

## Enabling Intelligent Data Capture for Intact Mass Analysis of Monoclonal Antibodies and Subunits using a Vion IMS QTof HRMS

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This is an Application Brief and does not contain a detailed Experimental section.

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### Abstract

Here we demonstrate the impact of enabling Intelligent Data Capture on data quality and file size for intact mAb and intact subunit analysis.

### Benefits

Real-time Intelligent Data Capture for reduced file size while maintaining data quality for intact protein analysis.

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### Introduction

High Resolution Mass Spectrometry (HRMS) analysis of intact proteins provides a rich dataset from which both quantitative and qualitative information can be derived. At the same time, managing these large datasets can be

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a challenge. The Vion IMS-QToF Mass Spectrometer has an option in data acquisition called “Intelligent Data Capture” (IDC). IDC employs adaptive background subtraction and data sweeping during data acquisition to remove background and noise peaks, resulting in improved signal to noise and reduced file sizes. A detailed description of IDC is explained in white paper “Intelligent Data Capture: Real-Time Noise Reduction for HRMS”.<sup>1</sup> In this application brief, the benefits of IDC for intact mass analysis of mAbs and subunits are presented.

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## Results and Discussion

### Part I. Trastuzumab Intact Protein Analysis

Trastuzumab (100 µg/mL) in ammonium acetate/BSA solution was prepared. Data was acquired by ESI-TOF-MS (+ve full scan mode) with or without IDC enabled. IDC intensity threshold (counts) of 5, 10, and 20 were evaluated. LC-MS conditions used for the acquisition were previously reported.<sup>2</sup> The observed and deconvoluted spectrum are shown in Figure 1. Results show that the protein charge state distribution, glycosylation profile, and deconvoluted spectra are essentially the same when comparing data acquired with and without IDC enabled. Acquiring with the default IDC counts of 5, there is a significant drop in Tof Valley peak from 15–20% to ~5%, resulting in near baseline separation of the glycoforms. Glycoform percent distribution and data file size reduction corresponding to each of the IDC counts are summarized in Table 1. Increasing the IDC counts from the default 5 to 20, does not significantly change the calculated glycoform distribution, nor the Tof valley profiles indicating the default counts is optimal. Similarly, comparing file sizes across IDC modes, a file size reduction of 39% was realized using the default IDC counts of 5, increasing the IDC counts to 10, and 20, reduced the file size further to 45% and 59% respectively. Overall, significant file size reduction was realized using IDC-enabled data acquisition with improved Tof valley profile and minimal effect on glycoform distributions.

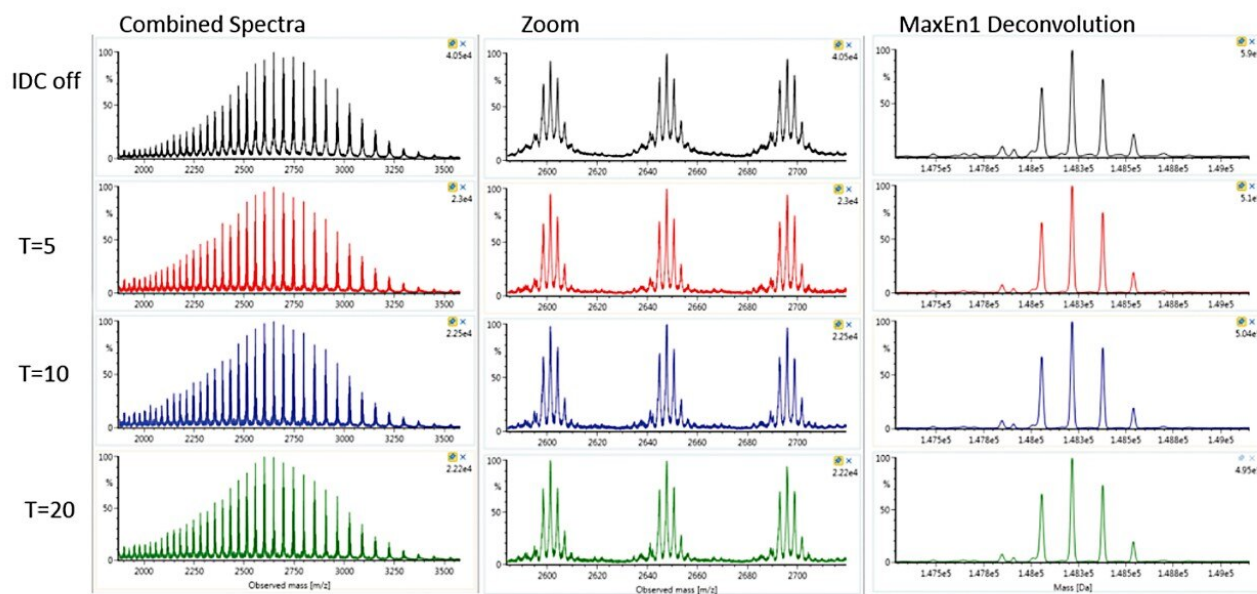


Figure 1. Combined MS spectra, zoom, and corresponding MaxEnt1 deconvoluted masses of trastuzumab using different IDC counts.

	Glycoform distribution				File size
mass	148056	148217	148379	148541	%reduction
no IDC	28.3%	35.4%	26.6%	9.7%	100%
IDC = 5	28.9%	37.6%	25.9%	7.6%	-39%
IDC = 10	27.1%	38.8%	26.8%	7.4%	-44%
IDC = 20	26.8%	38.8%	26.8%	7.6%	-59%

Table 1. Summary of glycoform distribution and file size reduction using different IDC counts.

## Part II. NIST mAb Intact Subunit Analysis

Waters NIST mAb Subunit Standard (p/n: 186008927) ~100 µg/mL in H<sub>2</sub>O was prepared. Data was acquired using ESI-TOF-MS (+ve full scan mode) with and without IDC enabled. IDC counts of 5, 10, and 20 were evaluated. LC-MS experimental conditions are described in a separate application note.<sup>3</sup> The observed and

deconvoluted mass spectra are shown in Figures 2–4 for each of the subunit scFc, LC and Fd of the reduced mAb. Results showed that Data Integrity was maintained with IDC enabled and across different IDC counts. There is significant file size reduction, at the default IDC counts of 5, the file size was reduced by 45%. Increasing the IDC counts to 10 and 20, reduced the file size further by 66 and 80% respectively.

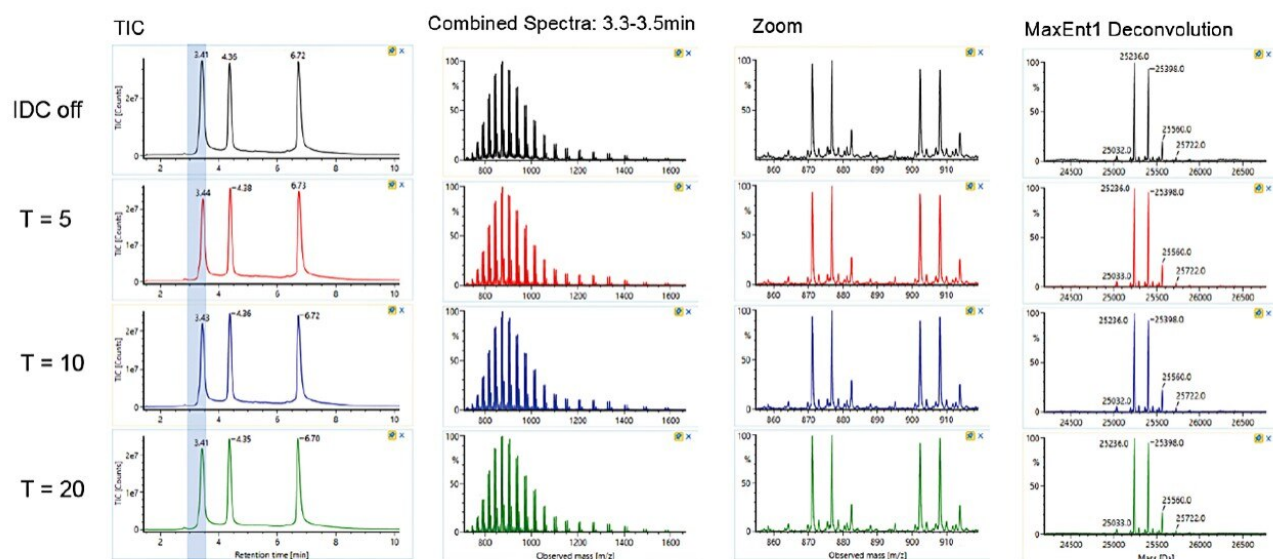


Figure 2. Combined MS spectra, zoom of  $m/z$  855–915, and corresponding MaxEnt1 deconvoluted masses for the mAb scFc.

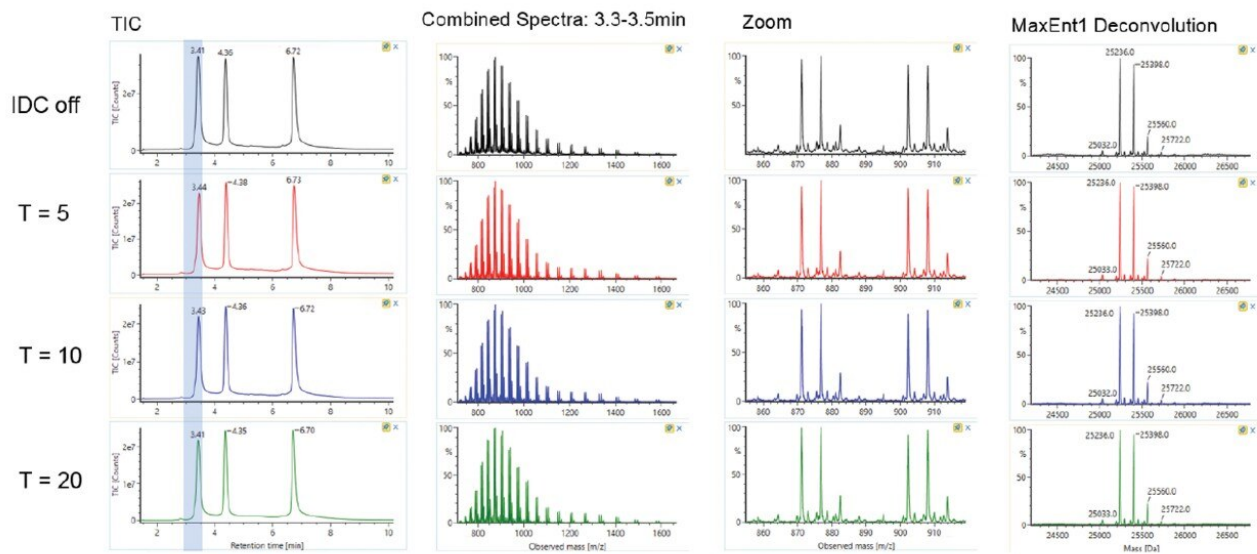


Figure 3. Combined MS spectra, zoom of  $m/z$  935–1020, and corresponding MaxEnt1 deconvoluted masses for the mAb LC.

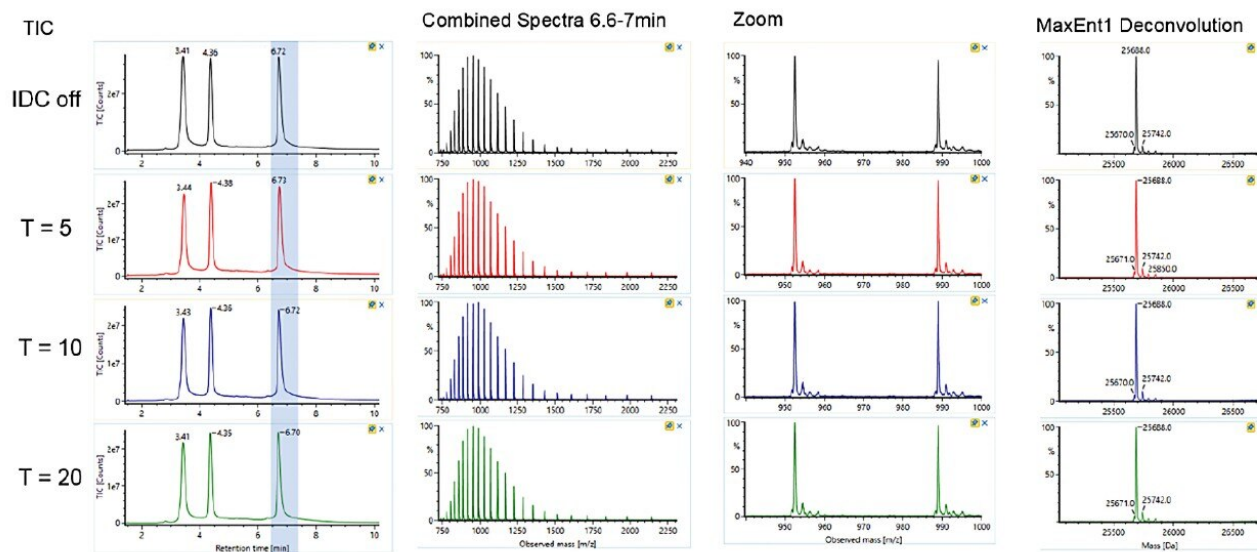


Figure 4. Combined MS spectra, zoom of  $m/z$  940–1000, and corresponding MaxEnt1 deconvoluted masses for the mAb Fd.

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## Conclusion

Enabling IDC for data acquisition is described for intact mass analysis of trastuzumab and NIST mAb subunits. Results show significant file size reductions of between 40 and 80% while maintaining mass spectral quality. The default IDC counts of 5 is optimal for routine acquisitions. The implementation of IDC during acquisition is flexible, employing either a default counts or user entered through promotable parameters. Overall, this implementation and resulting reduced file size will help to improve overall processing efficiencies of general LC-MS analysis.

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## References

1. Intelligent Data Capture: Real-Time Noise Reduction for High Resolution Mass Spectrometry, Waters white paper, 720006567EN.
2. High-Performance Vion IMS QTof for Monoclonal Antibody Intact and Subunit Mass Analysis, Waters application note, 720005906EN.
3. High Sensitivity Intact Monoclonal Antibody (mAb) HRMS Quantification, Waters application note, 720006222EN.

## Appendix. Enabling IDC in Vion data acquisition

### 1. Enabling IDC using the default counts

The IDC option can be found in Analysis Method on the page "Instruments\_Instrument\_Method Configuration\_Vion IMS QTof\_Experiment". An example of the page is shown in Figure A. Clicking the check box will toggle it on and off. The default IDC counts is 5. When a different IDC counts is desired, promotable parameters can be used and is described in the next section. The IDC is available for all analysis types and all acquisition modes.

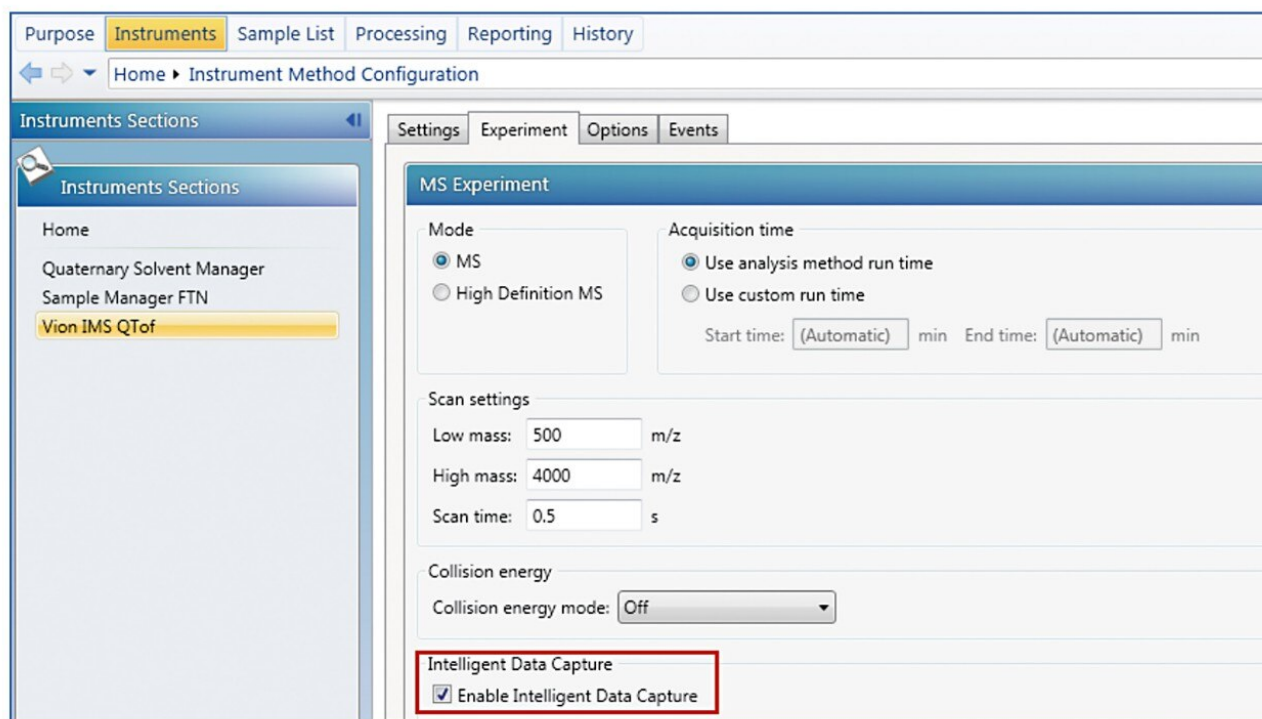


Figure A. Analysis Method page showing IDC option.

## 2. Enabling IDC as promotable parameters

When desired, IDC can be turned on or off and the counts varied from injection to injection. To enable this function, go to Analysis Method\_Sample List\_Sample List Setup, clicking on "Add/Remove Column" will display the dialog box shown in Figure B. Choose "Intelligent Data Capture" and "Intelligent Data Capture Intensity Threshold (counts)" will add these two columns to the list. IDC selection and IDC counts entered on these columns will override default counts in Figure A.



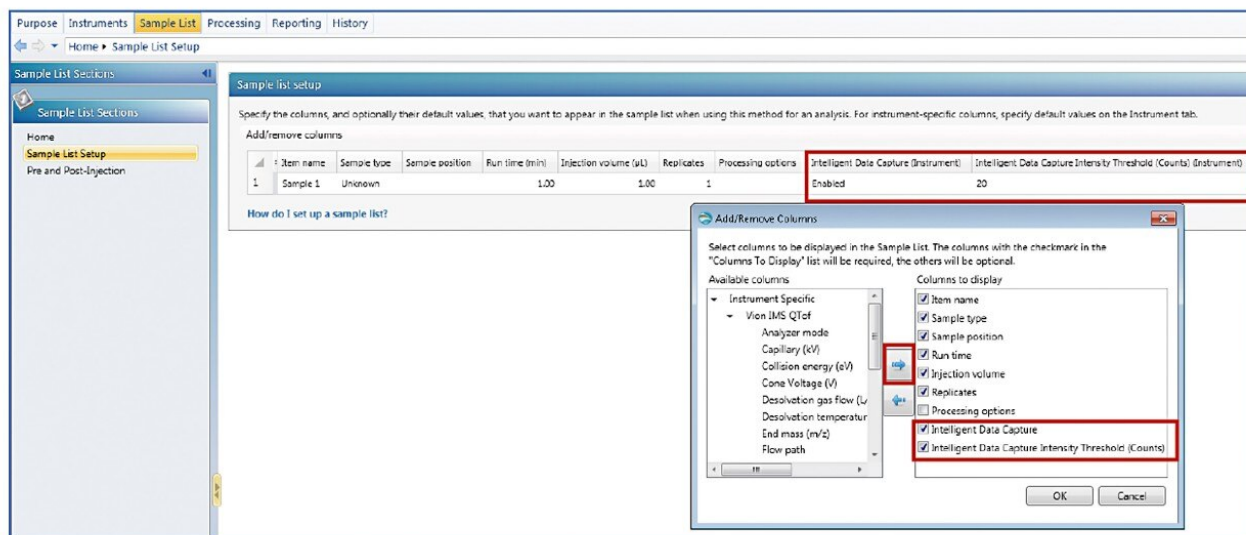


Figure B. Adding IDC as promotable parameters in analysis method page.

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