Digital Quadrature Heterodyne Detection for High-Resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

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The pursuit of ever higher mass-resolving power in Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) has driven a demand for higher magnetic field strength and longer time-domain ICR signal lifetime, with proportionate increase in data set size in direct-mode detection. Heterodyne-mode detection thus becomes increasingly important for achieving ultrahigh-mass resolution from a fixed maximum-size data set. Unfortunately, as conventionally performed (i.e., analog single phase), heterodyne detection reduces the S/N ratio by a factor of $2^{1/2}$ relative to direct-mode detection. Here, we restore the factor of $2^{1/2}$ by use of quadrature heterodyne detection. In addition, replacement of analog by digital heterodyning eliminates analog circuitry (and its associated noise). Finally, digital filtering of the time-domain discrete ICR signal not only eliminates the need for a bank of analog low-pass filters but also ensures that the Nyquist bandwidth and filter bandwidth are always matched, for optimal noise reduction. With these features, digital quadrature heterodyne detection becomes the detection method of choice for high-resolution FT-ICR MS.

Heterodyne detection[1] in Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS)[2–5] serves to reduce the frequency of the ICR signal and therefore the digitization rate required to satisfy the Nyquist sampling criterion.[1] As a result, a longer duration time-domain signal (and thus higher resolving power) may be acquired than by direct-mode detection for the same data set size. For example, at the National High Magnetic Field Laboratory (NHMFL), we have achieved a mass resolving power, $m/\Delta m_{50\%}$ (in which $\Delta m_{50\%}$ is the full width at half-maximum

magnitude-mode peak height), of 8 000 000 with a 9.4-T electrospray FT-ICR mass spectrometer.[6] In that example, heterodyne acquisition reduced the time-domain data size from 32 Mword to 32 Kword. Not only is 32 Mword prohibitively difficult to process, but most digitizers are also not capable of collecting such a large data set and therefore cannot offer that level of performance without heterodyne capability.

Ultrahigh-resolution FT-ICR MS is presently performed by analog single-phase heterodyne detection. The heterodyne process begins by mixing (effectively multiplying) an analog reference oscillator signal with the analyte time-domain ICR signal (obtained from the difference in charge induced on a single pair of opposed detector electrodes) to produce additional signals at sum and difference frequencies. Subsequent low-pass analog filtering removes the high-frequency components to leave only the "difference frequency" signal.[1] Therefore, the frequency of the original signal is effectively reduced, so that the signal may be digitized at a lower sampling frequency while still satisfying the Nyquist limit. A lower sampling frequency means fewer data points for a fixed time-domain signal duration or a longer detection period (and thus higher frequency-domain resolution) for a fixed time-domain data set size. Because the time-domain data are represented by a single data set, the time-domain data are treated as mathematically real; a mathematically complex Fourier transform then yields real and imaginary frequency-domain spectra, which are typically combined to yield a magnitude-mode frequency-domain spectrum that is then converted to a mass-to-charge (m/z) ratio spectrum. Heterodyne detection is conceptually equivalent to a slit-scanned spectrophotometer, in which the slit position is determined by the reference oscillator frequency and the slit width is the low-pass filter bandwidth.[7]

Although single-phase heterodyning effectively reduces the frequency of the ICR signal, its inability to distinguish between frequencies that lie above or below the reference frequency results in "foldover" of both signals and noise on either side of the reference frequency. Each ICR signal will produce two signals in the heterodyne spectrum, one at the correct frequency and a mirror image (signal foldover) with respect to the reference frequency. The signal foldover results in incorrect mass-to-charge

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ratios when the frequency-domain spectrum is converted to an m/z spectrum.[1] Signal foldover is generally not a problem, because the time-domain excitation waveform can be tailored so as not to excite ion cyclotron resonances above (or below) the reference frequency. However, for a low-pass filter with perfectly square cutoff at the Nyquist frequency, noise "folded over" from (say) above the reference frequency will reduce the signal-to-noise (S/N) ratio for signals below the reference frequency by a factor of $2^{1/2}$.

Quadrature heterodyne detection[8] offers a solution to the problem of spectral foldover. The incoming ICR signal (again detected by a single pair of opposed detector electrodes) is first split into two equal-amplitude components. Each component is then multiplied by reference oscillator signals of identical frequency but differing in phase by 90° (hence "quadrature" heterodyne). The two resulting data sets are treated as mathematically real and imaginary data. Subsequent complex Fourier transformation can then distinguish between signals of positive and negative frequency (corresponding to signal frequencies below and above the reference frequency). The resulting final spectrum is an exact representation of a segment of the original ICR spectrum shifted to lower frequency. Foldover of signal and noise observed in single-phase heterodyne detection is eliminated. The S/N ratio for quadrature heterodyne detection is therefore identical to that of direct detection and $2^{1/2}$ higher than the S/N ratio for single-phase heterodyne detection.

Despite its potential advantages and initial demonstrations,[9-11] analog quadrature heterodyne detection has not been implemented in FT-ICR MS. One reason is that even a small amplitude mismatch between the two analog channels produces "image" peaks, which are difficult to distinguish from true signals.[9] Moreover, if the dc levels of the two analog quadrature channels differ, then there will be a large peak at zero frequency (i.e., in the center of the frequency-domain spectrum). In FT-NMR spectroscopy, such artifacts may be eliminated by appropriate "phase-cycling", in which the phase of the reference signal is cycled (e.g., 0°, 180°, 0°, 180° with corresponding addition and subtraction of time-domain data from successive acquisitions to eliminate the zero-frequency peak; and a 0°, 90°, 180°, 270° with appropriate addition and subtraction to real and imaginary data sets to eliminate image peaks).[12] Phase cycling works well in FT-NMR because the sample is identical from one data acquisition to the next. However, successive FT-ICR signals typically require generating a *new* batch of ions each time (e.g., for liquid chromatograph/mass spectrometry), and any deviation in the number of ions of each m/z ratio from one ionization to the next will lead to image peaks that are *not* eliminated by phase cycling.

Here, we present digital quadrature heterodyne detection, previously demonstrated for FT-NMR spectroscopy.[13] By digitizing the time-domain ICR signal at a high sampling rate (20
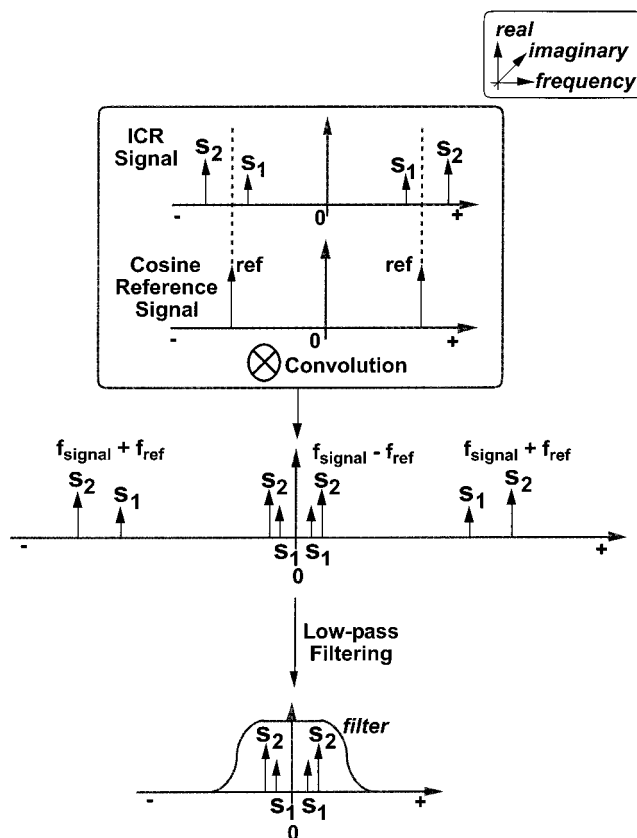
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Figure 1. Step-by-step procedure for heterodyne single-phase detection, represented in the frequency domain.

Msamples/s), and performing the sine and cosine reference signal multiplications digitally (followed by real-time digital filtering), we achieve the advantages of quadrature heterodyne detection while eliminating the dc offset and gain mismatch, and any associated noise of analog multiplication. We demonstrate experimentally the predicted $2^{1/2}$ enhancement in S/N ratio. In fact, the experimental S/N ratio improvement is actually greater than $2^{1/2}$, due to elimination of analog noise and noise resulting from mismatch between Nyquist and analog low-pass filter bandwidths.

THEORY

The implementation of single-phase heterodyne detection is most easily visualized in the frequency domain, as shown in Figure 1. The mixing (multiplication) process in the time domain corresponds to a convolution in frequency domain. A spectrum of two ICR signals (S_1 at a frequency below the reference frequency, and S_2 at a frequency above the reference frequency) is convolved with the reference spectrum to produce components at the sum and difference frequencies. After low-pass filtering, only the difference frequency components remain. The inability of the single-phase heterodyne experiment to distinguish between ICR signals whose frequencies lie above or below the reference frequency results in spectral foldover (i.e., each signal on one side of the reference frequency also appears as its mirror image on the other side of the reference frequency). Effectively, only half of the available bandwidth is used, because the other half yields no new information. Moreover, noise at frequencies (say) above the reference frequency is folded over into the other half of the spectrum, thereby increasing the rms noise by a factor of $2^{1/2}$,
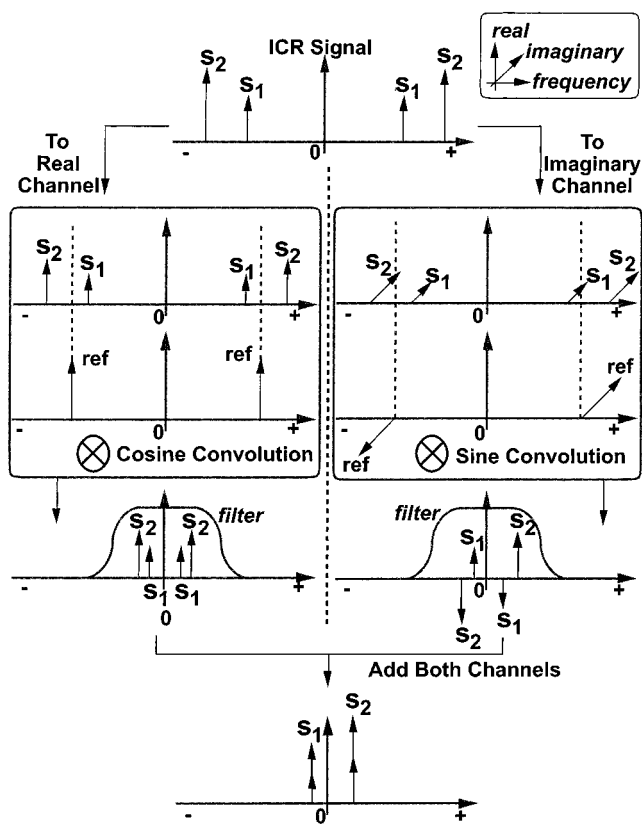
Figure 2. Step-by-step procedure for quadrature heterodyne detection, represented in the frequency domain.

and reducing the S/N ratio by the same factor, relative to direct-mode detection.

Figure 2 shows that spectral foldover is eliminated in quadrature heterodyne detection by distinguishing between signals whose frequencies lie above and below the reference frequency. First, the ICR signal is divided into two equal components henceforth treated as real and imaginary data. In the real channel, the spectrum is convolved with a cosine waveform, and in the imaginary channel, the signal is convolved with a sine waveform. The two reference waveforms (cosine and sine) have the same frequency but differ in phase by 90°. The data in both channels are subjected to low-pass filtering, and the convolved real and imaginary spectra are added to produce the final spectrum. The troublesome foldover peaks present in single-phase heterodyne detection are eliminated by that addition, because the foldover peaks will have the same amplitude but opposite phase in the two channels (shown in Figure 2). Adding the spectra from the two channels doubles the signal (thereby exactly compensating for halving the original signal when it was divided into two channels). As a result, the signal peak height in the quadrature heterodyne spectrum of Figure 2 is the same as that in direct mode. The noise level is also the same as for direct mode. The S/N ratio for quadrature heterodyne detection is thus the same as for direct detection and higher than for single-phase heterodyne detection by a factor of $2^{1/2}$.

"Image" peaks found in analog implementations of quadrature heterodyne detection[9] are readily understood from Figure 2. A difference in the gain between the two channels will result in incomplete cancellation of the foldover peaks when the spectra are added. Such a difference can arise from difference in the gain

during low-pass filtering or difference in the amplitude of the two reference waveforms. Image peaks will also result if the two reference waveforms are not perfectly orthogonal (i.e., do not differ in phase by exactly 90°,) as, for example, from different circuit path length in the two channels. Digital implementation avoids all of these problems. Finally, because the reference frequency may be placed in the center of the frequency range (rather than at one end of the range), quadrature heterodyne detection reduces the sampling rate by a factor of 2 compared to single-phase heterodyne detection. Thus, for a given time-domain acquisition period (and thus a given FT spectral digital resolution), quadrature heterodyne detection yields the same total number of data points as single-phase heterodyne detection, but stored as $N/2$ real and $N/2$ imaginary data rather than as N real data.

EXPERIMENTAL SECTION

Experiments were performed with a 9.4-T home-built electro-spray ionization (ESI) FT-ICR mass spectrometer described elsewhere.[14,15] Analog single-phase heterodyne data were collected from a single pair of opposed detection electrodes, with a commercial Odyssey data station (Finnigan FT-MS, Madison, WI). Digital quadrature heterodyne data (also from a single pair of opposed detection electrodes) were obtained with a MIDAS data station[16] equipped with an E1437A 20.48-MHz VXI digitizer (Hewlett-Packard, Palo Alto, CA).[17] Advanced HP E1437A device drivers compatible with National Instruments (Austin, TX) Lab-Windows CVI simplified the integration of the new digitizer into the MIDAS data station.

A 2 μM bovine ubiquitin (Aldrich, St. Louis, MO) solution in 50:50 methanol/water and 0.25% (v/v) acetic acid provided electrospray data to demonstrate signal foldover. A 128-Kword data set (K denotes 1024) was collected in analog single-phase heterodyne mode (40 kHz bandwidth), with the reference frequency set to 125 kHz. A 256-Kword data set was collected in digital quadrature heterodyne mode (±40 kHz bandwidth) with a reference frequency of 125 kHz. A Hanning apodization and single zero-fill were applied to each time-domain signal, followed by a fast Fourier transform (FFT) and magnitude calculation.

To determine the S/N advantage of digital quadrature (vs analog single-phase) heterodyne detection, we connected a function generator (model 2003, Global Specialties, New Haven, CT) to the excitation amplifier to ensure a stable excitation waveform for each scan. A ~2-V_{p-p}, 117.8-kHz (i.e., the ICR frequency at 9.4 T for the 7+ charge state of bovine ubiquitin) excitation was applied to the ICR cell transmitter electrodes during the detection period of 819 ms. The detected signal arises from leakage from the opposed transmitter electrodes to the orthogonal opposed receiver electrodes. To ensure that no systematic errors were introduced from the two different data systems, the Odyssey data system was configured to control both analog (Odyssey) and

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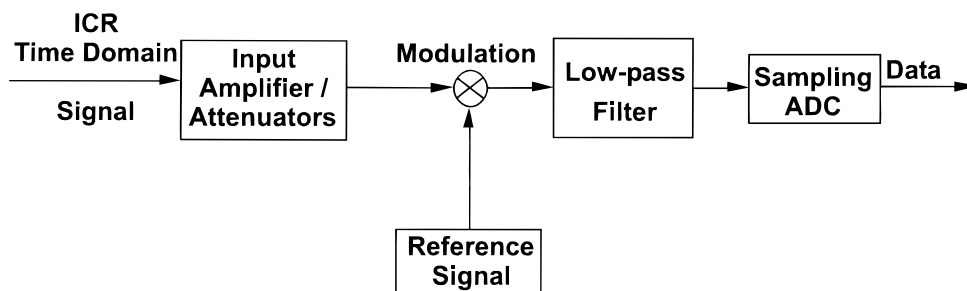
Figure 3. Block diagram of a typical implementation of analog single-phase heterodyne detection.
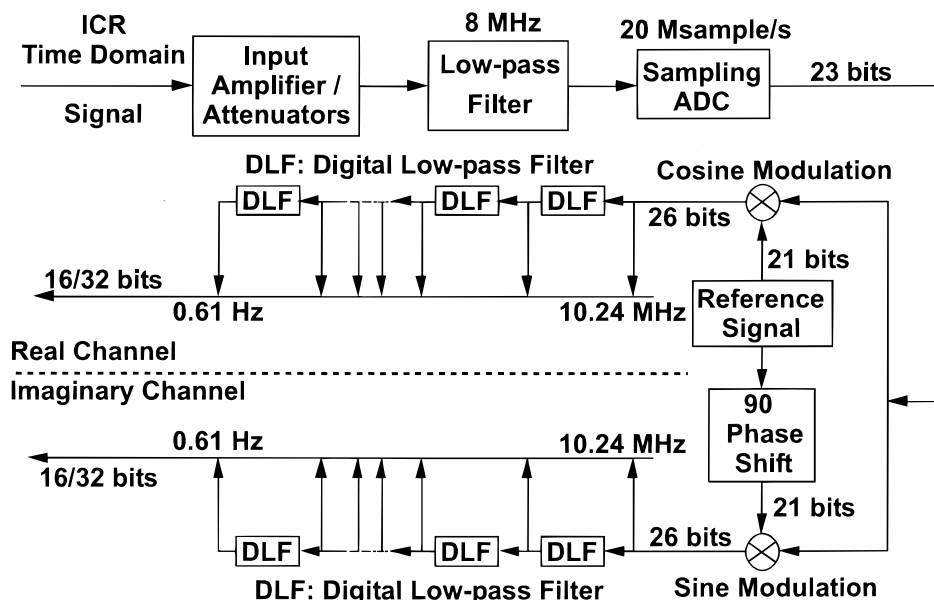

Figure 4. Block diagram of implementation of digital quadrature heterodyne detection (HP E1437A digitizer) with real-time digital filtering.

digital (MIDAS) heterodyne experiments. For digital quadrature heterodyne, an external trigger on the Odyssey data station triggered the HPE1437A digitizer.

For the purpose of S/N ratio determination, "signal" is defined as the magnitude-mode peak height minus the average magnitude-mode baseline noise. Magnitude-mode FT-ICR frequency-domain spectral noise is Rayleigh distributed (not Gaussian distributed)[18] and may be conveniently measured as the average spectral amplitude between 117.7 and 117.9 kHz. S/N ratio was measured for Nyquist bandwidths ranging from 160 to 1.25 kHz for analog single-phase heterodyne and from 160 kHz to 312.5 Hz for digital quadrature heterodyne detection. Decreasing the bandwidth and number of data points simultanously by successive factors of 2 resulted in a constant detection period of 819 ms. Noise spectra were collected by executing the identical experimental sequence with the function generator turned off. Each data set was processed by two zero-fills followed by FFT and magnitude calculation. Due to a large initial noise spike in the analog single-phase heterodyne time-domain signal, the first 14 data points were zeroed out for bandwidths from 10 to 1.25 kHz, to avoid baseline distortion in the resulting spectrum. We measured three signal scans and nine noise scans for each bandwidth to yield the reported average S/N ratio and its standard deviation.

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RESULTS AND DISCUSSION

Implementation of analog single-phase heterodyne detection is outlined in Figure 3. The ICR signal is first passed through an input amplifier or attenuator to match the maximum signal amplitude to the full range of the digitizer. A modulator mixes (i.e., effectively multiplies) an analog reference oscillator signal with an analyte ICR signal to produce signals at sum and difference frequencies, and the high-frequency components are then removed by low-pass analog filtering. The remaining difference frequency components may then be digitized at a much lower sampling rate than required for direct-mode acquisition.

Recently, we integrated into a MIDAS FT-ICR data station[16] a digitizer (Hewlett-Packard E1437A) (Figure 4) that is capable of performing digital quadrature heterodyne detection. After passing through a single fixed-bandwidth analog filter, the ICR signal is sampled at 20.48 Msample/s at 23 bit/sample. The digital data stream is multiplied by cosine and sine digital reference-frequency waveforms (0−10 MHz with 20 μHz resolution) to form real and imaginary discrete time-domain signals. The data from each channel is subjected to a series of 0−24 digital filters and decimation stages to produce the specified bandwidth. Each stage of decimation decreases the bandwidth by a factor of 2. For example, a decimation value of $N = 3$ corresponds to a bandwidth of $10.24 \text{ MHz}/2^N = 1.25$ MHz. The Nyquist (and filter) bandwidth
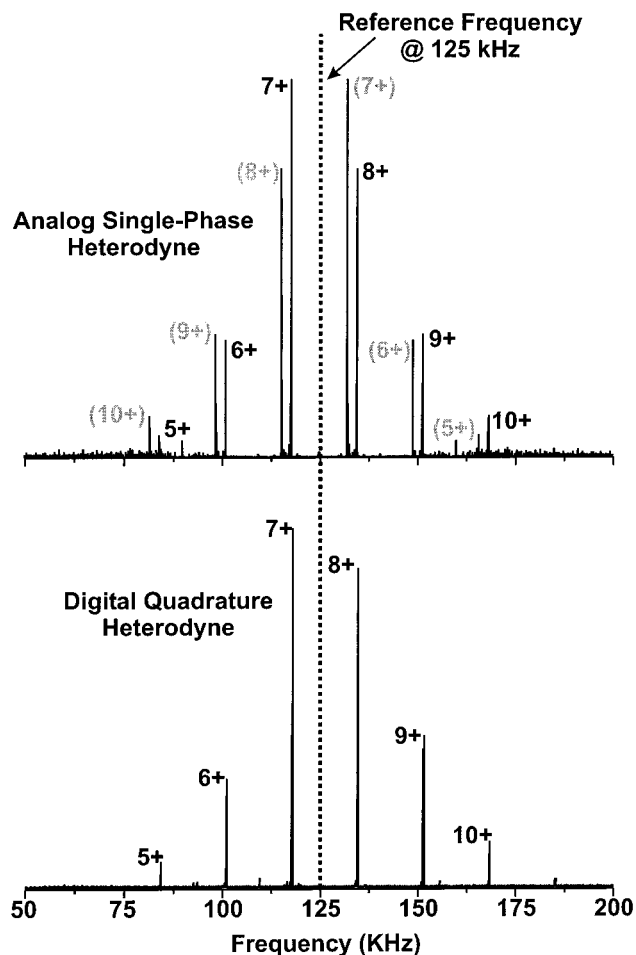
Figure 5. (Top) FT-ICR frequency-domain ICR spectrum of electrosprayed bovine insulin, resulting from analog single-phase heterodyne detection. Image peaks are denoted by gray charge-state labels in parentheses. (Bottom) The same spectrum, obtained by digital quadrature heterodyne, at the same reference frequency—note the elimination of image peaks and $2^{1/2}$ improvement (see next figure) in S/N ratio.

ranges from 10.24 MHz to 0.61 Hz ($N = 0-24$) in multiples of 2. Note that this procedure automatically matches the Nyquist frequency to the digital low-pass filter bandwidth. The final data are formatted in 16-bit (1 word/sample for each channel) or 32-bit (2 word/sample each channel) precision and stored to memory. The standard memory on the HP E1437A is 4 Mword and can be expanded up to 32 16-bit Mword.

Data are transferred from the digitizer over a National Instruments VXI to PCI MXI-2 interface at a rate of 430 Kword/s to the host PC. The (mathematically) complex data set is separated into real (cosine-modulated) and imaginary (sine-modulated) data. The data sets are processed by a complex FFT to give a frequency-domain spectrum. Not only is spectral foldover eliminated but the final spectrum also contains no image peaks[1,9] because the two channels are perfectly balanced in the digital processing. Performance is further enhanced by elimination of noise contributed by the analog heterodyne circuitry. One potential concern with digital processing is the accumulation of roundoff errors during digital processing. Here, those errors are minimized by the high precision of the discrete data (23 bit/word for the ADC and 21 bit/word for the reference sinusoids).
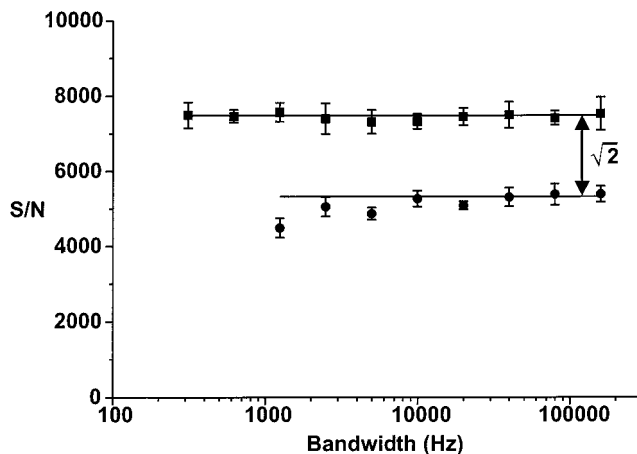
Figure 6. Comparison of S/N ratio vs bandwidth for analog single-phase heterodyne (●) and digital quadrature heterodyne detection (■). Quadrature heterodyne detection produces the same S/N ratio as direct-mode detection (not shown). The S/N ratio for heterodyne single-phase detection is reduced by a factor of $2^{1/2}$ compared to that for quadrature heterodyne or direct-mode detection.

Performance comparisons of (conventional) analog single-phase heterodyne vs digital quadrature heterodyne FT-ICR MS are shown in Figures 5 and 6. Figure 5 shows the effect of signal foldover for an ESI/FT-ICR mass spectrum of bovine ubiquitin plotted in the frequency domain (to make it easier to locate image peaks). Single-phase heterodyne detection at a reference frequency of 125 kHz produced the charge-state distribution in Figure 5 (top). The reference frequency is situated between the ICR frequency for the 7+ and 8+ charge states and therefore leads to folded-over signals denoted by gray charge-state labels in parentheses. (Because the left half of the spectrum is the mirror image of the right half, only half of the spectrum is usually displayed.) Digital quadrature heterodyne detection eliminates signal foldover and yields the true charge-state distribution (Figure 5 (bottom)). Figure 5 also shows the S/N ratio enhancement of digital quadrature heterodyne detection, as documented quantitatively in the next figure.

Figure 6 presents experimental evidence for the S/N ratio advantage of digital quadrature heterodyne over analog single-phase heterodyne detection. To maximize stability in successive data acquisitions, we performed "receive during transmit" detection of the small leakage from a constant-amplitude 117.8-kHz transmitter signal to the detector electrodes. Separate noise spectra were collected by turning the function generator off during data acquisition. The noise level was determined as the average of the baseline noise magnitude[18] from 117.7 to 117.9 kHz (i.e., the segment within which the signal was measured) for each scan.

Direct-mode detection produced a S/N of 7723 ± 306 with MIDAS (with HP E1437A digitizer and real-time digital filtering) compared to 7253 ± 265 for Odyssey (analog filtered) data acquisition (not shown in Figure 6). The slight difference may be attributed to digital vs analog filtering.

In accord with theoretical predictions, the S/N ratio dropped by a factor of $2^{1/2}$ on switching from direct-mode detection to single-phase heterodyne detection (Figure 6, circles). In fact, the S/N loss was somewhat higher than a factor of $2^{1/2}$ at some frequencies, presumably due to mismatch between the Nyquist

frequency and the nearest available analog filter frequency in analog single-phase heterodyne detection. The factor of $2^{1/2}$ is restored on switching from single-phase heterodyne to quadrature heterodyne detection (Figure 6, squares).

The present description, based entirely on postprocessing, has the goal of restoring the S/N ratio for heterodyne detection back to the S/N ratio that would be obtained with direct-mode detection of the same ions. In all of the present discussion, the ICR signal is obtained from the difference in induced charge on a *single* pair of opposed detection electrodes. Such experiments *cannot* distinguish between positive and negative ions of the same mass-to-charge ratio, because ions of either sign will produce the same detected signal. However, positive and negative ions may be distinguished by a related experiment we hereby designate as "physical" quadrature detection on two orthogonal pairs of opposed detection electrodes. Image current induced on one pair of electrodes is digitized to give a time-domain signal stored as mathematically real data. Simultaneously, the other (orthogonal) pair of electrodes generates a second time-domain signal stored as mathematically imaginary data. Complex FFT of the resulting mathematically complex time-domain data yields a frequency-domain spectrum in which ions of opposite sign appear at frequencies of opposite sign. The various relations between the present "computational" quadrature detection and the different (but related) "physical" quadrature detection will be discussed in a future paper.

CONCLUSION

We demonstrate, both theoretically and experimentally, that noise foldover in conventional single-phase heterodyne detection reduces the FT-ICR S/N ratio by a factor of $2^{1/2}$ compared to direct detection. Quadrature heterodyne detection recovers that loss in S/N ratio by eliminating noise foldover. In addition, the elimination of signal foldover simplifies spectral interpretation. By performing quadrature heterodyne detection digitally, we eliminate the dc offset and "image" peak problems associated with analog implementation of quadrature heterodyne detection. Digital filtering further improves performance, by ensuring an optimal match between Nyquist bandwidth and low-pass filter bandwidth.