproducibility. a) Representative intra-instrument reproducibility scatterplot, one of 36 total runs shown above. b) The median and mean Pearson correlation coefficients of phenotype frequencies within the same instrument runs (n=36) and c) between replicates (n=3) acquired on multiple instruments (n=3)

Conclusion

The Hyperion XTi Imaging System demonstrates exceptional reproducibility both in terms of instrument sensitivity over time and biological consistency across instruments. With a **4.5% CV** over 60-plus days and inter-instrument correlation values nearing **0.999**, researchers can confidently generate reproducible, high-quality Imaging Mass Cytometry data for robust spatial proteomic insights.

Learn more at **standardbio.com**

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CORPORATE HEADQUARTERS
2 Tower Place, Suite 2000
South San Francisco, CA 94080 USA
Toll-free: 866 359 4354 in the US and Canada
Fax: 650 871 7152
standardbio.com

SALES
North America | +1 650 266 6170 | info-us@standardbio.com
Europe/Middle East/Africa/Russia | +33 1 60 92 42 40 | info-europe@standardbio.com
Latin America | +1 650 266 6170 | info-latinamerica@standardbio.com
Japan | +81 3 3662 2150 | info-japan@standardbio.com
China (excluding Hong Kong/Macau) | +86 21 3255 8368 | info-china@standardbio.com
All other Asia-Pacific countries/India/Australia | +1 650 266 6170 | info-asia@standardbio.com

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