Quantitative SIMS Imaging of Agar-based Microbial Communities

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Supporting Information

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Figure S1. Microscopic evaluation of AQ/AQNO distributions during preparation of a *P. aeruginosa* PAO1C colony biofilm for SIMS imaging. The same aggregates are visible in both (**a**) hydrated and (**b**) dehydrated samples, and are stationary with respect to one another during drying. Subfigures (**c**) and (**d**) are magnified regions of (**a**) and (**b**), respectively (as indicated by the white boxes), and (**e**) and (**f**) are acquired from agar without bacteria.
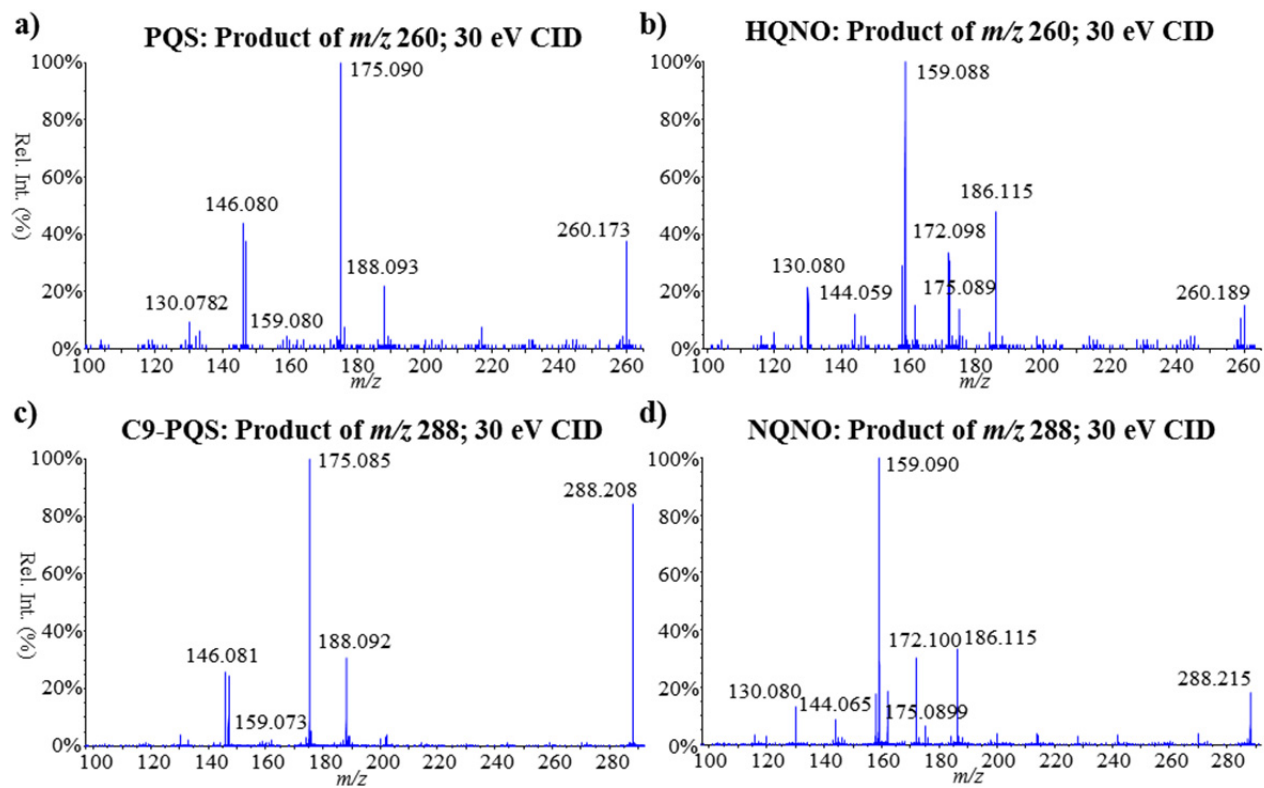
Figure S2. C_{60}-SIMS product ion spectra for (**a**) PQS, (**b**) HQNO, (**c**) C9-PQS, and (**d**) NQNO standards. Notice that despite a strong base peak of m/z 159.07 for both HQNO and NQNO, and m/z 175.07 for PQS and C9-PQS, the corresponding peak is also present in the isomer at a relatively low intensity.

a)

PQS (m/z 260 →175.07 ± 0.05)

$y = 5.643E-04x^2 + 7.549E-03x - 7.771E+00$
$R^2 = 9.802E-01$

Average Intensity vs Surface Density [pmole × cm^{-2}]

PQS: m/z 260 → 175.07

5 mm

10x

5 mm

Deposition Quantity [fmole]

40 200 400 1000 2000 3000 4000 6000 8000

MS^2 Intensity: 750, 600, 450, 300, 150, 0

Saturated, 60, 45, 30, 15, 0

b)

HQNO (m/z 260 → 159.07 ± 0.05)

$y = 1.171E-04x^2 + 1.539E-01x - 2.442E+00$
$R^2 = 9.964E-01$

Average Intensity vs Surface Density [pmole × cm^{-2}]

HQNO: m/z 260 → 159.07

5 mm

10x

5 mm

Deposition Quantity [fmole]

40 200 400 1000 2000 3000 4000 6000 8000

MS^2 Intensity: 365, 300, 225, 150, 75, 0

Saturated, 30, 23, 15, 8, 0

Figure S3. Calibration curves and product ion images for (**a**) PQS and (**b**) HQNO dehydrated FAB agar. Each data point on the calibration curve corresponds to the average ion intensity from three replicate deposition lines. Only the deposition quantities highlighted with blue text in the ion images (i.e., 1000–8000 fmol for PQS and 200–8000 fmol for HQNO) were used to construct the calibration curves. The error bars represent the standard deviation of three replicate deposition lines.

a)

C9-PQS (m/z 288 → 175.07 ± 0.05)

$y = 3.577E\text{-}03x^2 - 4.963E\text{-}02x$
$R^2 = 9.954E\text{-}01$

b)

NQNO (m/z 288 → 159.07 ± 0.05)

$y = 3.118E\text{-}03x^2 + 3.742E\text{+}00x - 6.109E\text{+}01$
$R^2 = 9.979E\text{-}01$
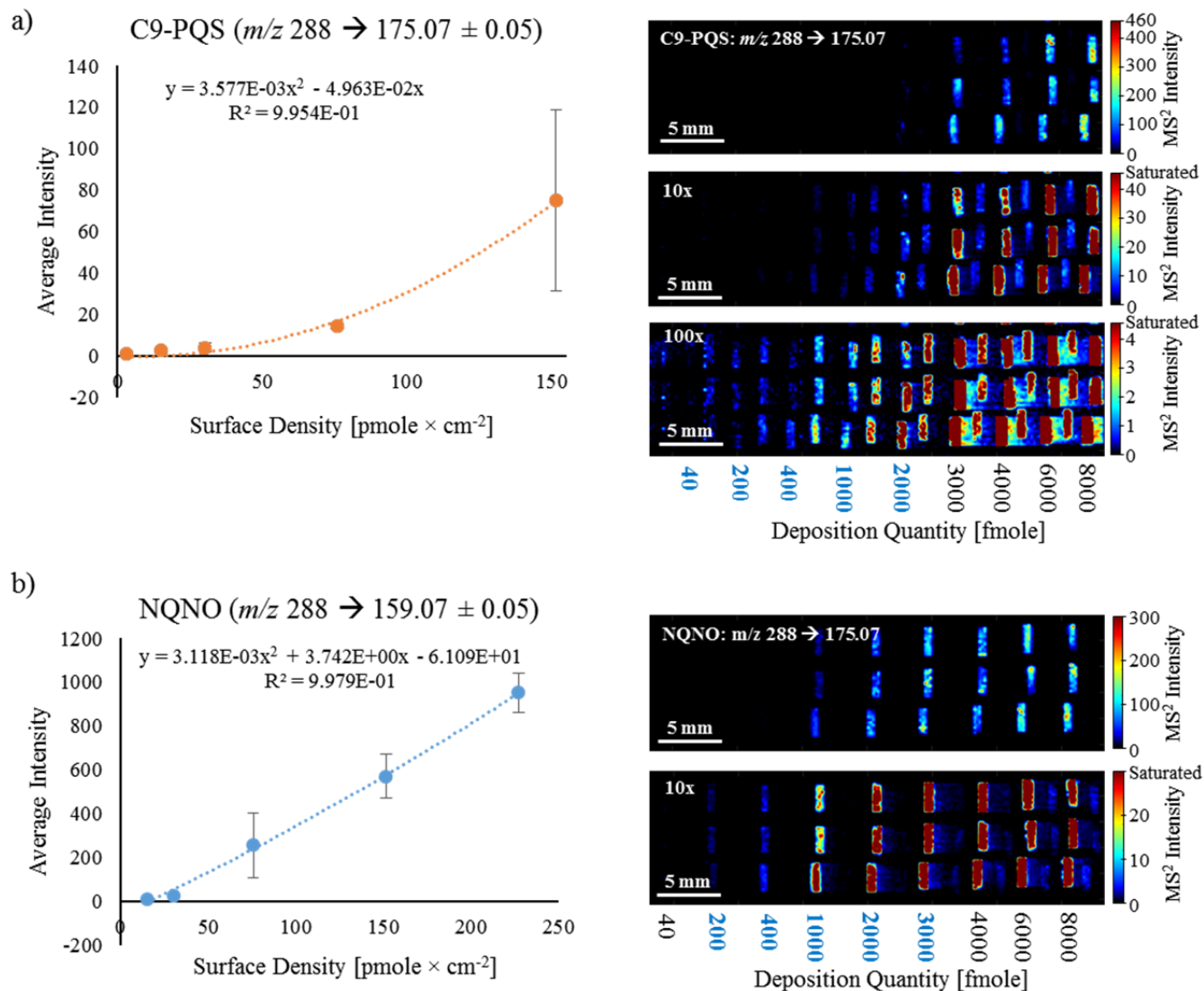
Figure S4. Calibration curves and product ion images for (**a**) C9-PQS and (**b**) NQNO on dehydrated FAB agar. Each data point on the calibration curve corresponds to the average ion intensity from three replicate deposition lines. Only the deposition quantities highlighted with blue text in the ion images (i.e. 40–2000 fmol for C9-PQS and 200–3000 fmol for NQNO) were used to construct the calibration curves. The error bars represent the standard deviation of three replicate deposition lines.
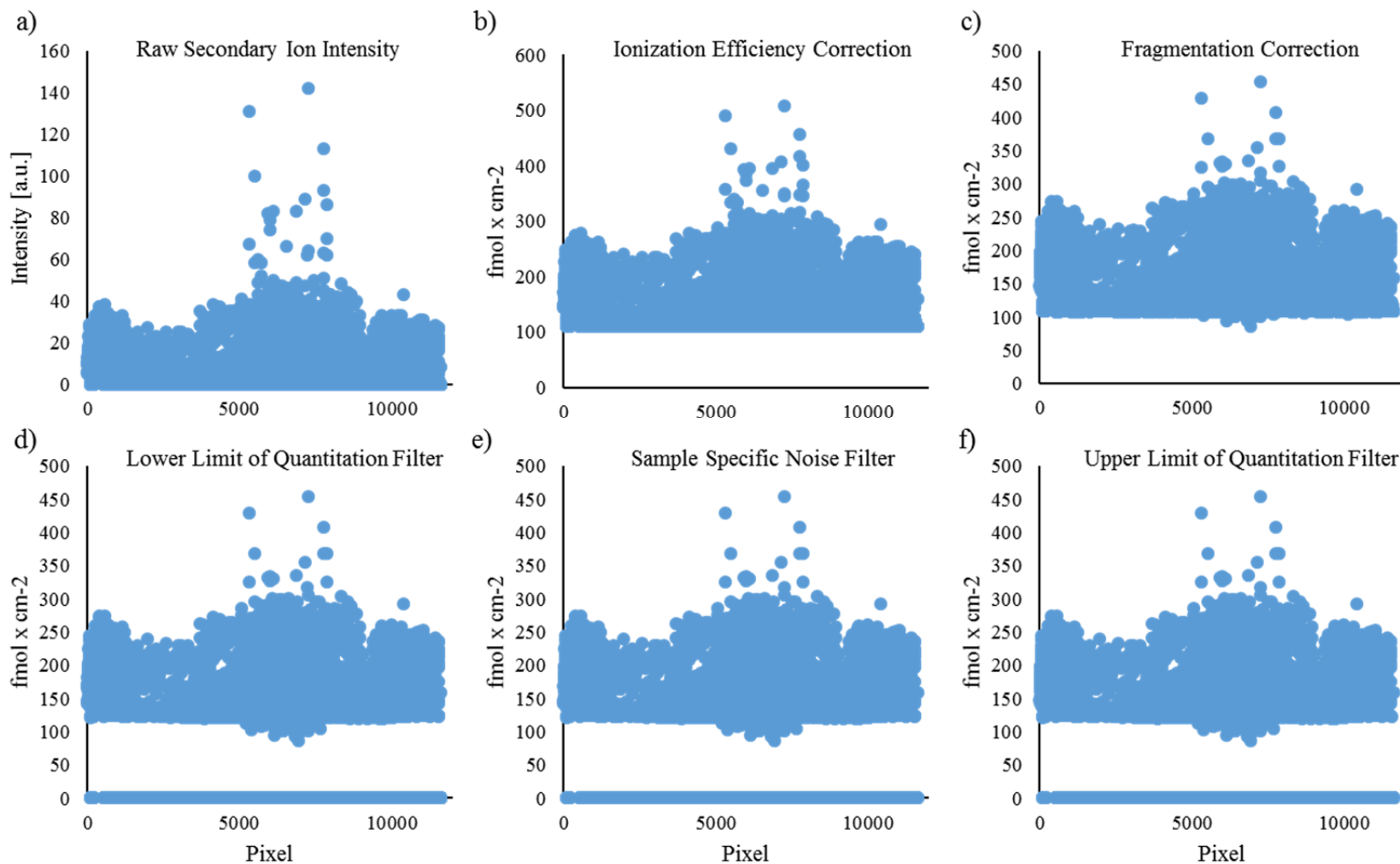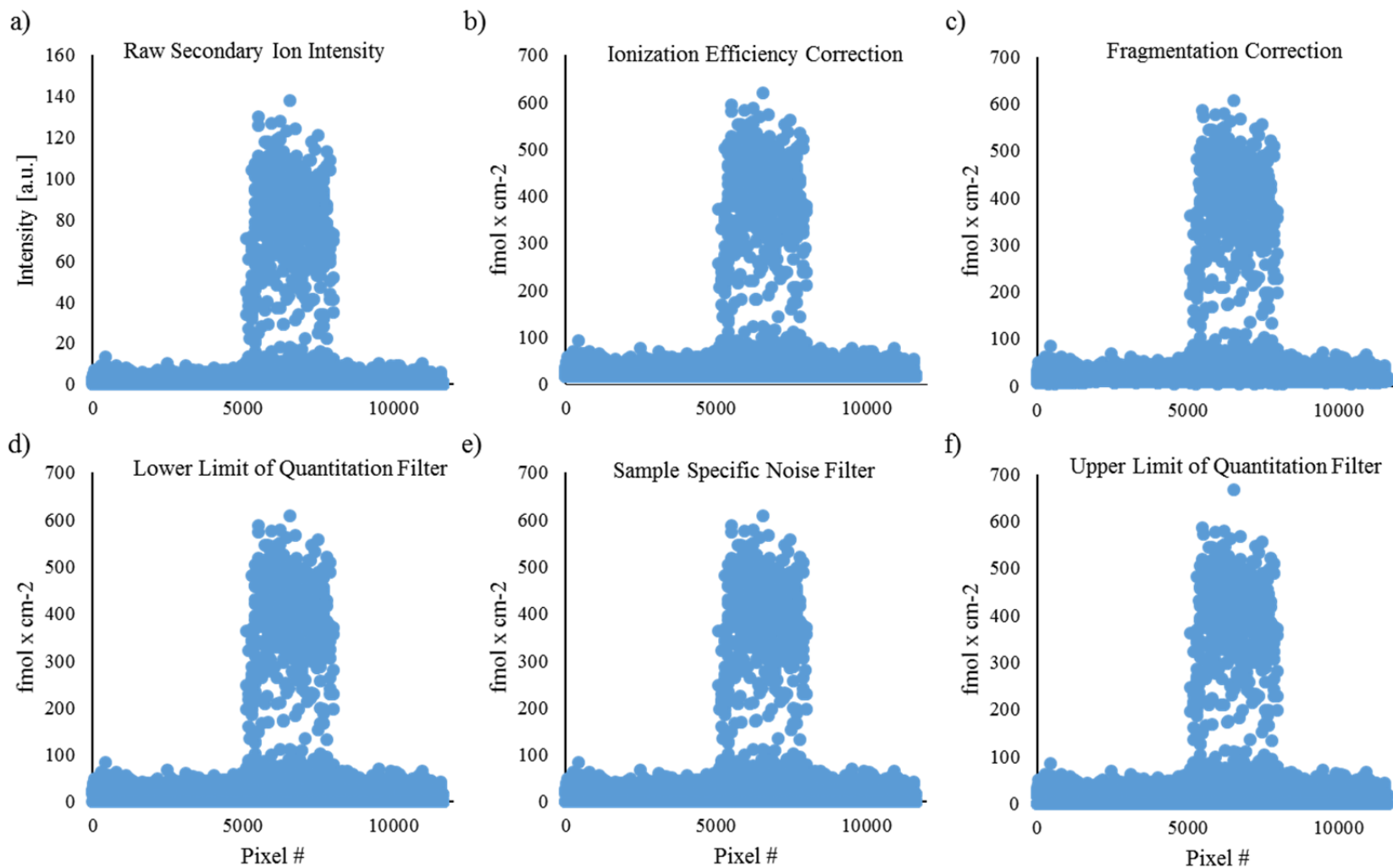
Figure S5. Quantitative analysis of PQS in the 72 h *P. aeruginosa* PAO1C colony biofilm from **Figure 4**. Each dot is a single pixel from the ion image. (**a**) Raw secondary ion intensity, (**b**) surface density corrected for ionization efficiency, (**c**) surface density corrected for interference from the common fragment, (**d**) surface density following removal of all values lower than the LLOQ, (**e**) surface density following removal of all values lower than the sample-specific background noise, and (**f**) surface density following assignment of all values greater than the ULOQ to 110% of the ULOQ.
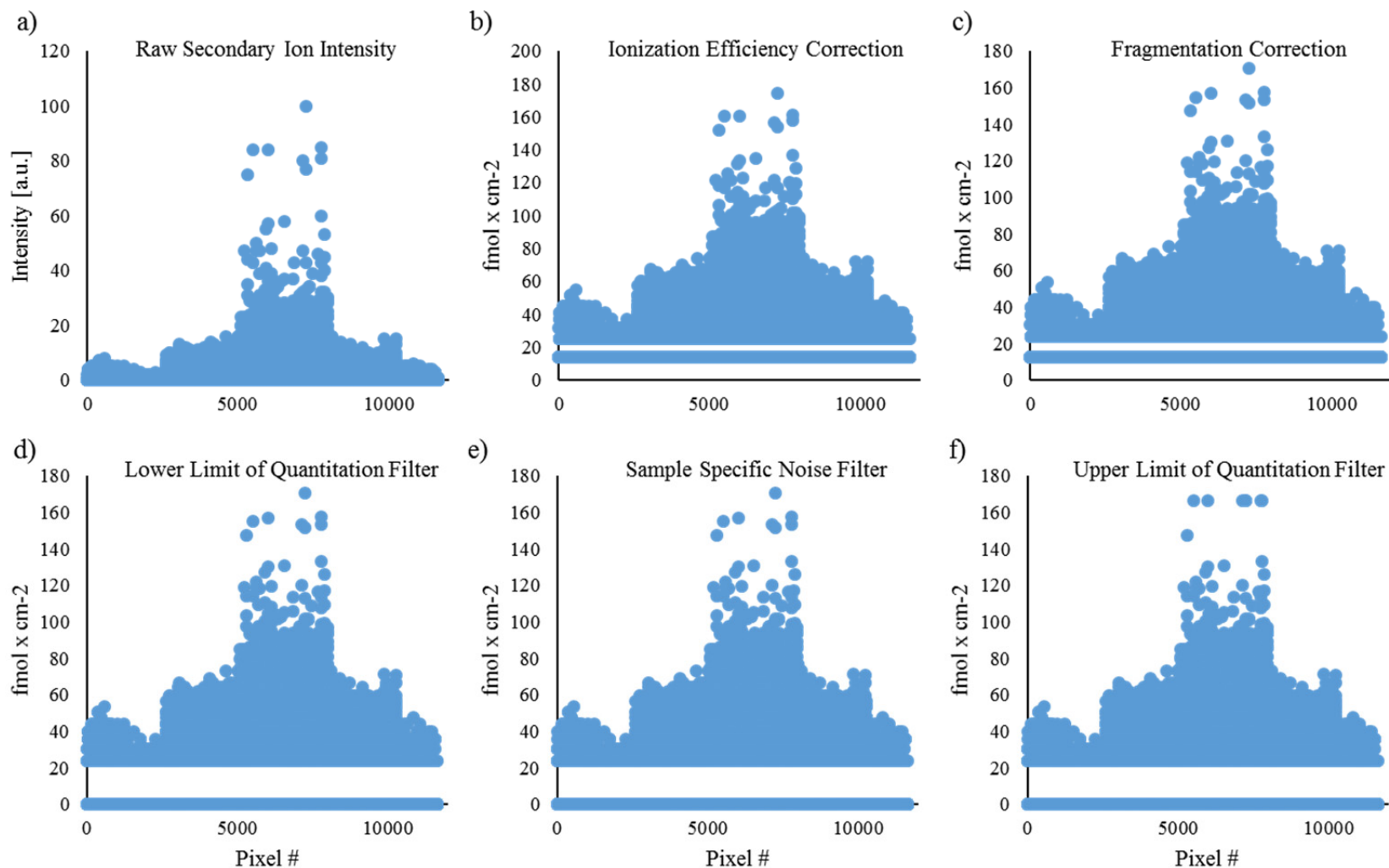
Figure S6. Quantitative analysis of HQNO in the 72 h *P. aeruginosa* PAO1C colony biofilm from **Figure 4**. Each dot is a single pixel from the ion image. (**a**) Raw secondary ion intensity, (**b**) surface density corrected for ionization efficiency, (**c**) surface density corrected for interference from the common fragment, (**d**) surface density following removal of all values lower than the LLOQ, (**e**) surface density following removal of all values lower than the sample-specific background noise, and (**f**) surface density following assignment of all values greater than the ULOQ to 110% of the ULOQ.
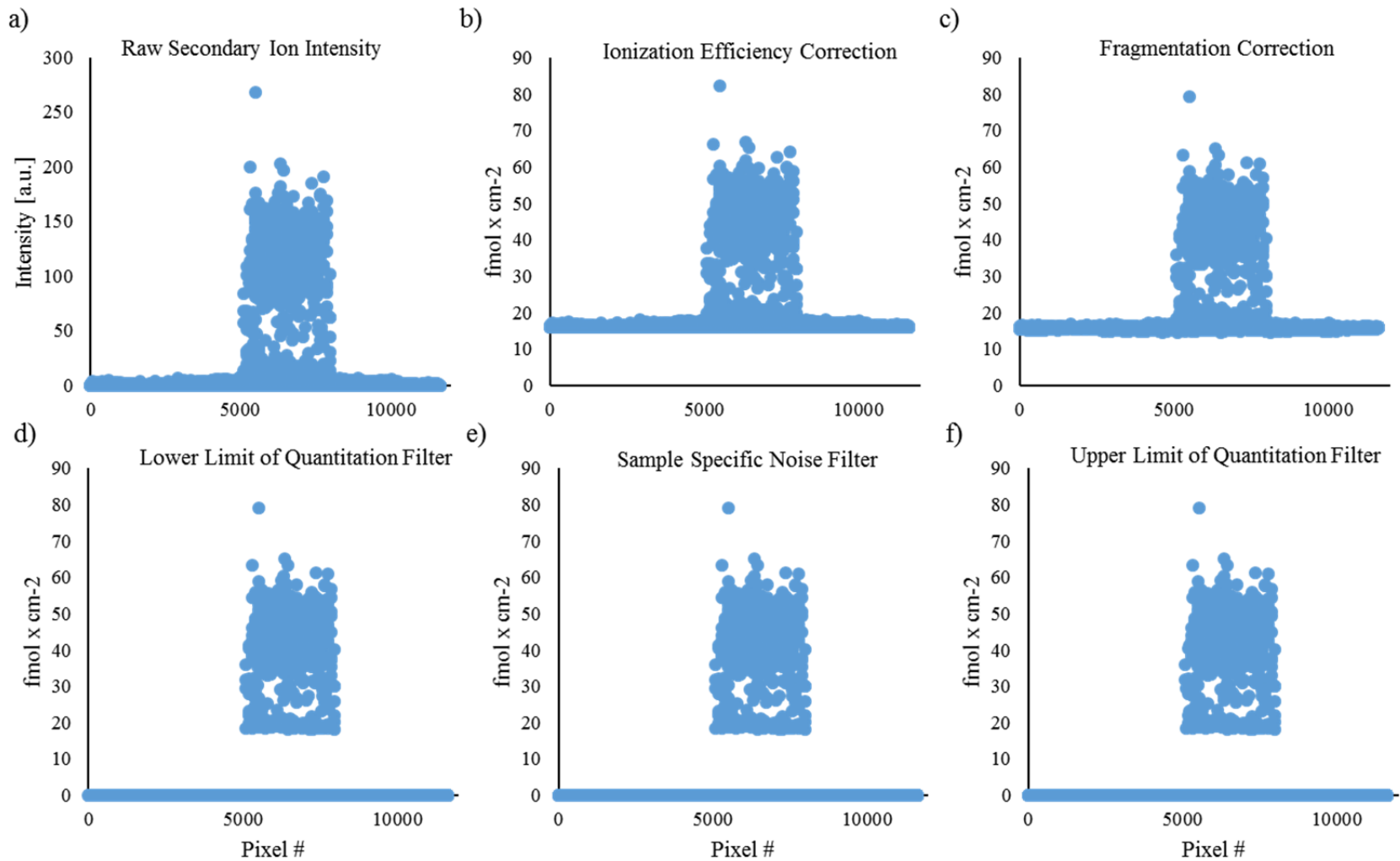
**72 h PAO1C, C9-PQS:** *m/z* **288 → 175.07 ± 0.05**

Figure S7. Quantitative analysis of C9-PQS in the 72 h *P. aeruginosa* PAO1C colony biofilm from **Figure 4**. Each dot is a single pixel from the ion image. (**a**) Raw secondary ion intensity, (**b**) surface density corrected for ionization efficiency, (**c**) surface density corrected for interference from the common fragment, (**d**) surface density following removal of all values lower than the LLOQ, (**e**) surface density following removal of all values lower than the sample-specific background noise, and (**f**) surface density following assignment of all values greater than the ULOQ to 110% of the ULOQ.

Figure S8. Quantitative analysis of NQNO in the 72 h *P. aeruginosa* PAO1C colony biofilm from **Figure 4**. Each dot is a single pixel from the ion image. (**a**) Raw secondary ion intensity, (**b**) surface density corrected for ionization efficiency, (**c**) surface density corrected for interference from the common fragment, (**d**) surface density following removal of all values lower than the LLOQ, (**e**) surface density following removal of all values lower than the sample-specific background noise, and (**f**) surface density following assignment of all values greater than the ULOQ to 110% of the ULOQ.
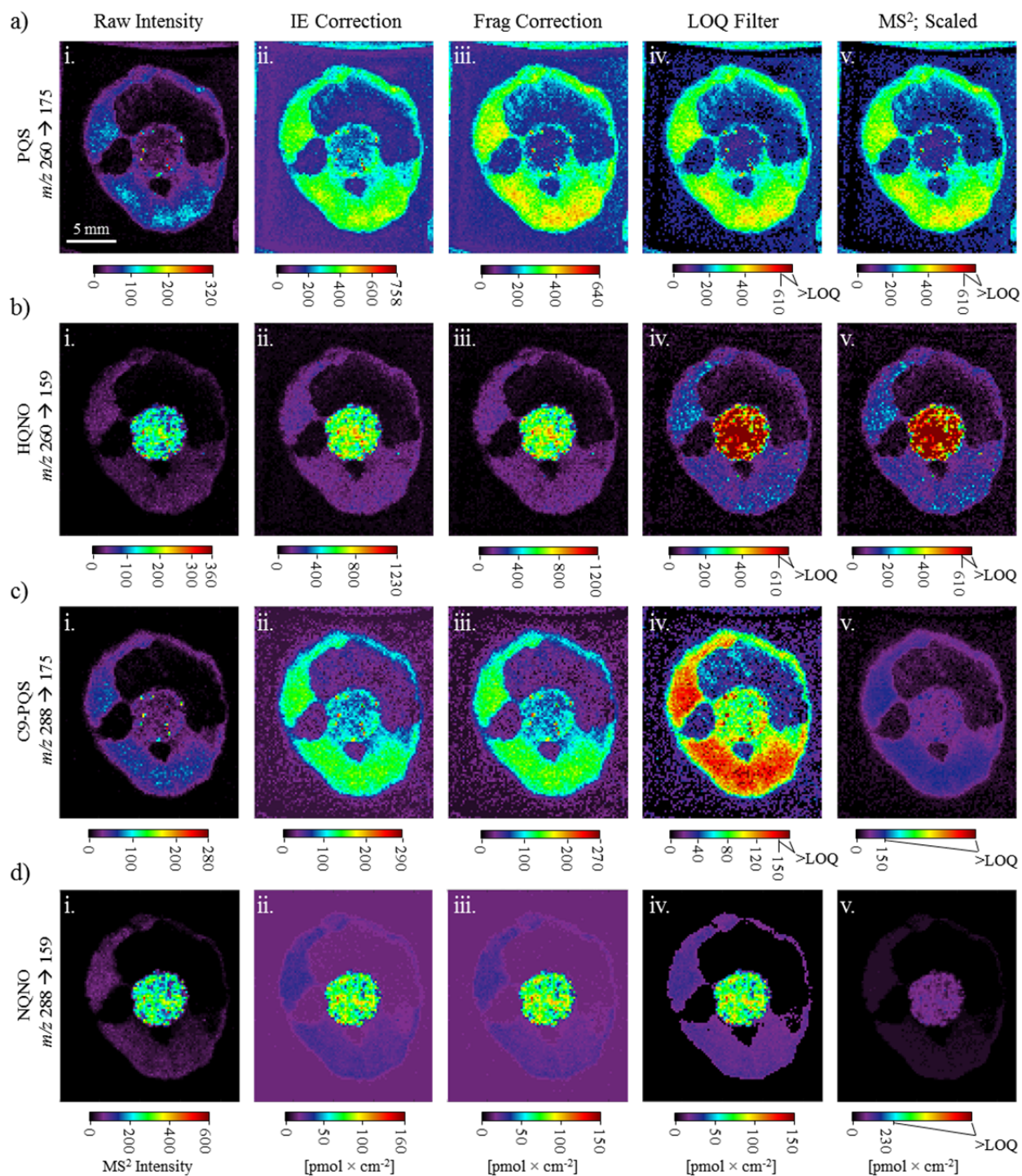
Figure S9: SIMS product ion images for (**a**) PQS, (**b**) HQNO, (**c**) C9-PQS, and (**d**) NQNO in a 72 h *P. aeruginosa* PAO1C colony biofilm. Images in column **i** show the uncorrected (raw) secondary ion intensity. Column **ii** shows the same images following correction for differences in ionization efficiency. Images in column **iii** have been adjusted to remove the artificial ion intensity arising from common product ions. Images in column **iv** have been modified to remove intensities above and below the upper and lower limits of quantitation. The final series of images in column **v** are scaled to a common maximum density of 610 pmol x cm^{-2} to facilitate direct analyte-to analyte comparisons. High intensity regions outside of the agar shown in **a–d** arise from the chemical background of the copper tape.