

Aliasing

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Problem:

I have been approached many times by graduate students and post-docs with some variation of the following question:

“In my HMBC there is this strange peak around -5 ppm. There are no carbons at that chemical shift in my 1D ^{13}C . Also, I know I have a carbonyl in my molecule. There is a peak around 210 ppm in my 1D ^{13}C . I don't see any cross-peaks to it in my HMBC. What is wrong with the HMBC?”

Answer:

This issue, called “aliasing”, is well known in multidimensional NMR spectroscopy. The core issue to understand is what happens to peaks outside the spectral window. Recall that the spectral window is defined by 2 parameters: the carrier frequency (also called the “offset”), which determines the center of the spectral window, and the sweep width, which determines the size of the window. As an example, Fig. 1 shows a 1D ^{13}C NMR spectrum (with ^1H decoupling) of 1% 3-heptanone in CDCl_3 with two different spectral windows. In blue, the carrier frequency (called “o1p” in the Bruker software) equals 105 ppm and the sweep width (called “sw”) equals 230. These parameters result in a spectral window from 220 to -10 ppm. Note the presence of the downfield carbonyl peak at ~212 ppm. In red, the carrier frequency equals 95 ppm and the spectral width equals 210 ppm. These parameters result in a spectral window from 200 to -10 ppm. The carbonyl peak is not present in this spectrum. Hence, for 1D NMR spectroscopy, resonances outside the spectral window are not observed in the spectrum. This behavior is in line with the expectations of people familiar with other types of spectroscopy, for example UV-Vis spectroscopy.

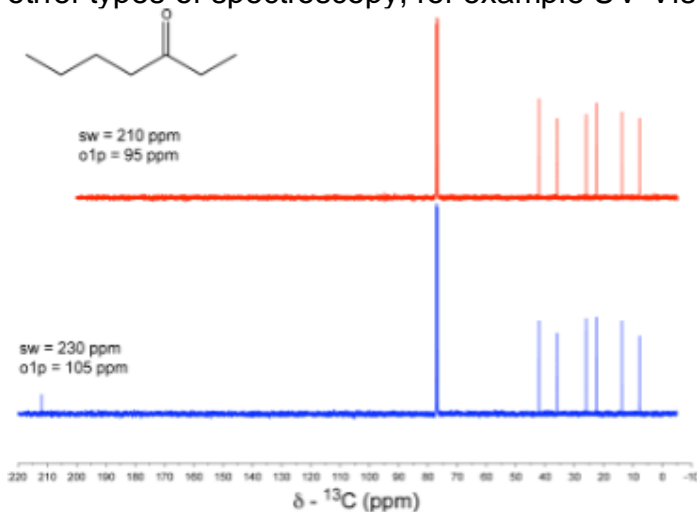


Figure 1. Effect of sweep width and offset on 1D ^{13}C NMR spectra with ^1H decoupling of 1% 3-heptanone in CDCl_3 : Bottom (blue) – sweep width = 230 ppm and offset = 105 ppm, resulting in a spectral window of -10 to 220 ppm; Top (red) – sweep width = 210 ppm and offset = 95 ppm, resulting in a spectral window of -10 to 200 ppm. Molecular structure of 3-heptanone inset.

The behavior of resonances outside the spectral window in the indirectly acquired dimension in multidimensional NMR, on the other hand, is very different (see FAQ on Multidimensional NMR for details about the acquisition of 2D data). Fig. 2 shows a 2D ^1H - ^{13}C HMBC of 1% 3-heptanone with different spectral windows in the indirectly acquired dimension. In the blue spectrum, the carrier frequency equals 105 ppm and the spectral width equals 230 ppm. The spectral window is large enough to encompass all ^1H and ^{13}C correlations and cross peaks between the carbonyl carbon and alkyl protons are present at the bottom of the figure. In the red spectrum, the carrier frequency equals 95 ppm and the spectral width equals 210 ppm. Note that there are no cross peaks to the carbonyl carbon at 212.2 ppm in the ^{13}C dimension because the peak is outside the spectral window. However, note that there are additional peaks at 2.2 ppm in the ^{13}C dimension. We know that no carbon in the molecule has this chemical shift (see Fig. 1). So why do we see cross peaks?

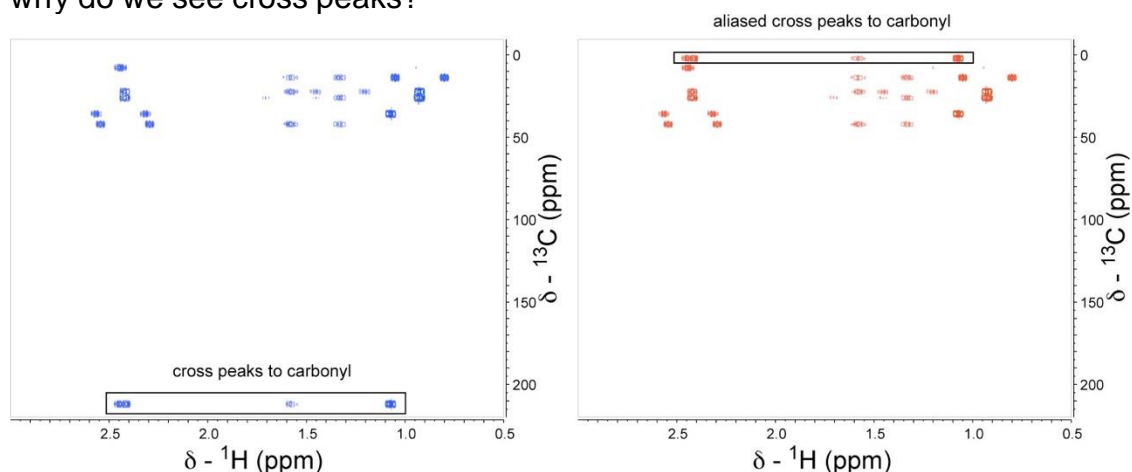


Figure 2. Effect of spectral width and offset on indirectly acquired dimension of 2D ^1H - ^{13}C HMBC spectra of 1% 3-heptanone in CDCl_3 : Left (blue) spectral width = 230 ppm and offset = 205 ppm; Right (red) spectral width = 210 and offset = 95 ppm. Cross peaks between alkyl ^1H and carbonyl ^{13}C denoted by box and annotation.

The answer is these peaks arise due to “aliasing” of the resonance outside the spectral window of the indirectly acquired dimension. Aliasing results in the appearance of new peaks at a frequency equal to the chemical shift of the unaliased peak minus the spectral width. For the aliased crosspeaks in Fig. 2, the observed chemical shift, 2.2 ppm, matches well with the value calculated from this formula, $212.2 - 210$ ppm.

Why does peaks outside spectral window get aliased (advanced topic)?

In NMR spectroscopy, the response of sample to the pulse or series of pulses is a sum of exponentially decaying sinusoids (e.g. sines or cosines). The Fourier Transform (see FAQ on Fourier Transform) untangles this complex signal into an NMR spectrum with frequency (usually displayed as Chemical Shift in units of ppm) on the x-axis. It is important to understand that the data acquired on the

spectrometer is a sum of exponentially decaying sinusoids with time (in units of seconds) on the x-axis. These sinusoids oscillate at frequencies (measured in Hz) relative to the frequency of the carrier. These oscillations are recorded as a digital, rather than analog, signal. In other words, the signal is sampled at a certain number of discrete data points. The time between these data point is called the dwell time. The dwell time defines the maximum and minimum frequency (aka the sweep width) that can be recorded digitally according the formula

$$\text{sweep width} = \text{dwell time}^{-1} \quad \text{Equation 1}$$

known as the Nyquist theorem. The Nyquist theorem a consequence of the fact that it required two data points per oscillation to define a sinusoid. Signals with frequencies outside the sweep width are sampled improperly and appear at a frequency equal to their unaliased frequency minus the sweep width.

Figure 3 describes this effect pictorially. The real and imaginary components of an NMR signal with a frequency of 1 Hz and no relaxation is depicted as a solid line in Fig. 3A (see FAQ on Quadrature Detection for details on real and imaginary components). Circles represent discretely sampled data points. In other words, the actual data acquired on the instrument would be a list of x and y with x being the time for each data point and y being the amplitude at time x. In this case the dwell time (time between data points) equals 167 ms, so the sweep width equals 6 Hz (1/0.167 s). For this sweep width the spectral window ranges from -3 Hz to 3 Hz.

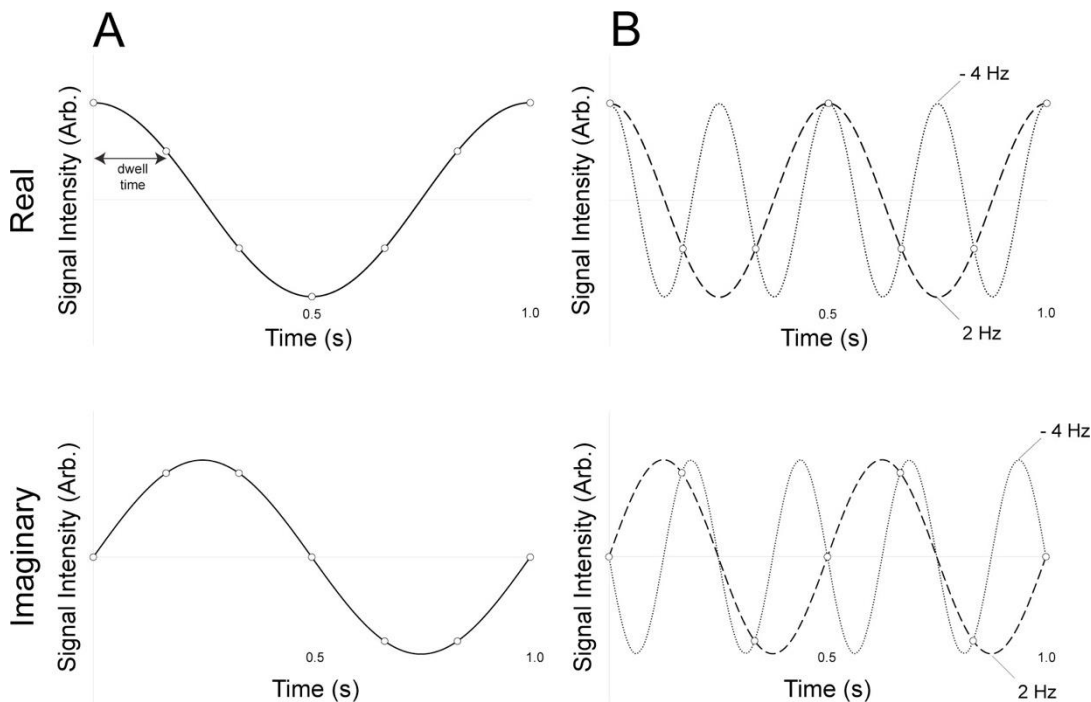


Figure 3. Real (top) and imaginary (bottom) channel for NMR signal with frequency of 1 Hz (A, solid line), 2 (B, long dash) and -4 Hz (B, short dash). Data points for discrete acquisition with dwell time equal 167 ms (sweep width equals 6 Hz) indicated by open circle.

The real and imaginary components of an NMR signal with a frequencies of 2 and -4 Hz are depicted with long and short dashed line, respectively, in Fig. 3B. Circles represent discretely sampled data points for a dwell time of 167 ms (sweep width of 6 Hz). Note that the 2 Hz signal is properly digitized. After the Fourier Transform this signal would appear in the spectrum at a frequency of 2 Hz. Based on the dwell time, we would expect the signal at -4 Hz to be aliased. To understand why this signal is aliased, look at the value of the -4 Hz signal at each data points. The amplitude of the -4 Hz and 2 Hz signal are equal at all discretely sampled data points. For instance, at time equals 500 ms, the amplitude of both the 2 and -4 Hz signal is at it's maximum on the real channel and zero on the imaginary channel. Note that the amplitude for the sinusoids with frequencies 2 and -4 Hz are equal at every discretely sampled data point.

One may wonder why signals outside the spectral windows of indirectly acquired dimensions in 2D spectra (aka the y-dimension of the 2D) are aliased (Fig. 2), whereas 1D spectra are not aliased (Fig. 1)? The answer is that modern NMR spectrometers are built with digital filters that eliminate any signals outside the spectral window in the directly acquired dimension to avoid having noise aliased into the spectrum. In other words, 1D NMR is an exception to the rules about aliasing (i.e. aliased frequency = unaliased frequency – spectral width) we learned in this section!

How do I avoid aliasing:

At the KU NMR core lab we have set default parameters for two dimensional experiments to maximize sensitivity and resolution of organic compounds. Some molecules have nuclei with chemical shifts outside the common range. These signals might be aliased in the multidimensional experiments. There is no reason to fret, though. Because the underlying causes of aliasing are well-understood and the effects of aliasing are predictable, it is perfectly acceptable to use an aliased spectrum for spectral assignment or even publish papers with aliased spectra! In protein NMR it is common to alias signals intentionally in order to increase resolution while decreasing acquisition time. If you are insistent that you cannot analyze an aliased spectrum, the only option is to re-record with an appropriate spectral window.