Optimization of the Astral detection system: Enabling high linear dynamic range and single ion sensitivity for deep proteome coverage

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Abstract

Purpose: Optimization of high-dynamic range detector to achieve deep proteome coverage with the Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer.

Methods: A novel method based on detuning the Astral asymmetric ion mirror system [3] is used, whereby single ion distributions are created to measure the single ion detector response and determine the number of charges per peak.

Results: Astral detection system is calibrated and optimized to achieve optimum analytical performance.

Introduction

As single-cell proteomics and other MS domains move toward studies using lower sample loads and large cohorts, researchers increasingly require instrumentation with higher acquisition speed, dynamic range, and sensitivity. The HRAM Astral™ analyzer (Figure 1) [1, 2] was designed specifically with these competing demands in mind. Optimization of the ion detection system becomes therefore key to provide sensitivity without compromising dynamic range.

The detection system is comprised of a post-acceleration electrode stack, El-Mul's MTOF ion detector (Figure 2a and 2b), a preamplifier and an analog-to-digital converter (ADC). The electrode stack accelerates ions with an attractive potential of several kilovolts on the conversion dynode of the detector to increase the number of released secondary electrons and therefore the detection efficiency. The secondary electrons are accelerated on to the surface of a scintillator resulting in the release of photons which are further guided on a photomultiplier tube with a gain of ca. 105. The signal from the last dynode of the photomultiplier-tube is fed into the dual-channel preamplifier with a gain ratio of 1:10 to maximize the dynamic range. Both output signals are fed to a commercial 14-bit ADC for further processing.

Figure 1 shows a schematic drawing of the Astral analyzer and the primary ion trajectory through the analyzer. Trapped ions are extracted into the Astral analyzer and travel along the drift direction back-and-forth between the two ion mirrors until they reach inflection point and return backwards. At the end of their return path, they traverse the post accelerator and arrive at the detector (Figure 2a). Figure 2b shows a detailed view on the components of the ion-to-photon detector. The detector exhibits a time response of ca. 1.6 ns to allow for high resolution measurements.
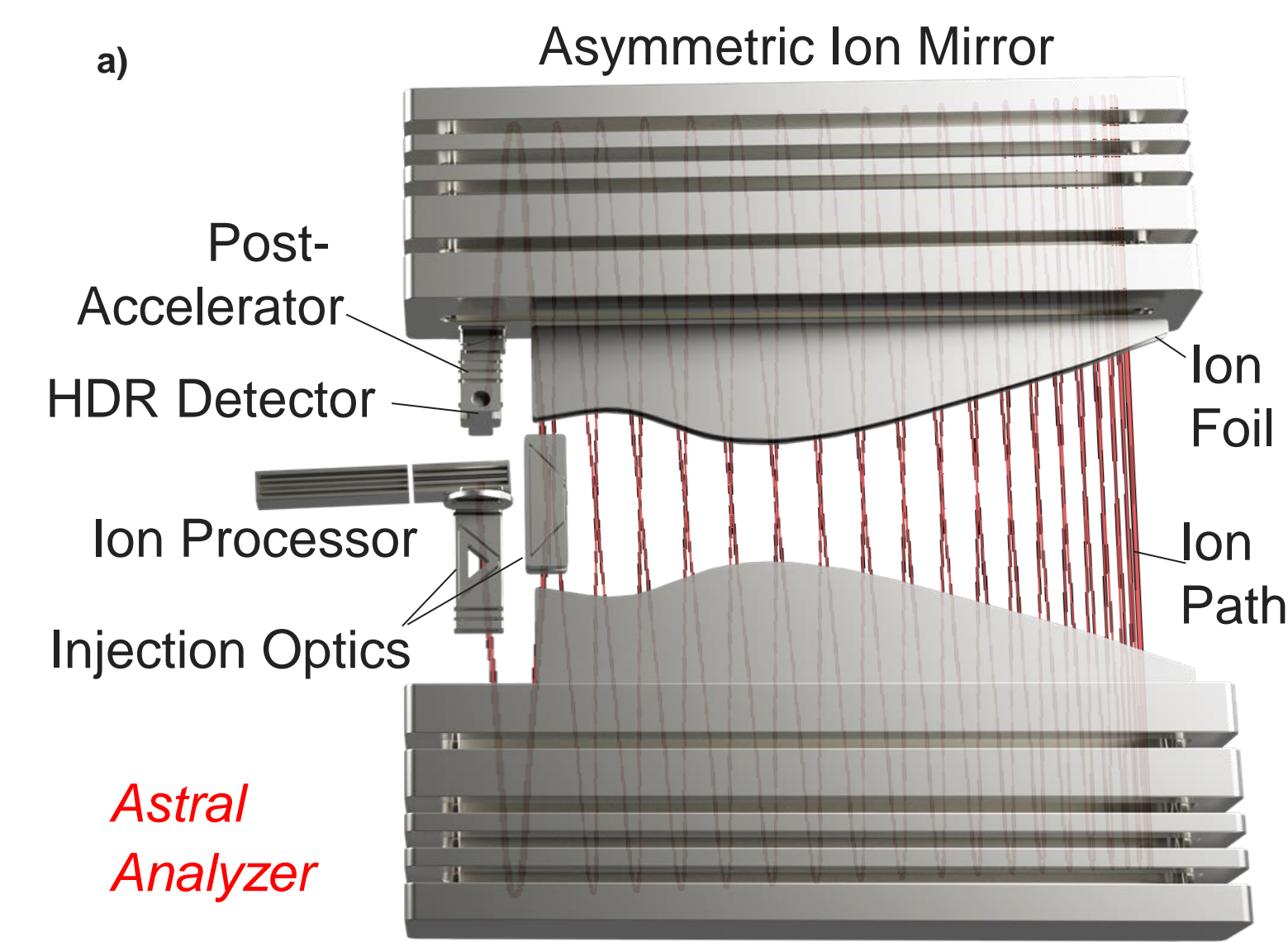

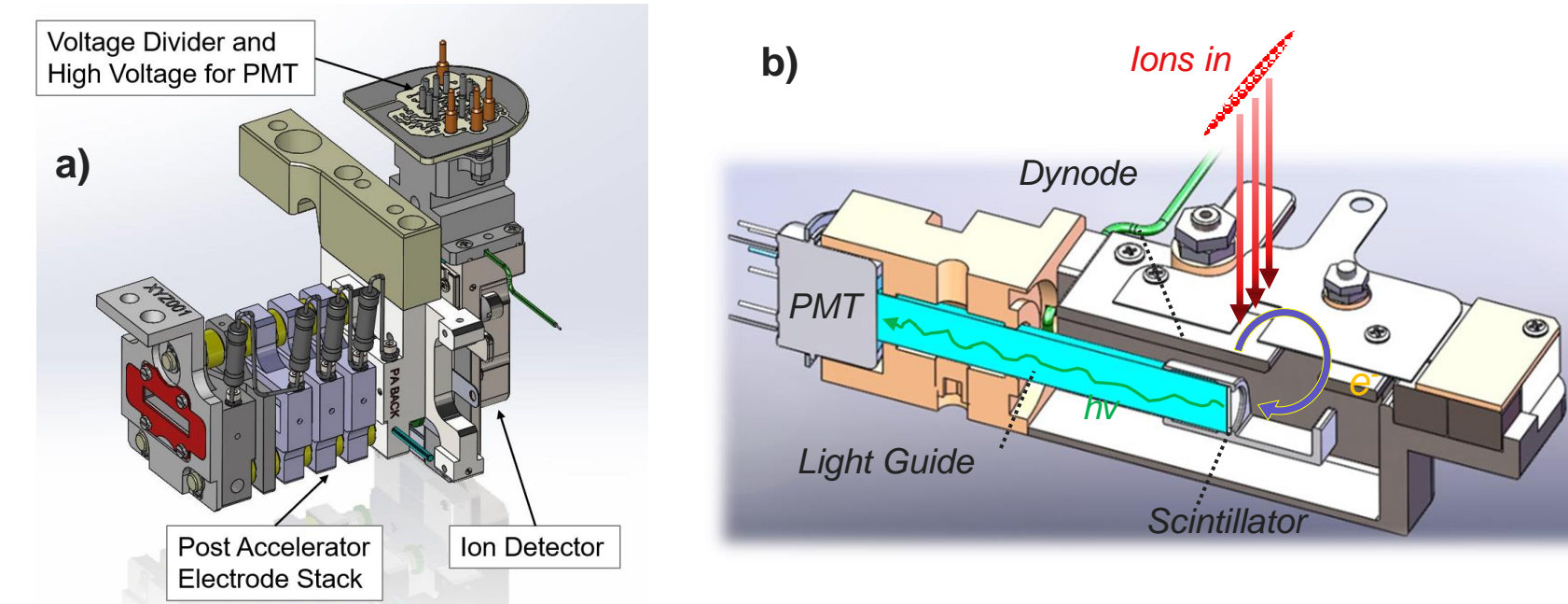Figure 1. Schematic drawing of the Astral analyzer and the primary ion trajectory.

Experimental

The detection system of the Astral mass analyzer is calibrated using a novel method based on detuning the ion mirror system [3] which defocuses the ion package to create single ion distributions of electrospray-ionized ions of m/z 525 from Thermo Scientific™ Pierce™ Flexmix™ calibration solution (Figure 3). As a result, the so-called single ion area is determined as function of the detector voltage to measure the number of charges in a peak. The detector voltage is optimized to allow for efficient single ion detection in combination with high linear dynamic range. Both parameters also depend on the ADC detection threshold, which is calibrated in a separate step based on the electronic noise band.

The following criteria are optimized in the process:
- Very low probability for detecting electronic noise peaks
- High detection probability for single ions
- High dynamic range of at least 1:1000
- Very similar instrument-to-instrument performance

The effect of the single ion detection probability on the analytical performance on the Orbitrap Astral MS was further tested in LC-MS runs with HeLa and different sample loads.

Results

Detector Calibration

Figure 3 shows the single ion distribution of m/z 525 when defocusing the ion mirror system of the Astral analyzer [3]. As the voltage of the most-repulsive electrode is reduced, ions travel a longer path through the analyzer and thus arrive later at the detector. Therefore, the defocused single ions of m/z 525 appear at a different mass-to-charge ratio when using the standard mass calibration. The mean area of the single ion peaks is defined as the single ion area. This parameter is measured as function of the detector voltage (Figure 4). The voltage is adjusted to reach a mean single ion area of 15 mV*ns which corresponds to peak amplitudes of around 10 mV.
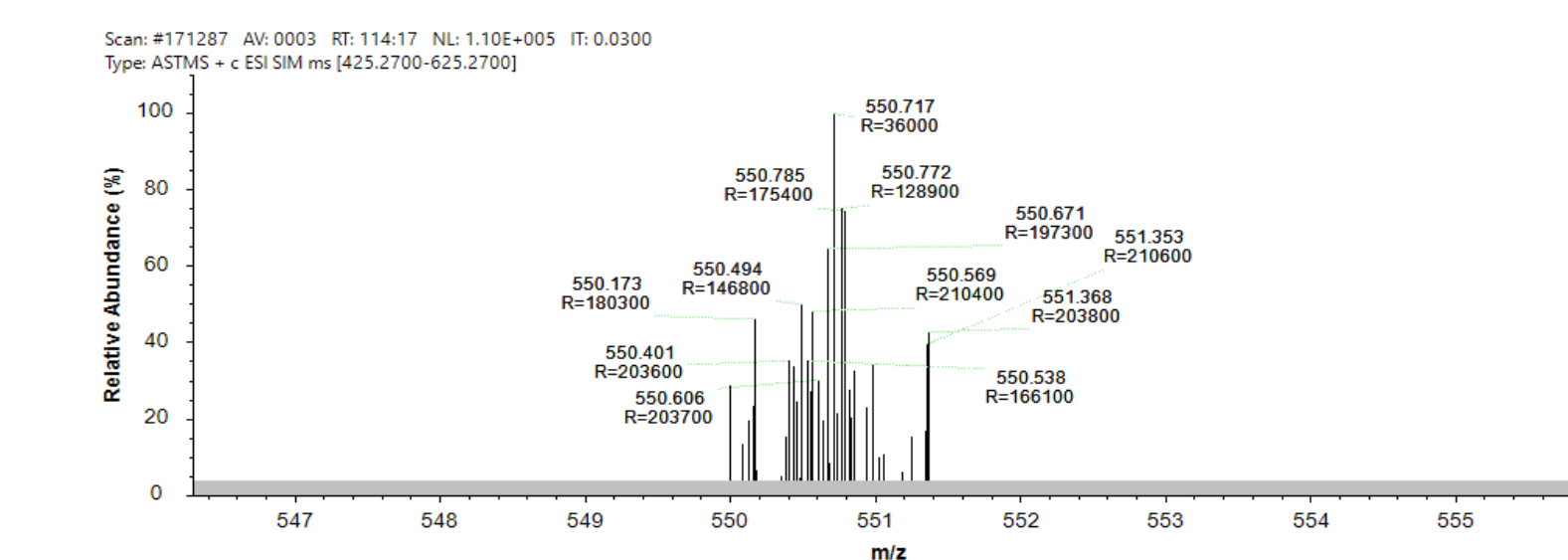

Figure 3. Top: Defocused single ion distribution of m/z 525. Due to the defocusing, the flight path through the Astral analyzer increases, shifting the measured arrival time.
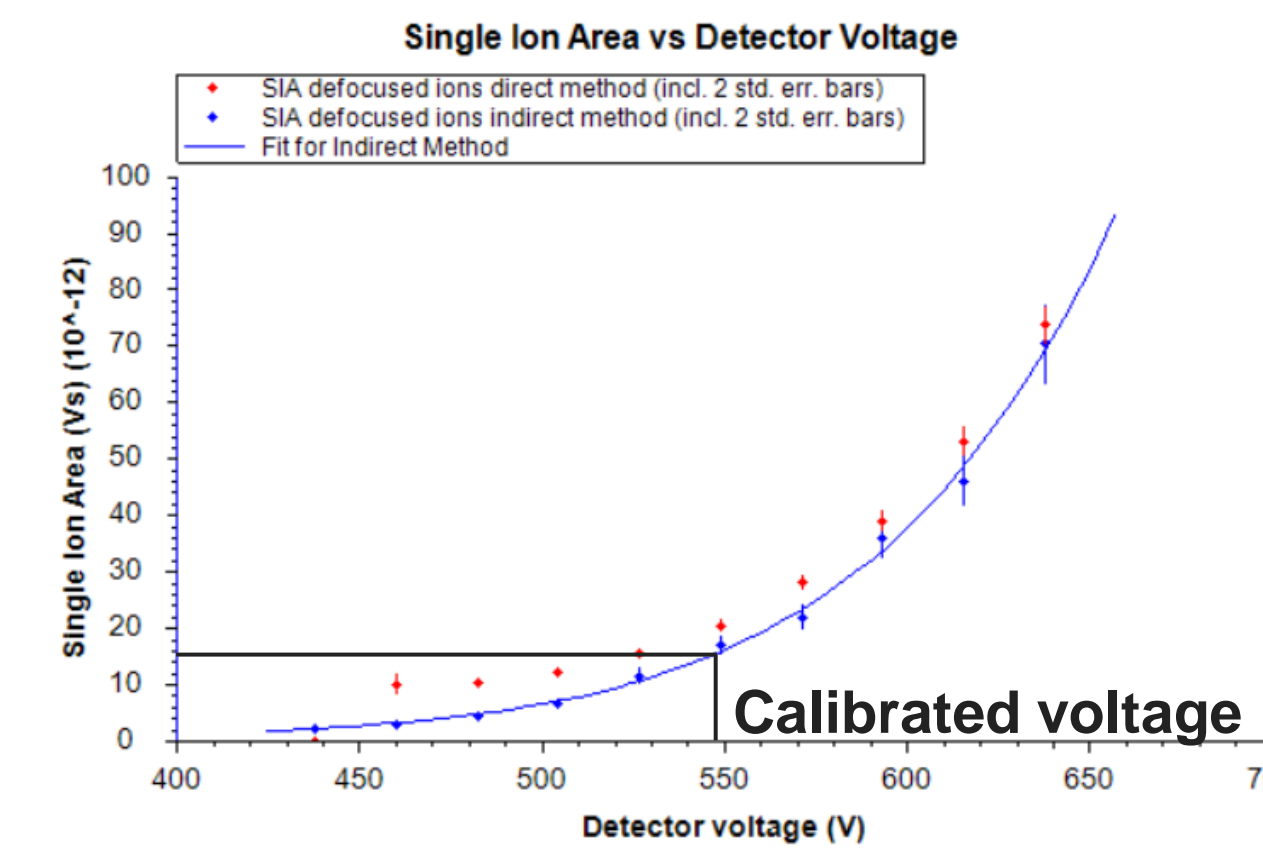
Figure 2. a) 3D-model of the post acceleration electrode stack and the HDR detector of the Astral analyzer. b) Detailed drawing of the HDR detector.

Figure 4. Median single ion area as function of the detector voltage. The voltage is adjusted to reach a single ion area of 15 mV*ns.

Detection Threshold

Figure 5 shows the ADC calibration. To reduce electronic noise in Astral spectra, the peak detection threshold of the ADC is set well above the noise band with typical values of 0.63 mV for the noise band (1σ) and around 3.8 mV for the threshold (6σ). As one mass spectrum corresponds to around 4 million ADC sampling points, the 6σ setting corresponds to less than one noise peak detected by the ADC per 100 mass spectra. With additional data processing, the number of electronic noise peaks is further reduced.
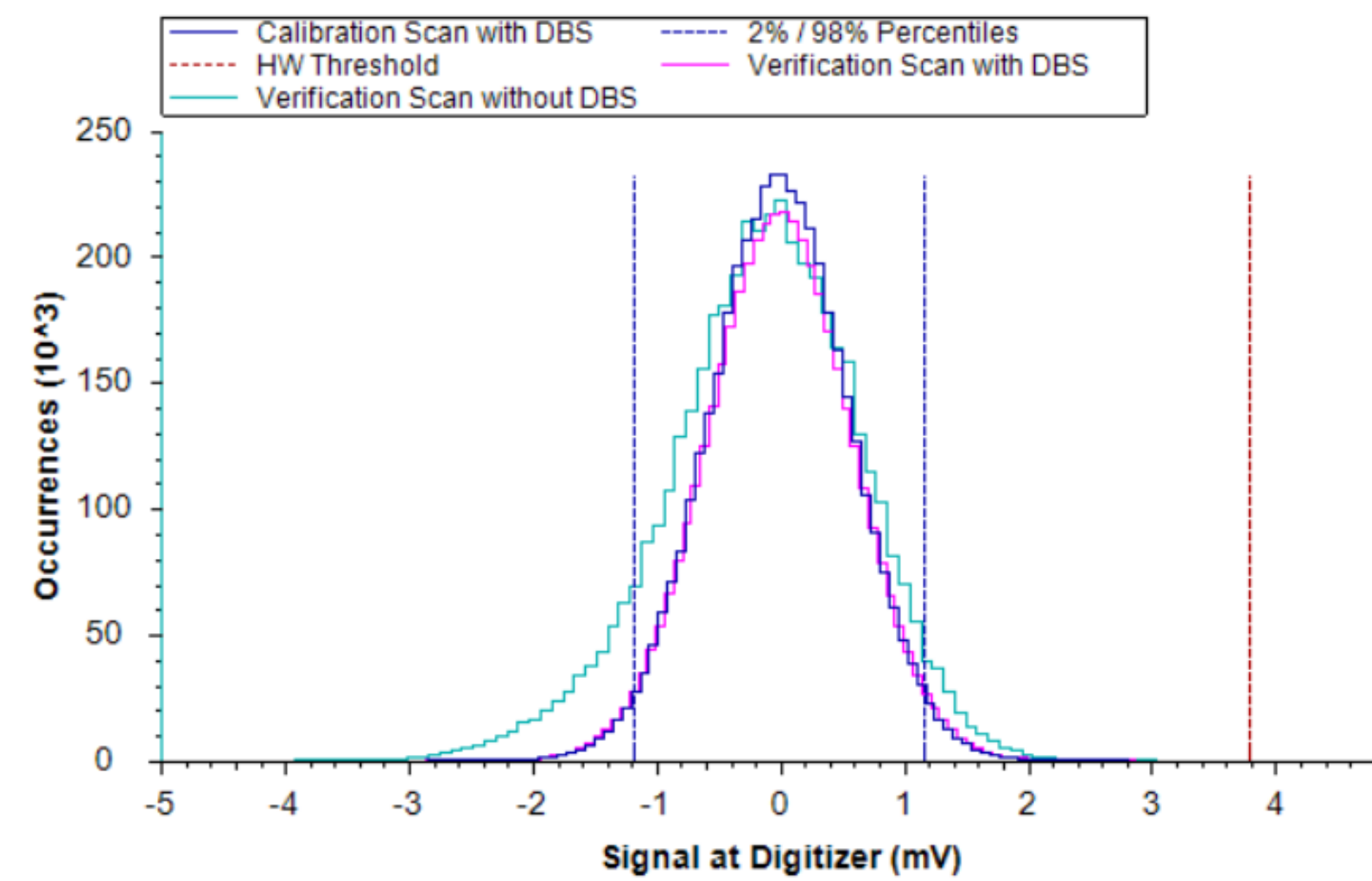

Figure 5. Calibration of the Astral ADC showing the electronic noise distribution and the applied detection threshold to reduce the number of detected noise peaks.

Signal to Noise Ratio

The SNR can be determined using the combined data of the measured noise distribution and the average amplitude of the single ions. Assuming white noise of 1σ = 0.63 mV and a single ion peak width (FWHM) of 2 ns, the area of the noise distribution for a single ion is around 5.8 mVns. As the single ion area is calibrated to 15 mVns, we get single ion SNR well above 2:

$$\text{Single Ion SNR} = 15 \text{ mVns} / 5.8 \text{ mVns} = 2.6$$

The single ion SNR value can be further used to calculate the number of charges in a peak.

$$\text{Number of Charges in Peak} = \text{SNR of Peak} / \text{Single ion SNR}$$

Single Ion Detection Probability

The single ion detection probability is a function of the applied threshold (Figure 5) and the intensity of the single ion peaks which is itself a function of the PMT voltage (Figure 6). For the detector, a voltage is set that typically corresponds to about 85% single ion detection probability as this allows for very efficient single ion detection while guaranteeing the linear dynamic range of at least 1:1000. Increasing the detector voltage further would boost the detection efficiency but at the cost of reduced linear dynamic range of the system.
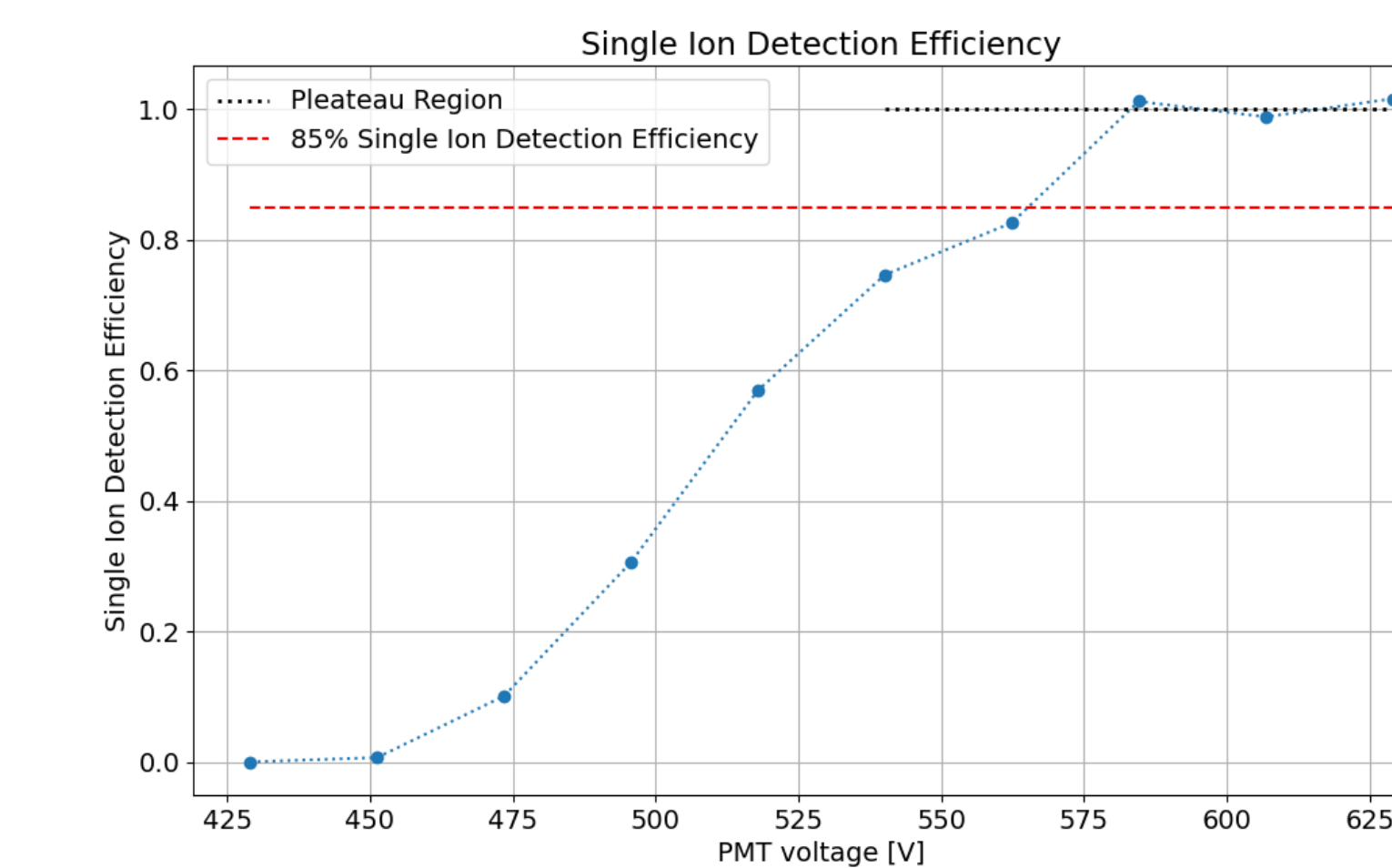

Figure 6. Single ion detection probability as function of the PMT voltage. The Detection probability reaches a plateau at which all single ions get detected.

Effect on Analytical Performance

The effect of the single ion detection probability has been tested in LC-MS runs using HeLa with different sample loads (Figure 7). The detection probability is varied from 0.7 to 0.98 by adjusting the detector voltage. For higher sample load, the number of identified peptides stays rather constant over the detector voltage. On the other hand, for lower loads we see a drop in identifications for a detection probability of 0.7 compared to higher values. A detection probability of 85% seems still to be a good trade-off between single ion detection efficiency and dynamic range.
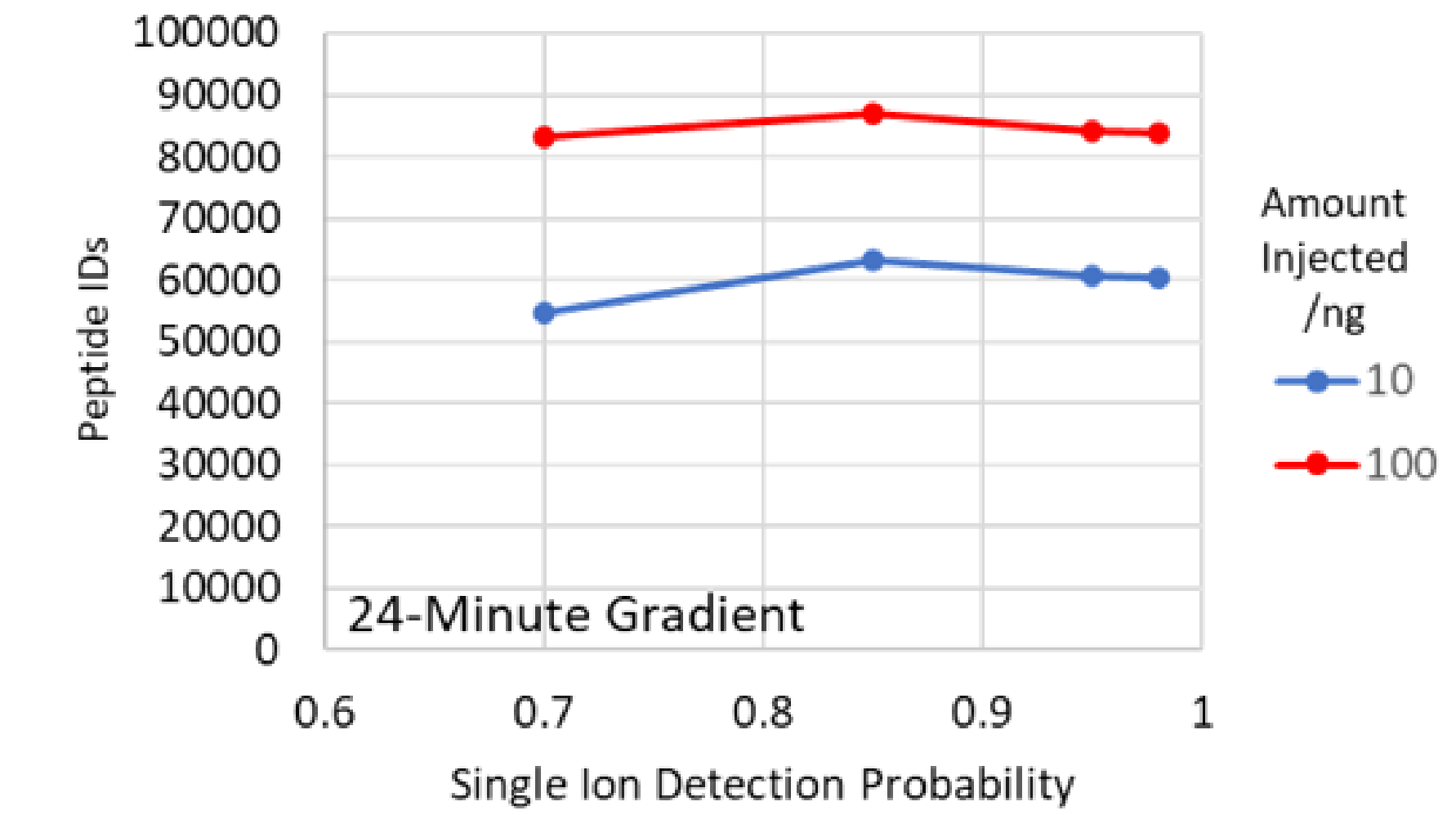

Figure 7. Peptide IDs as function of the single ion detection probability for different HeLa sample loads. At 100ng sample load, the identified peptides do not depend on the single ion detection probability, while we can see a drop in IDs for the 10ng sample at the lowest detection probability of 0.7.

Conclusions

The proper calibration and optimization of the Astral detection system is key in achieving high dynamic range spectra while maintaining efficient single ion detection. With a single ion signal-to-noise ratio of 2.6, we could clearly show that measured peaks are well separated from electronic noise. The detector is further optimized to maximize analytical performance.

References

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3. Stewart H., Petzoldt J., Hagedorn B., Grinfeld D., (2022). Method of gain calibration, United States Patent US20220367165A1, Patent Pending.

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