Appendix S1: Error propagation equations

1. Ratio calculations from ion counts

The ion counts (N) that were measured in this study for calculating isotope ratios include $^{12}C^-$, $^{13}C^-$, $^{14}N^{12}C^-$, $^{15}N^{12}C^-$, $^{12}C_2^-$ and $^{13}C^{12}C^-$. These result in the following measured isotope ratio (R_m) calculations assuming isobaric interferences (e.g. $^{12}C^1H^-$ for $^{13}C^-$; $^{14}N^{13}C^-$ for $^{15}N^{12}C^-$; $^{13}C_2{}^-$, $^{12}C^{13}C^1H^-$ and $^{12}C_2{}^1H_2^-$ for $^{14}N^{12}C^-$,) are fully resolved.

$$^{\frac{13}{12}}R = \frac{N(^{13}\mathrm{C})}{N(^{12}\mathrm{C})}$$

$$^{\frac{25}{24}}R = \frac{N(^{12}\mathrm{C}) \cdot N(^{13}\mathrm{C}) + N(^{13}\mathrm{C}) \cdot N(^{12}C)}{N(^{12}\mathrm{C})^2} = 2 \cdot \frac{N(^{13}\mathrm{C})}{N(^{12}\mathrm{C})} = 2 \cdot {}^{\frac{13}{12}}R$$

$$^{\frac{27}{26}}R = \frac{N(^{15}\mathrm{N}) \cdot N(^{12}\mathrm{C}) + N(^{12}\mathrm{C}) \cdot N(^{15}\mathrm{N})}{N(^{14}\mathrm{N}) \cdot N(^{12}\mathrm{C}) + N(^{12}\mathrm{C}) \cdot N(^{14}\mathrm{N})} = \frac{N(^{15}\mathrm{N})}{N(^{14}\mathrm{N})} = {}^{\frac{15}{14}}R$$

2. Error from shot noise

Even if detection, amplification and signal conversion in isotope ratio measurements were completely free of noise and fractionating effects, there would still be a limit to the maximum attainable precision of isotopic data. This limit is posed by shot noise, a consequence of the discrete nature of electronic charge (whether it is carried by electrons or ions). The statistical error from shot noise is rarely a concern in standard bulk isotope measurements, but due to the low number of ions detected and limited chance for reanalysis from measuring individual cells in secondary ion mass spectrometry, this error estimate provides an important constraint on precision. This is particularly relevant for small analytical targets, in measurements of ions from low abundance elements, ions with low ionization efficiency or with rare minor isotopes.

Error from shot noise in ion counts and raw isotope ratios

The number of ions N observed at a detector in a fixed time interval t follows a Poisson distribution (i.e., a discrete probability distribution of independent events occurring with an average rate of a). The corresponding probability mass function is $f(N) = P(X = N) = \frac{(at)^N e^{-at}}{N!}$. The expected value $E[N] = \bar{N}$ (mean) of a Poisson distribution is $\bar{N} = at$ (for a fixed time interval t). The variance $Var[N]$ of a Poisson distribution is identical to the expected value, and hence the standard deviation σ_N scales with the square root of $\bar{N}$ $\left(\sigma_N = \sqrt{Var[N]} = \sqrt{\bar{N}}\right)$. In secondary ion mass spectrometry, analytical targets are typically analyzed in several passes over the same area generating ion images that consist of multiple planes. For small targets and/or low ion currents, individual passes

rarely collect a sufficient number of ions to constrain isotope ratios with high precision so the planes from the same target are usually aligned and ion counts summed across them. Statistically, this amounts to an equivalent Poisson distribution for the counting time across all planes, or as demonstrated by standard error propagation simply

$$N_T = \sum_{i=1}^{n} N_i$$

$$\sigma_{N_T} = \sqrt{\sum_{i=1}^{n} \sigma_{N_i}^2} = \sqrt{\sum_{i=1}^{n} \sqrt{N_i}^2} = \sqrt{N_T}$$

with n the number of planes and N_i the ion count for a specific ion in each plane. This counting error ($\sigma_{N_T} = \sqrt{N_T}$) is propagated readily to the resulting isotope ratio $R = \frac{N_{Tm}}{N_{TM}}$ (m=minor,M=major isotope) by standard error propagation:

$$R = \frac{N_{Tm}}{N_{TM}}$$

$$\sigma_R = \sqrt{\left(\frac{\partial R}{\partial N_{Tm}} \cdot \sigma_{N_{Tm}}\right)^2 + \left(\frac{\partial R}{\partial N_{TM}} \cdot \sigma_{N_{TM}}\right)^2} = \sqrt{\left(\frac{1}{N_{TM}} \cdot \sigma_{N_{Tm}}\right)^2 + \left(-\frac{N_{Tm}}{N_{TM}^2} \cdot \sigma_{N_{TM}}\right)^2}$$

$$= R \cdot \sqrt{\left(\frac{\sigma_{N_{Tm}}}{N_{Tm}}\right)^2 + \left(\frac{\sigma_{N_{TM}}}{N_{TM}}\right)^2} = R \cdot \sqrt{\frac{1}{N_{Tm}} + \frac{1}{N_{TM}}}$$

For details on σ_R , see Fitzsimons et al. 2000 and Hayes 2001.

Error propagation for instrument fractionation correction

Secondary ion mass-spectrometry can have signification isotopic fractionation associated with the measurement itself. Such fractionation may cause significant loss in measurement accuracy and distort quantitative interpretation of the data. Due to the large enrichments in stable isotope labeling applications above naturally occurring isotope abundances, the errors introduced by instrument fractionation are frequently not corrected for. While overall trends and relative patterns in data from the same analytical session are usually preserved and can be interpreted correctly, not taking instrument fractionation into account is particularly problematic for accurately comparing data across multiple instrument sessions because instrument fractionation factors can (and usually do) change over time and with changes in tuning parameters. In order to accurately compare all data across the multi-year experimental effort of this study, we estimated an instrument isotope fractionation factor for each analytical session from the deviation in isotope ratio of all unlabeled ROIs relative to the expected ratio (approximately natural abundance, specifically ${}^{13C}R_{nat} = 0.011237$ and ${}^{15N}R_{nat} = 0.003677$), and corrected all measured ratios in each session by the session's nanosims fractionation factor:

$$\alpha_{NS/true} = \frac{R_{unlabeled}}{R_{nat}} = \frac{\left(\frac{\sum N_m}{\sum N_M}\right)_{unlabeled}}{R_{nat}}$$

$$\sigma_\alpha = \sqrt{\left(\frac{\sigma_{R_{unl}}}{R_{nat}}\right)^2 + \left(\frac{R_{unl} \cdot \sigma_{R_{nat}}}{R_{nat}^2}\right)^2} \approx \frac{\sigma_{R_{unl}}}{R_{nat}} = \frac{\sum N_m / \sum N_M}{R_{nat}} \cdot \sqrt{\frac{1}{\sum N_m} + \frac{1}{\sum N_M}}$$

assuming negligible error on the natural abundance ratio. The resulting fractionation correction for the measured isotope ratios (R_m) of all analyzed samples in a session is then the standard correction and associated error propagation:

$$R_{corr} = \alpha_{NS/true} \cdot R_m$$

$$\sigma_{R_{corr}} = \sqrt{\left(R_m \cdot \sigma_\alpha\right)^2 + \left(\alpha_{NS/true} \cdot \sigma_{R_m}\right)^2} = R_{corr}\sqrt{\left(\frac{\sigma_\alpha}{\alpha_{NS/true}}\right)^2 + \left(\frac{\sigma_{R_m}}{R_m}\right)^2}$$

Error propagation for conversion to fractional abundance

Lastly, due to the high isotope enrichments associated with tracer experiments, it is more intuitive to compare fractional isotope abundances rather than isotope ratios. Fractional abundances also allow accurate mass-balance calculations which introduce significant error in δ or isotope ratio space for such enriched samples. The fractional abundances and associated error propagated from shot noise and instrument fractionation error estimates are then calculated as follows:

$$F = \frac{R_{corr}}{1 + R_{corr}}$$

$$\sigma_F = \sqrt{\left(\frac{\partial F}{\partial R_{corr}}\sigma_{R_{corr}}\right)^2} = \frac{\sigma_{R_{corr}}}{\left(1 + R_{corr}\right)^2}$$

3. Counting statistic considerations

Increasing sample dwell time

The number of secondary ions per pixel is a function of the total sputtering yield (Y), the useful yield (τ), the primary beam current (IP), the atomic concentration (CA), and the dwell time (td, time per pixel) (Slodzian et al. 1992). Assuming τ, CA and Y are fixed, the only way to improve counting statistics (e.g., the number of secondary ions per pixel) is to increase the number of primary ions striking the surface (IP) or increase the dwell time. Since nanoSIMS is a destructive technique, small biological targets disappear as they are analyzed. Increasing the intensity of the primary beam not only decreases spatial resolution making submicron viral particle measurements more challenging, but also sputters through the sample faster. Therefore, by analyzing the same spatial area repeatedly and combining the data across each frame, we can increase the total ion counts per viral particle and reduce error (Fig. S3)

4. References

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