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EXtended ACquisition Time (EXACT) NMR – A Case for ‘Burst’ Non-Uniform Sampling

Ikenna E. Ndukwe,^[a] Alexandra Shchukina,^{[b],[c]} Krzysztof Kazimierczuk,^[b] Carlos Cobas,^[d] and Craig P. Butts^{*[a]}

Dedication ((optional))

Abstract: A strong case exists for the introduction of burst Non-uniform sampling (NUS) in the direct dimension of NMR spectroscopy experiments. The resulting gaps in the NMR Free Induction Decay can reduce the power demands of long experiments (by switching off broadband decoupling for example) and/or be used to introduce additional pulses (to refocus homonuclear coupling for example). The final EXtended ACquisition Time (EXACT) spectra are accessed by algorithmic reconstruction of the missing data points and can provide higher resolution in the direct dimension than is achievable with existing non-NUS methods.

In Fourier Transform NMR, the achievable resolution is limited by the properties of the measured Free Induction Decay (FID) signal. In particular, nuclear spin relaxation rates and digital resolution of acquired datasets both limit the observed linewidth in the frequency spectrum – the former usually being an intrinsic or extrinsic property of a particular sample, while the latter arises from practical considerations such as experiment times for multi-dimensional NMR, or hardware duty cycle for broadband decoupling methods. Numerous approaches have been developed to address these practical limitations, including pre-transform apodisation functions and reference deconvolution procedures,^[1] linear-prediction of the FID,^[2] frequency- and time-domain deconvolution,^[3] non-Fourier transform methods,^[4] non-uniform sampling (NUS),^[5] and ‘ultrafast’ single scan methods^[6] for the former and low power decoupling waveforms for the latter. In the context of this manuscript, NUS is of especial relevance. NUS has become almost ubiquitous in recent years, employed in the indirect dimension(s) to increase resolution and/or speed-up acquisition of multidimensional NMR spectra. By skipping acquisition of increments in the indirect dimension(s) of NMR spectra, and subsequently reconstructing these with appropriate algorithms,^[5, 7] multi-dimensional datasets can be measured up to orders of magnitude faster with little loss in data quality in

ideal cases. On the other hand, application of NUS in one-dimensional NMR spectra, or the direct dimension of multi-dimensional NMR spectra, has been limited to algorithm testing where the selection of sampled data points is randomized and/or weighted to aid fidelity of the reconstructed data.^[8]

Herein we highlight the opportunities provided by ‘burst’ NUS^[9] in the direct dimension of NMR experiments, where multi-point data sampling (‘data chunks’) is interspersed with periods where the receiver is gated off and all data points thus have zero intensity (‘gaps’) in the FID. The missing data points in these gated gaps can be reconstructed with algorithms analogous to those used for existing NUS applications, such as variants of Compressed Sensing (CS)^[10] or Maximum Entropy (MaxEnt) methods.^[11] Crucially however, these gap periods provide the opportunity to modify experimental outcomes during the FID measurement, such as incorporating homo/heteronuclear decoupling, without perturbing measured data points. Conceptually this is similar to the interleaved acquisition approach used in ‘real-time’ broadband homonuclear decoupling techniques (‘Pureshift’)^[12] where pulses are inserted between successive data chunks during the acquisition. With existing real-time techniques the resulting gaps are excised from the FID, artificial shortening it, causing line-broadening and discontinuity artefacts in the final spectrum. Herein we propose that the missing data point periods *not* be excised, hence the approach is termed EXACT (EXtended ACquisition Time) NMR, but instead are algorithmically reconstructed after acquisition. The advantages of EXACT modification are demonstrated through application to real-time band-selective homonuclear decoupling in the EXACT Pureshift EXSIDE experiment where EXACT gives narrower lineshapes than the equivalent non-EXACT approach, and also to the EXACT HSQC where >1 second acquisition times are now achievable without risk of sample heating or damage to the NMR probe or electronics caused by broadband heteronuclear decoupling.

The EXACT concept is illustrated for a simple ¹³C{¹H} pulse-acquire sequence (Figure 1a) comprising the acquisition of chunks of data points interspersed with receiver-gated gaps (Δ) where the intervening data points are stored with zero intensity in the FID. This sequence was acquired and the resulting 50% burst-sampled FID (Figure 1b, acquired with data chunk length of 0.0160 s and Δ of 0.0160 s each) was Fourier transformed to give the expected artefactual spectrum (Figure 1c) with symmetrical intraband aliases^[13] around each peak. The intraband aliases appear at positions whose bandwidth (31.25 Hz) from the causal peaks are inverse ratios of the time interval between the middle points of adjacent chunks in the EXACT FID (0.032 seconds). The magnitudes of these aliases depends inversely on the relative sizes of the chunks and gaps within the respective time intervals (see SI for details).

[a] Mr I. E. Ndukwe and Dr C. P. Butts,
School of Chemistry
University of Bristol

Cantocks Close, Bristol, BS8 1TS, UK
E-mail: Craig.Butts@bris.ac.uk

[b] Dr A. Shchukina and Dr K. Kazimierczuk
Centre of New Technologies
University of Warsaw

Banacha 2C, 02089, Warszawa, Poland

[c] Dr A. Shchukina
Institute for Spectroscopy
Russian Academy of Sciences
Fizicheskaya 5, 142190, Moscow, Troitsk, Russia

[d] Dr Carlos Cobas
Mestrelab Research S.L.
Feliciano Barrera 9B – Bajo, 15706 Santiago de Compostela, Spain

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Figure 1. (a) Burst-sampled ^{13}C $\{^1\text{H}\}$ sequence schematic, $d1$ =relaxation delay (b) Experimental burst-sampled $^{13}\text{C}\{^1\text{H}\}$ FID for strychnine, $\Delta = 0.016$ s, chunk duration = 0.016 s (c) corresponding Fourier transformed spectrum with insets of zoomed regions of interest (d) corresponding IST-reconstructed spectrum with insets of the same regions.

The detailed phase and lineshapes of the aliases can be predicted from the burst-sampled schema (Figure 2a) analogous to that used in Figure 1b. Fourier transformation of this burst-sampled schema gives rise to the point spread function (PSF) shown in Figure 2b. Convolution of the PSF with a perfect Lorentzian peak gives the predicted aliased spectrum for each resonance which matches the peak lineshapes in the Fourier transformed spectra (Figure 1c) of the experimental EXACT ^{13}C FID shown in Figure 1b (more details in SI).

The desired spectrum can, in principle, be generated from the burst-sampled NUS data by reconstruction of the missing data points with algorithms such as Compressed Sensing (CS) or Maximum Entropy (MaxEnt). Among many algorithms of CS, iterative soft thresholding (IST) is known to be fast and effective for NMR data.^[10a, 10b] IST starts from a frequency domain spectrum convolved with the PSF and attempts to deconvolve the artefact pattern by an optimisation process where the highest peaks are iteratively thresholded until the residuals are below a user-defined threshold (L^2 -norm). The task is relatively easy in the case of random NUS, when noise-like artefacts are evenly distributed, but is more problematic when regularity of sampling is introduced which is the case here for burst-sampled data.

The IST reconstruction of the EXACT ^{13}C spectrum is shown in Figure 1d. All of the strychnine resonances are accurately reconstructed. However, the 1:1:1 triplet of the CDCl_3 solvent peak at 77 ppm still shows residual artefacts in the reconstructed spectrum (Figure 1d inset) as the triplet peaks are 32 Hz apart ($^1J_{\text{CD}}$) which nearly matches the aliasing bandwidth (31.25 Hz) causing coincidental overlap of peaks with aliases from adjacent resonances in the convoluted point spread

function. The resulting intensities of real peaks are thus reduced or modulated by this overlap. As IST relies on initial iterative peak-searching (as do most NUS reconstruction methods), the cancellation/modulation of peak intensities from coincidental overlap of aliases and real peaks makes it challenging to reconstruct the spectrum with existing IST algorithms. This highlights a limitation of such approaches, especially with regularly sampled data such as that shown in Figure 1b, but this coincidental overlap of aliased and real peaks can be reduced experimentally by partial randomization of the chunk and gap periods in the burst-sampled schema and/or increasing the sampling density.^[14] Consequently in subsequent applications reported here, more randomised burst-sampled FIDs are used to minimise such issues.

The acquisition of EXACT FIDs provides an opportunity to manipulate the underlying NMR experimental methods in potentially valuable ways. For example, EXACT acquisition allows one to turn off heteronuclear decoupling during the Δ delays, and thus reduce the high duty cycles in broadband X-decoupled NMR methods e.g. HSQC. Broadband heteronuclear decoupling, routinely applied over bandwidths of tens of thousands of Hz, e.g. >25 kHz for ^{13}C decoupling over >200 ppm on a 500 MHz NMR spectrometer, puts high power demands on the spectrometer. This brings a high risk of sample over-heating and probe damage which thus normally limits the length of acquisition times for which heteronuclear-decoupled HSQC FIDs are acquired – typically <500 ms for broadband ^{13}C -decoupling. With EXACT acquisition however, the continuous ^{13}C -decoupling waveforms can be eliminated during the Δ delays and replaced with a pair of 180° ^{13}C pulses (Figure 3a) placed symmetrically within the Δ period. In this example, the first pulse is placed just after the gating-off of the receiver and ^{13}C -decoupling, with the second pulse applied after $\Delta/2$, as shown in Figure 3a. These ^{13}C pulses effect refocussing of heteronuclear coupling but do not perturb ^1H chemical shift evolution between data chunks, meaning the acquired (^1H) data chunks in the EXACT HSQC match those which would be acquired in a standard continuously ^{13}C -decoupled HSQC FID.

Figure 2. The computed spectrum (far right) of a singlet resonance arising from convolution of a perfect Lorentzian peak with the point spread function of a burst sampling schema analogous to that used in Figure 1b.

Figure 3. (a) EXACT HSQC pulse sequence (b) Standard ^{13}C -decoupled HSQC spectrum of strychnine, 400ms t_2 acquisition period (c) IST Reconstructed EXACT HSQC spectrum of strychnine, 800ms t_2 acquisition period. Insets in (b) and (c) show zooms of correlations to carbons at 60–65ppm. Full details of all parameters used can be found in the SI.

To demonstrate this, an EXACT-HSQC was acquired with a 800ms second FID (8000 data points over a 10 kHz ^1H spectrum width), 55% sampled in F_2 (4400 acquired data points) and a 1 second relaxation delay between 4 scans and 96 t_1 increments. This was acquired using the pulse sequence shown in Figure 3a where the chunk size was varied within the FID from 15–88 ms and with gaps of $\Delta=22$ ms or 25 ms between data chunks. The resulting EXACT HSQC FID is shown in Figure S8. IST Reconstruction of the FID gave a spectrum (Figure 3c) which matches well with a standard HSQC spectrum acquired with a fully sampled 400ms F_2 FID (4000 data points, Figure 3b). Crucially, the EXACT HSQC data has comparable numbers of acquired (and continuously decoupled) F_2 data points to the HSQC sequence (4400 vs 4000), but spread over longer F_2 acquisition times (800 ms vs 400 ms). The EXACT acquisition imposes lower power demands on the hardware— duty cycles are ~24% and ~29% respectively with equal relaxation delays – so does not raise the possibility of sample heating. The contour lineshapes of the 800 ms EXACT HSQC also compare well with the 400 ms standard HSQC (see insets in Figures 3c and 3b). In fact, we have successfully conducted HSQC measurements with acquisition times as long as 1.5 seconds, using longer inter-chunk delays ($\Delta \approx 60$ ms), with no sign of sample heating or power-handling concerns on the NMR instrument, however there was no tangible resolution benefit to measuring data in F_2 beyond 1 second as T_2 relaxation in this experiment becomes limiting. It should be noted that detailed inspection of the EXACT HSQC F_2 lineshapes does show slight reconstruction artefacts and phase distortions within the peaks which currently mask the highest resolution details of the lineshapes enabled by the longer F_2 acquisition time. These artefacts become more pronounced at higher levels of sparsity or with decreased randomness of the sampling schemes and work to optimise algorithms and acquisition schema are in progress.

EXACT acquisition can be used to obtain substantial linewidth improvement in HOBS-style (HOBS - HOModecoupled Band-Selective, also known as ‘BASH’ - Band-selective homonuclear)^[12a, 12b, 15] decoupled spectra such as our recently reported Pureshift EXSIDE experiment.^[16] These ‘real-time’ homodecoupling experiments employ band-selective ^1H J -refocussing pulse elements, interleaved between data chunks,

to effect the desired decoupling of a region-selective spectrum from all nuclei outside that region. However, the excision of these J -refocussing periods from the FID artificially shortens the FID and introduces stepped reductions in FID amplitude between the stitched chunks caused by spin relaxation and/or B_1 inhomogeneities during these excised periods. This artificial shortening of the FID means that existing HOBS-style methods, including the Pureshift EXSIDE, give singlets with linewidths typically >3Hz. For example, in our recent report of Pureshift EXSIDE,^[16] a 15Hz-wide ^1H multiplet in F_2 of an EXSIDE^[17] spectrum (Figure 4b) was collapsed to 4Hz (Figure 4c) by incorporating a HOBS-style band-selective decoupling during acquisition of the FID. The resulting reduction in linewidth gave a corresponding increase in sensitivity, however this linewidth was limited to 4Hz by the artificial shortening of the FID from excision of the J -refocussing periods. An EXACT acquisition, on the other hand, retains the data points during the J -refocussing periods, keeping the full length of the FID, avoiding artificial shortening of the FID and so should reduce line broadening in HOBS-style spectra.

We demonstrate the EXACT modification to HOBS by incorporation into the Pureshift EXSIDE. The EXACT Pureshift EXSIDE pulse sequence (Figure 4a) comprises an initial EXSIDE sequence, with the EXACT acquisition block comprising data chunks interspersed with J -refocussing periods ($\Delta \approx 0.012$ s) where band-selective inversion pulses are applied to all passive (unobserved) spins during the J -refocussing periods while leaving the active (observed) spin untouched. This refocuses homonuclear coupling evolution to the passive spins, but allows chemical shift evolution of the active spin throughout the J -refocussing period. The resulting IST-reconstructed spectrum (Figure 4d) shows the anticipated narrowing of the homodecoupled F_2 -linewidths to 2.3 Hz, approximately half of the value achieved with the non-EXACT Pureshift EXSIDE. In line with previous reports for real-time HOBS-style Pureshift methods, the actual linewidth observed is affected by the choice of acquisition chunk length (ideally $<1/(3^n J_{\text{HH}})$), the length of the J -refocussing period and the nature of the inversion pulse applied, however in all conditions we have tested, we find that the EXACT Pureshift EXSIDE linewidths are ~50% of the corresponding non-EXACT linewidths.

Figure 4. (a) EXACT Pureshift EXSIDE pulse sequence (b) EXSIDE spectrum of H22 of strychnine (linewidth ≈ 15.5 Hz). (c) Pureshift EXSIDE spectrum of H22 of strychnine (linewidth ≈ 4.5 Hz), (d) EXACT Pureshift EXSIDE spectrum of H22 of strychnine (linewidth ≈ 2.3 Hz). Full details of all parameters used can be found in the SI.

In summary, EXACT NMR removes current limitations on the length of FIDs in experiments such as HSQC and HOBS-style decoupling experiments. In the former, extending the F_2 acquisition times of broadband-decoupled FIDs potentially allows measurement of smaller and more precise $^nJ_{\text{HH}}$ – a valuable parameter for stereochemical analysis of organic compounds – without danger to NMR hardware or the samples being studied. In the latter, band-selective J -refocussing elements can be introduced into the acquisition period without the need for data point excision from the acquired FID, affording much narrower F_2 lineshapes than existing methods. The EXACT method is not limited to just the techniques described here, but is restricted to techniques which allow unperturbed chemical shift evolution of active spins during the FID gaps *i.e.* pulses cannot be applied to the active spins during this period in the method as outlined here. The burst-sampled FIDs that the EXACT method produces are also not as readily reconstructed with existing reconstruction algorithms as traditional NUS data. As a result, we have limited the sampling densities and randomised sampling schemes to conservative ranges compared to existing NUS applications in F_1 of NMR spectra, but we anticipate that this will improve with more discriminating data processing algorithms.

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Author(s), Corresponding Author(s)*

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NMR – A Case for 'Burst' Non-
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Just to be EXACT: Burst non-uniform sampling in the direct dimension of some NMR experiments, followed by algorithmic reconstruction of the missing data points, allows Extended Acquisition Times (EXACT) to provide higher resolution NMR data than is achievable for existing methods.