

## Estimating Detector Gain:

A simple experiment can be performed to characterize any ion counting mass spectrometer in terms of signal-to-noise, where noise is defined as the precision obtained over many measurements of a nominally constant signal. This experiment involves infusing a peptide standard, collecting many MS/MS spectra, and performing an analysis similar to that which the instrument vendor uses to determine the number of ions in the ion trap. This analysis parallels previous work reported on gas chromatography-mass spectrometry instruments using selected ion monitoring.

The analysis proceeds as follows. We assume that instrument noise sources can be divided into two groups: (1) noise dependent solely on the statistics of the arrival of ions at the detector, i.e., shot noise, described formally by Poisson statistics; and (2) all other noise sources, including noise due to the electron multiplier itself, pulse counting electronics and associated circuitry, and digitization errors. The signal variance due to these two groups can be written as

$$\sigma_T^2 = \sigma_p^2 + \sigma_c^2 \quad (1)$$

where  $\sigma_T^2$  is the total variance in a repeated measurement,  $\sigma_p^2$  is the variance due to Poisson (shot) noise, and  $\sigma_c^2$  contains the variance due to all other noise sources.

We acquire many nominally identical spectra (1000 in this case), and compute intensity ratios for several of the most prominent ions relative to a single particular ion. Assuming the mass analyzer and attendant ion optics and detection systems perform adequately, by far the greatest source of instability in electrospray mass spectrometry is that of the ESI source itself.

Measuring the ratio of the intensities of two different ions in the same spectrum ensures that the multiplicative contribution of noises sources which affect all peaks in a spectrum – the common mode noise, which includes electrospray instability – will cancel.

In ion trap mass spectrometers, automatic gain control (AGC) is used to keep the number of ions (charges) in the trap approximately constant by varying the time during which ions are allowed to accumulate in the trap. This time is called the ion injection time, as discussed in the Introduction. The measured peak area is therefore  $A = \alpha(N/t)$ , where  $N$  is the number of ions in the peak, and  $t$  is the ion injection time, which is the same for all ions in a given spectrum. For other instrument platforms this could be the dwell or scan time. The factor  $\alpha$  is included to account for inaccuracies in the instrument calibration. Because  $\alpha$  and  $t$  are the same for all ions in a spectrum, the ratio of peak areas for any two ions  $a$  and  $b$  in a spectrum is  $R = Na/Nb$ .

The variance  $\sigma_R^2$  in repeated measurements of  $R$  can be computed theoretically (for small variations in  $Na$  and  $Nb$ ) by using the propagation of errors:

$$\sigma_R^2 = \sigma_{Na}^2 \left( \frac{\partial R}{\partial Na} \right)^2 + \sigma_{Nb}^2 \left( \frac{\partial R}{\partial Nb} \right)^2 \quad (2)$$

By reducing the partial derivatives in Eq (2) we get:

$$\sigma_R^2 = R^2 \left( \frac{\sigma_{Na}^2}{Na^2} + \frac{\sigma_{Nb}^2}{Nb^2} \right) \quad (3)$$

We can now simplify this equation by assuming that the variance in the intensity measurements is dominated by Poisson (shot) noise. The variance of any set of Poisson-distributed events is equal to the number of events,  $\sigma^2 = N$ . Substituting this relation for the variances on the right-hand-side of Eq (3) results in the Poisson-limited noise:

$$\sigma_p^2 = R^2 \left( \frac{1}{Na} + \frac{1}{Nb} \right) \quad (4)$$

Since the number of ions in the peak is  $N = At/\alpha$  (as defined above), Eq (4) can be rewritten as

$$\sigma_p^2 = \bar{R}^2 \left( \frac{1}{A_a t} + \frac{1}{A_b t} \right) \alpha \quad (5)$$

where the bars indicate averages over repeated measurements. Inserting Eq (5) into Eq (1), we have

$$\sigma_T^2 = \bar{R}^2 \left( \frac{1}{A_a t} + \frac{1}{A_b t} \right) \alpha + \sigma_c^2 \quad (6)$$

In this equation, the measured quantities are  $R$  (ratio of peak areas),  $\sigma_T^2$  (variance in the ratio of peak areas),  $A_a$  (average peak area of ion  $a$  over many measurements),  $A_b$  (average peak area of ion  $b$  over many measurements), and  $t$  (ion injection time). The unknown quantities are  $\alpha$  and  $\sigma_c^2$ . Thus this equation is of the form  $y = mx + b$ , with  $m = \alpha$  and  $b = \sigma_c^2$ . Taking measurements of many different ratios over many mass spectra allows us to perform a linear regression to obtain  $\alpha$  and  $\sigma_c^2$ . Because the instruments under consideration here use automatic gain control, we anticipate that  $\alpha$  will be near 1, indicating good instrument calibration. We further anticipate that  $\sigma_c^2$  will be small, indicating that instrument noise is limited by Poisson (shot) noise.

To carry out the analysis described above, we have infused a solution of Glu[1]-fibrinopeptide-B into the mass spectrometer and continuously acquired 1000 fragmentation spectra of the 2+ charge state. We extracted individual signals for 10 different y-ions, y2 through y11, and then computed the peak areas, ratios of peak areas (relative to y11, the base peak in the spectrum),

and the variance in the ratio measurements for each of the 9 measured ratios. We then performed a linear regression on the resulting data. This linear regression often results in a  $R^2$  value in excess of 0.99. On ThermoFisher instruments that use ions per second as their measure of intensity we expect to obtain a value for  $\alpha$  near 1. We speculate that there may be some systematic error in calibration leading to a value of  $\alpha$  different from 1. In all cases, however, the magnitude of  $\sigma_c^2$  is small compared to other terms in Eq (6). This result indicates that non-Poisson noise sources are small in magnitude in each mass spectrometer and strongly suggests that noise in each instrument is dominated by Poisson-limited noise. This procedure and analysis could be used on a routine basis to check the quality and consistency of the calibration routine, as well as to check for the presence of any non-Poisson-limited noise which could indicate problems with the instrument hardware or detection electronics.