5. Experimental Content

1) Understand the basic physical theory and experimental configuration of a PNMR system. Learn to explain related physical phenomena in PNMR using classical vector model.
2) Learn to use signals of spin echo (SE) and free induction decay (FID) to measure T_2 (spin-spin relaxation time). Analyze the influence of magnetic field homogeneity on NMR signal.
3) Learn to measure T_1 (spin-lattice relaxation time) using reverse recovery.
4) Qualitatively understand the relaxation mechanism, observe the effect of paramagnetic ions on nuclear relaxation time.
5) Measure T_2 of copper sulfate solution at different concentrations. Determine the relationship of T_2 with the change of concentration.
6) Measure the relative chemical displacement of the sample.

6. Experimental Procedure

1) Apparatus connection

For the RF Transmitting Unit (on the back panel):

Use the WHITE (**note: must be the white serial cable**) 9-core serial cable to connect the "Signal" port to the serial port of the computer. Use the 2-core cable to connect "Mod Field" port to the "Mod Field" port of the constant temperature unit. Connect the "Amplifier Power" port to "Amplifier Power" port of the constant temperature unit using the 5-core cable. Connect the "RF Signal (O)" port to the "RF Signal (I) port of the constant temperature unit using the lockable BNC cable. Finally plug in the power cord.

For the Signal Receiving Unit (on the back panel):

Use the BLACK (**note: must be the black serial cable**) 9-core serial cable to connect the "Cont. Temp." port to the "Cont. Temp" port of the constant temperature unit. Use the 4-core cable to connect "Heater Power" port to the "Heater Power" port of the constant temperature unit. Use a BNC cable to connect "Pre-Amp (I)" port to the "Pre-Amp (O)" port of the constant temperature unit. Use the BNC to audio converting cable to connect the "Resonance Signal" port to the audio/microphone socket of the computer. Plug in the power cord.

2) Warm-up the apparatus

Turn on the power switches of the two electric units. The current electromagnet temperature is displayed on the Constant Temperature Unit, which is generally equivalent to the local indoor temperature at that time. The temperature increases after a period of time, which shows that the heater is working. After 3 - 4 hours (the time may be different at different seasons or different locations), the temperature will be controlled and stable at 36.50 °C. (Sometimes it changes between 36.44 °C and 36.56 °C, which is normal).

Open the acquisition software, click the "Continuous Acquisition" button, the computer controls the RF signal. The frequency is generally 20.000 MHz. The initial values are generally set at: pulse interval 10 ms, first pulse width 0.16 ms, second pulse width 0.36 ms. Then carefully adjust "Mod Field" amplitude (on the RF Transmitting Unit) to change the magnetic

field with a small range. When it is adjusted to an appropriate value, the FID signal can be observed in the acquisition software interface (spin echo signal can also be observed when adjusted properly), then adjust the "Cont. Field" (for magnetic field uniformity") on the receiving unit, the change of the tail wave of FID signal can observed.

3) Measurement of apparent lateral relaxation time T_2^*

Adjust the pulse interval to the maximum (60 ms), and adjust the second pulse width to 0 ms. Only the first pulse is kept, carefully adjust the "Mod Field" and "Const Field" using both Coarse and Fine knobs, and adjust the first pulse width within a small range (adjust it around 0.16 ms) to maximize the coda wave. Use the software to measure the apparent lateral relaxation time T_2^* through exponential fitting. Change different samples (such as glycerine samples, oil samples, etc.) for comparison and record their values.

4) Measurement of lateral relaxation time T_2 with spin echo (SE signal)

On the basis of the previous step, find the pulse width of 90° pulses (as the first pulse). Adjust the pulse interval to 10 ms, and adjust the second pulse width to be twice the first pulse width (*for the actual apparatus, it is not exactly twice width relationship*) as the 180° pulse, carefully adjust the "Mod Field" and "Const Field" using both Coarse and Fine knobs to maximize the spin echo signal.

Using the software to measure the echo signal magnitudes at different pulse intervals, do exponential fitting to obtain the lateral relaxation time T_2, which is compared with the apparent lateral relaxation time T_2^* to analyze the effect of magnetic field uniformity on the lateral relaxation time. Change different samples for comparison.

5) Measurement of lateral relaxation time of hydrogen nuclei in copper sulfate solutions of different concentrations and analyze the relationship between the relaxation time and the concentration change. (Optional)

The measurement process is the same as the previous step. Measure the transverse relaxation times of five different concentrations of copper sulfate solutions. Use fitting method to find their relationship.

6) Learn to measure the longitudinal relaxation time with the inversion recovery method

The inversion recovery method is to use the 180° - delay - 90° pulse sequence to measure the longitudinal relaxation time T_1. The method is similar to the spin echo method. Adjust the first pulse as the 180° pulse, adjust the second pulse as 90° pulse, change the pulse interval (delay) to measure the amplitudes of the coda waves at different pulse intervals. Do curve fitting to get the longitudinal relaxation time T_1.

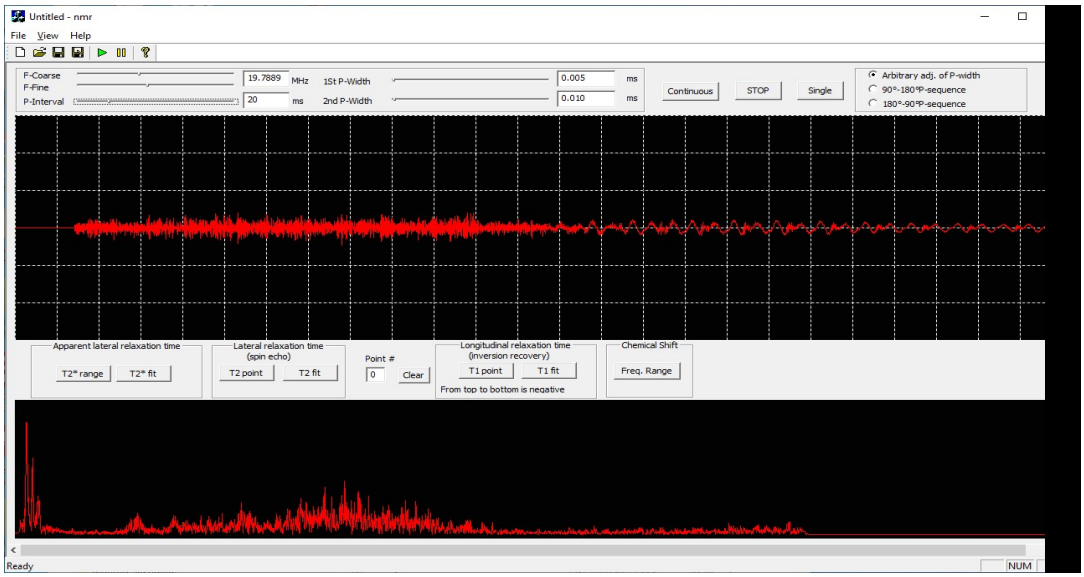
7) Measurement of relative chemical shift of the sample

On the basis of achieving the FID signal of glycerine, replace it with the xylene sample. Use the software to analyze the relative chemical shift of xylene (the frequency difference between the two peaks of the xylene spectrum is about 100 Hz).

7. Installation and Use of Software

7.1. Software Installation

Usually, you only need to copy the application file folder “LEAI-13 PNMR V3.0” to your computer, and double click the file “nmr.exe” to run it. If the software cannot be opened directly in some computers, first copy the mscomm32.ocx file to the folder WINDOWS-SYSTEM32 of the system disk (usually Disk C) of the computer. Then, type regsvr32 mscomm32.ocx in the application “Run under Start menu” and press Enter.



7.2. Cable Connections

For the RF Transmitting Unit (on the back panel):

Use the WHITE (**note: must be the white serial cable**) 9-pin serial cable to connect the “Signal” port to the serial port of the computer, which controls the DDS signal generator to send out pulse signals. Use the 2-pin cable to connect “Mod Field” port to the “Mod Field” port of the constant temperature unit. Connect the "Amplifier Power” port to “Amplifier Power” port of the constant temperature unit using the 5-pin cable. Connect the “RF Signal (O)” port to the “RF Signal (I) port of the constant temperature unit using the lockable BNC cable. Finally plug in the power cord.

For the Signal Receiving Unit (on the back panel):

Use the BLACK (**note: must be the black serial cable**) 9-pin serial cable to connect the “Cont. Temp.” port to the “Cont. Temp” port of the constant temperature unit. Use the 4-pin cable to connect “Heater Power” port to the “Heater Power” port of the constant temperature unit. Use a BNC cable to connect “Pre-Amp (I)” port to the “Pre-Amp (O)” port of the constant temperature unit. Use the BNC to audio converting cable to connect the "Resonance Signal” port to the audio/microphone socket of the computer. This cable sends the FID signal and the spin echo signal to the computer. Plug in the power cord.

If using a RS232 to USB adapter cable, please firstly run the driver installation program in the CD directory\USB to 232 driver\PL2303 Windows Driver\PL2303_Prolific_Driver Installer_v1.12.0.exe, and then connect the USB plug head to the computer.

Before running the software, the serial communication port must be set to COM1 by following these steps: Right click on Strat → Device Manager → Prolife USB-to-Serial Comm Port (Com#). If Com # is 1, stop here. If Com# is not 1, then right click on USB-to-Serial Comm Port (Com#) → Properties → Port Settings → Advanced →Comm Port Number, on the Comm# list, select Com 1, then click OK.

7.3. Use of Software

1) Configuration of Microphone:

After the BNC to audio converting cable is connected the "Resonance Signal" port of the Signal Receiving unit with the audio/microphone socket of the computer, the computer will install the driver automatically.

After the driver is installed successfully, please set the 5-USB Sound Device as the default (Right click on Strat → Settings → System → Sound) and make sure that the microphone device is turned on (i.e. in recording state, not muted). Set the volume (i.e. signal amplitude) of this device to a medium value (e.g. 50) by clicking Properties (P).

2) Signal Acquisition

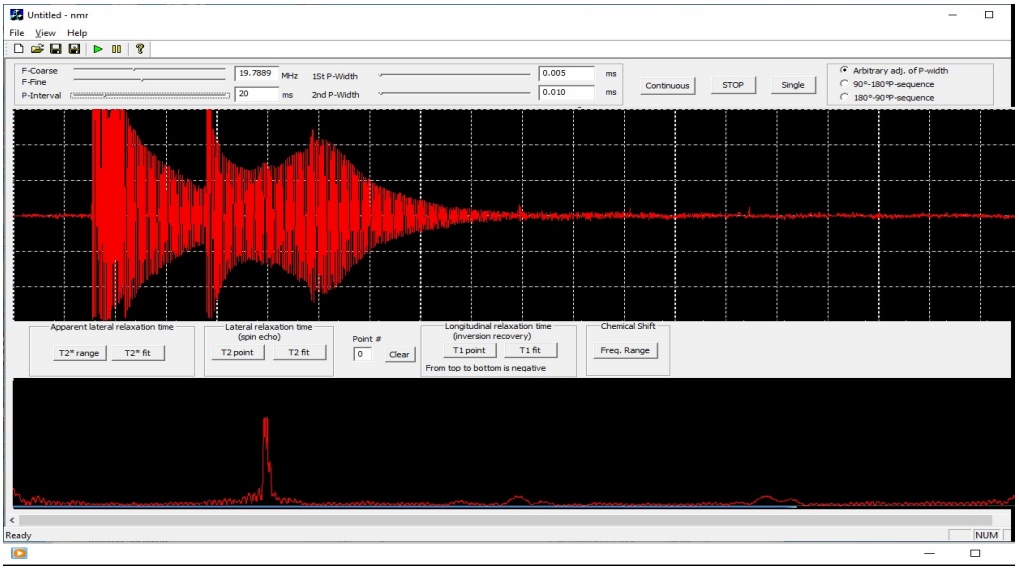
a) Click "**Continuous"** (i.e. Continuous Acquisition) button on the program panel.

b) Set **Resonance Frequency** to **20 MHz** (use both "**F-Coarse**" (coarse) and "**F-Fine**" (fine) adjustment bars). It is the resonance frequency of hydrogen nuclei at the constant magnetic field.

c) Set the values of the **"1st P-Width" (First Pulse Width)** and the **"2nd P-Width" (Second Pulse Width)** to the reference values given on the electro-magnet unit. (you can also adjust these values according to your will) to obtain the proper FID signal and the spin echo signal (usually through 90° - τ - 180° pulse sequence to observe the spin echo signal).

d) In addition, the "Cont. Field" (for magnetic field uniformity") on the receiving unit can be finely adjusted to maximize the 90° pulse coda signal.

e) After the signal is acquired, the pattern will be displayed on the panel.



To find the pulse width (duration) for acquiring the optimal 90° and 180° pluse sequence, the following steps can be tried:

There are 3 modes for the adjustment of 1st and 2nd pulse widths: (1) Arbitrary adjustment (both pulse widths can be adjusted arbitrary). (2) 90° - 180° pulse sequence (1st pulse width arbitrary adjustable, the 2nd pulse width changes with the 1st pulse at 2 times width) and (3) 180° - 90° pulse sequence (2nd pulse width arbitrary adjustable, the 1st pulse width changes with the 2nd pulse at 2 times width).

Under "**Arbitrary adj. of P-width**" mode, start with the width of both the 1st and 2nd pulses set to 0 (i.e. pulse off), slowly increase the 1st pulse width and observe the effect on the signal. Pulse width determines the time allowed for the RF to rotate the vector μ . The longer the RF is on, the farther the vector rotation.

As the pulse width increases, you will notice that the initial height of the FID signal rises to a maximum, indicating a 90° pulse. At this time, the corresponding pulse width is the optimal 90° pulse width.

Then, increase the pulse width further, the FID signal decreases to close to 0 (i.e. the minimum). At this time, the corresponding pulse width is the optimal 180° pulse width (it is about twice the 90° pulse width).

Under a 180° rotation, there is no x-y magnetization and thus no FID signal. Continuing to increase the pulse time shows the signal increase again to repeatthe above effects.

3) Measurement of apparent lateral relaxation time T_2^*

a) Under "**Continuous"** acquisition mode, adjust parameters to achieve proper FID signal and spin echo signal (see Step 2).

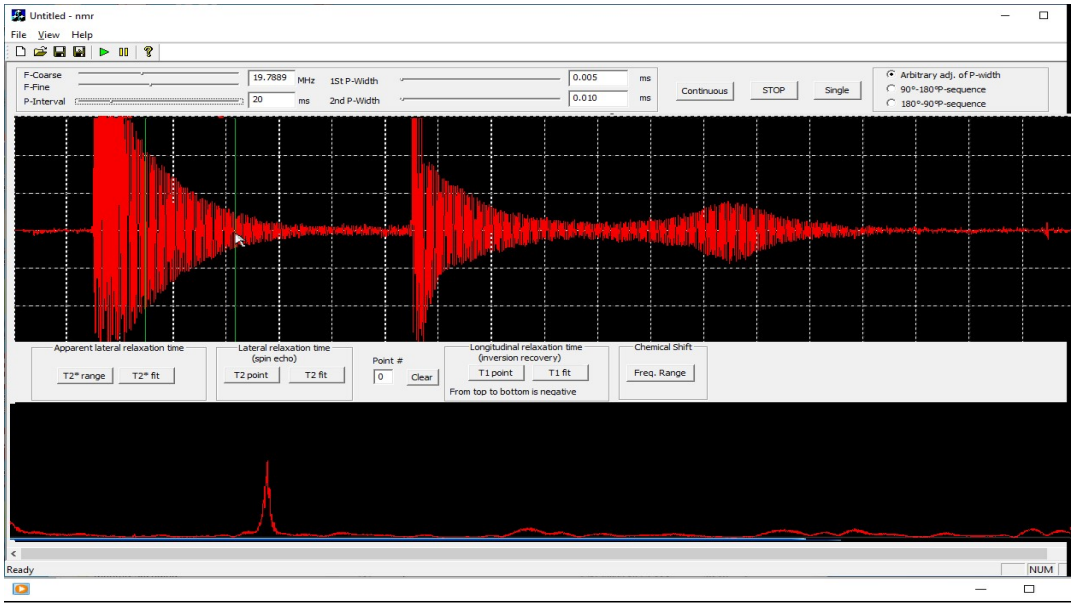
b) Adjust **"P- Interval"** to a suitable value, for example, greater than 25 ms.

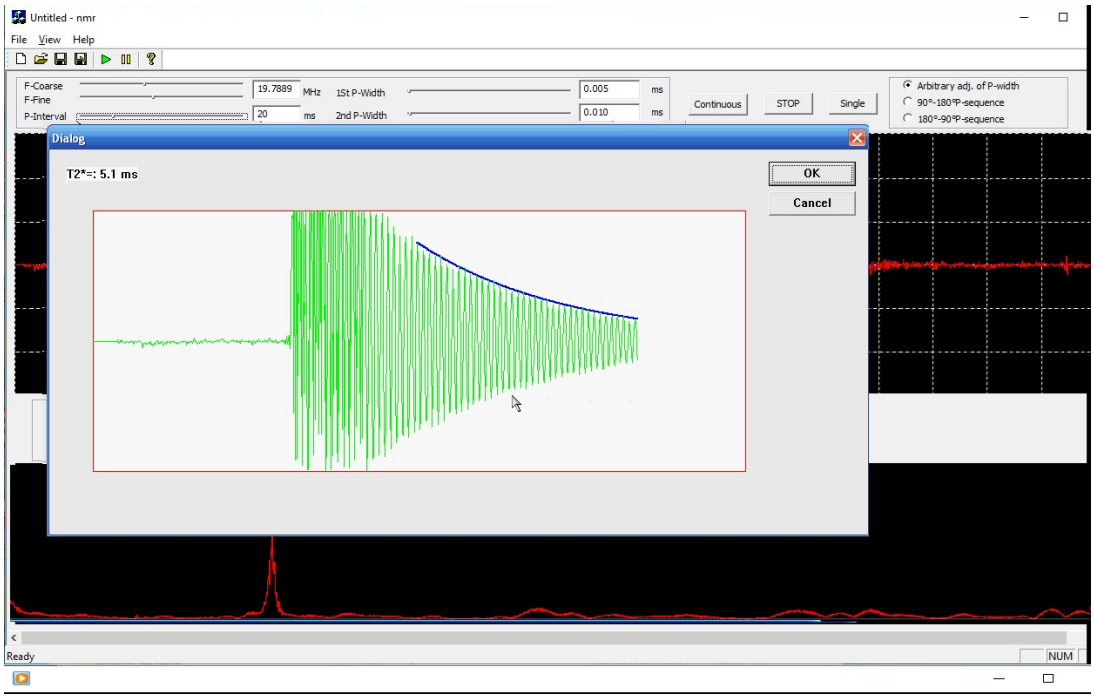
c) Click the "**Stop**" button.

d) Click the "**T_2* Range**" button in the frame of "**Apparent Lateral Relaxation Time**".

e) Select an appropriate range (i.e. a segment) of the 90° pulse coda (FID signal) by firstly moving the mouse to the start point of the interested segment, left-click there and hold (a vertical green line marks the start point), then moving the mouse to the end point of the segment and release the mouse there (a vertical green line marks the end point there).

f) Then click "**T_2* Fit**" to get the apparent transverse relaxation time T_2*. (The software automatically do exponential fitting).





Theory of measurement of apparent lateral relaxation time T_2^*

After the impact of a 90° pulse, the characteristic time for the spins to lose a non-thermal equilibrium x-y magnetization is called the *lateral relaxation time* T_2. If the external magnetic field across the sample is not perfectly homogenous, spins in different physical locations will precess at different rates. This means that the precession of the individual spins is no longer in phase. Over time, the phase difference between the precessions of the individual nuclei increases and the magnitude of the FID signal decreases. The time for this loss of signal, which is not sololy due to relaxation processes but mixed with the influence of magnetic field inhomogeneity, is called *apparental lateral relaxation time* T_2*.

To determine the apparent lateral relaxation time T_2*, the software does a curve fitting operation to the envolop of the FID signal.

4) Measurement of lateral relaxation time T_2 with spin echo (SE signal)

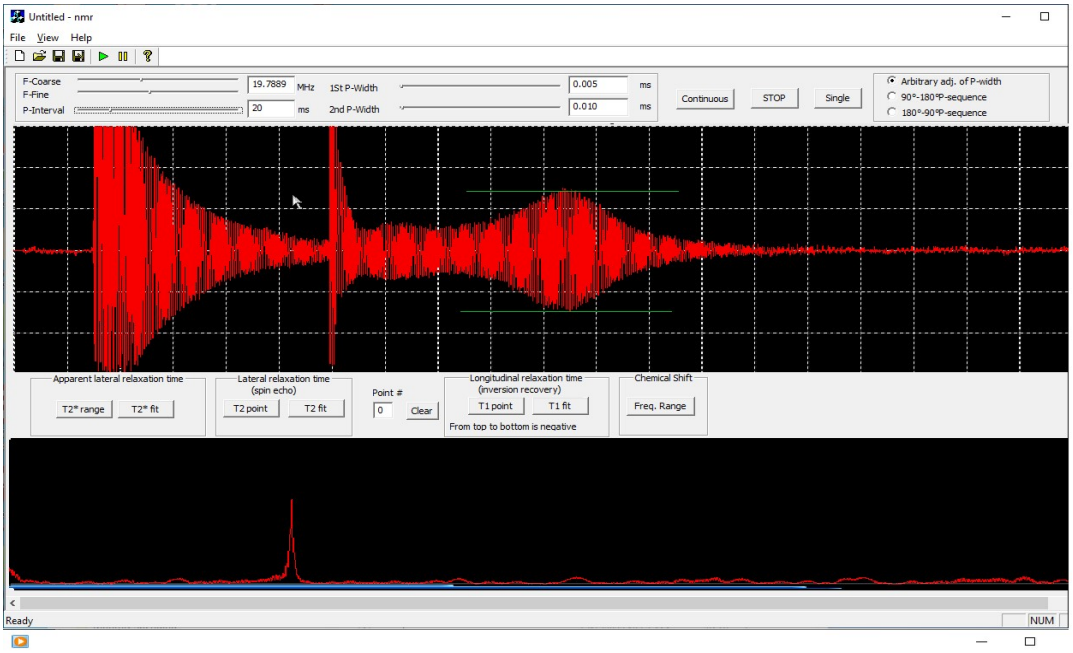
a) Under "**Continuous**" acquisition mode, adjust the widths of the first pulse (90°) and the second pulse (180°) (usually the width of the 180° pulse is about twice the width of the 90° pulse), as well as the magnitudes of the modulation field and homogeneity field, so that an appropriate spin echo signal is achieved.

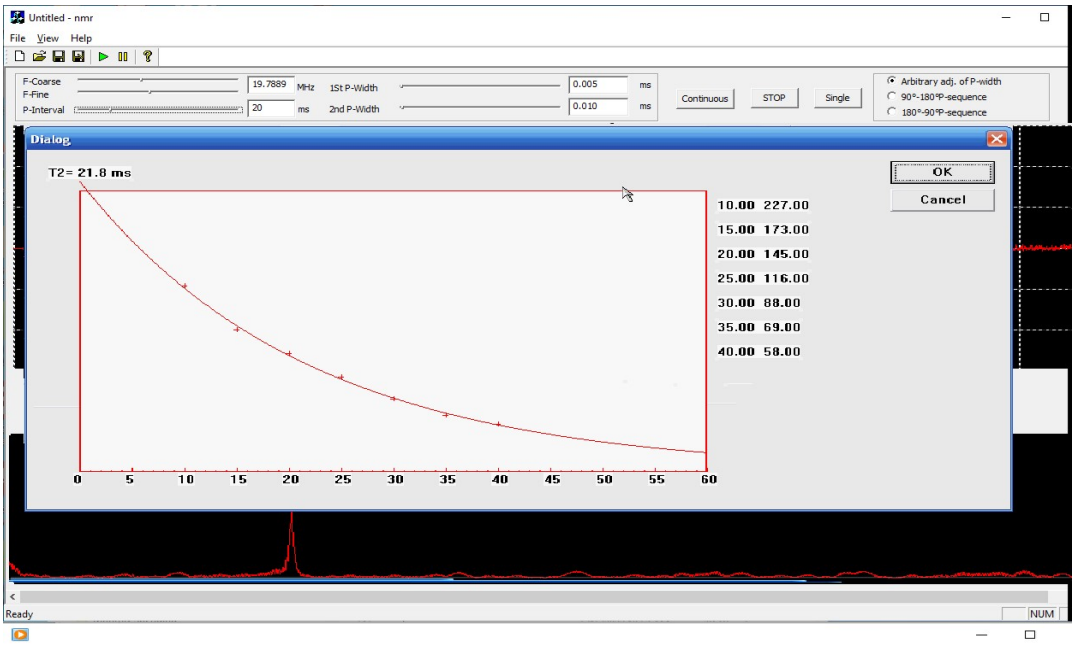
b) Under different "**P-Interval**" values, measure the amplitude of spin echo signal. Detailed steps are as follows:

- Start from a small "**P-Interval**", e.g. 10 ms, click the "**T_2 Point**" (data point at current pulse interval) button in the frame of "**Lateral Relaxation Time**".
- Move the mouse to the top (i.e. the maximum height) of the spin echo signal, left-click there and hold (a horizontal green line marks the maximum height), then move the mouse to the bottom (i.e. the minimum height) of the spin echo signal and release the mouse there (a horizontal green line marks the minimum height). The value in the "**Point #**" (point number) cell increases one.

- Adjust the "**P-Interval**" to different values, e.g. 15 ms, 20 ms, 25 ms, … repeat the above procedure to acquire their amplitudes of the spin echo signal under different pulse intervals. (it is recommend to take at least 6 points).

g) Then click "**T_2 Fit**" to get the transverse relaxation time T_2. (The software automatically do exponential fitting). Click "**Clear**" button to zero "**Point #**".





Theory of measurement of lateral relaxation time T_2 with spin echo

To dertmine the real lateral relaxation time T_2, we need to compensate for the apparent decay of the x-y magnetization due to inhomogeneity in an external magnetic field. The external inhomogeneity creates a variation in the nuclei precession times around an average. The

introduction of a 180° pulse allows the spins to regroup before again dephasing. This creates a spin echo which allows us to measure the true T_2.

Details please read Instruction Manual Section 3, (3) 4).

5) Measurement of longitudinal relaxation time T_1 with the inversion recovery method.

a) Under "**Continuous"** acquisition mode, adjust the widths of the first pulse (180°) and the second pulse (90°) (usually the width of the 180° pulse is about twice the width of the 90° pulse).

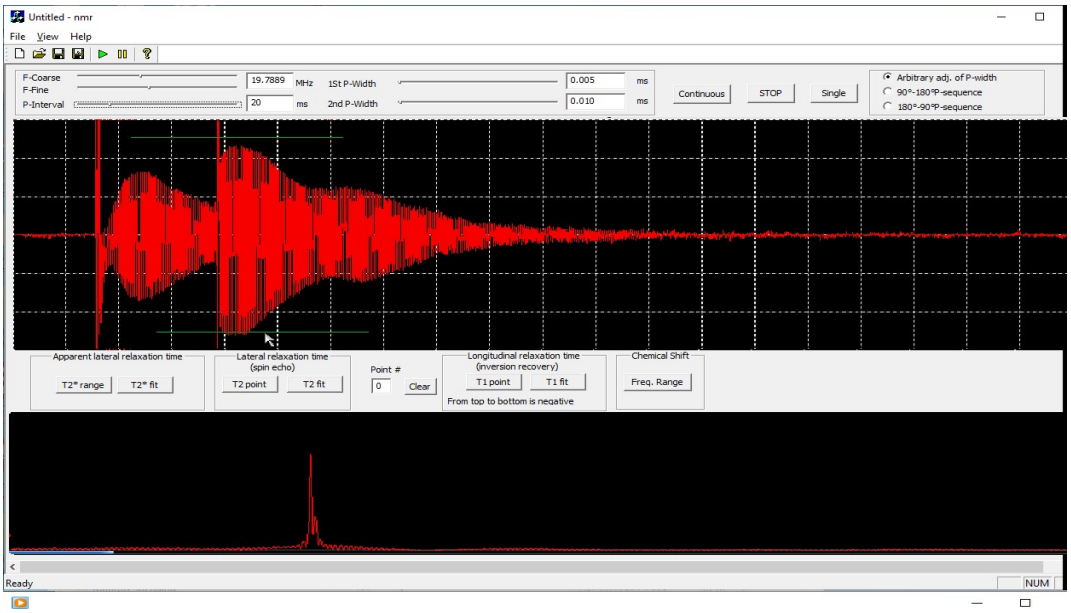
Note 1: here the first pulse is the 180° pulse and the second pulse is the 90° pulse. It is to measure the magnitude change of the second pulse coda with the change of pulse interval.

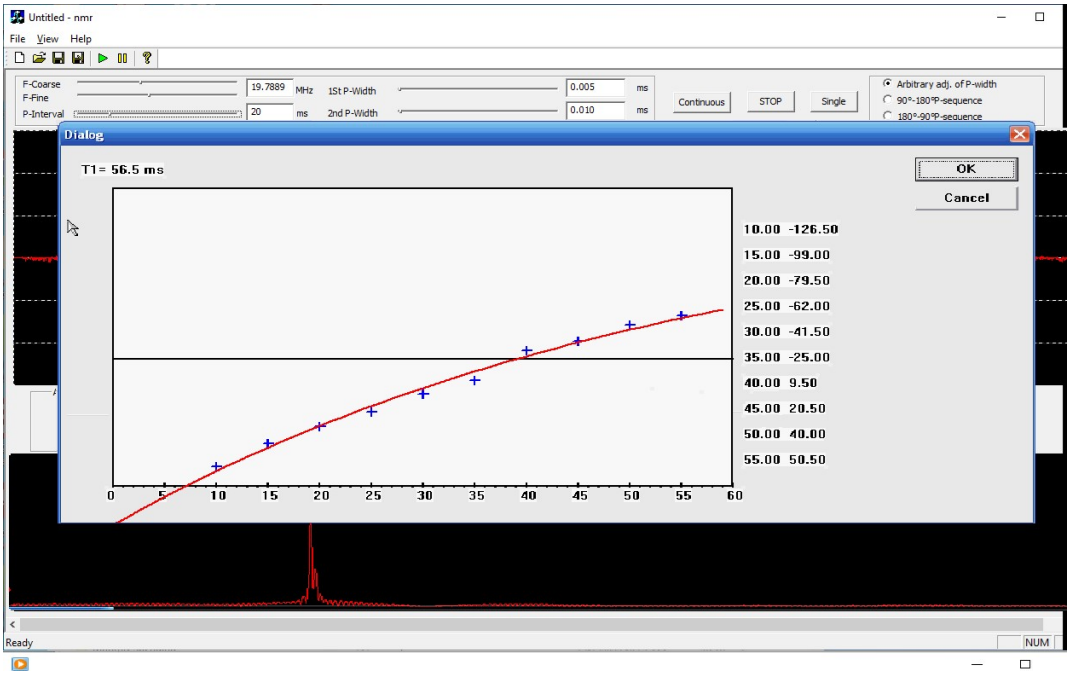
b) Under different "**P-Interval**" values, measure the amplitude of the second pulse coda signal. Detailed steps are as follows:

- Start from a small "**P-Interval**", e.g. 10 ms, click the "**T_1 Point**" button in the frame of "**Longitudinal Relaxation Time**".
- Move the mouse to the top (i.e. the maximum height) of the 2nd pulse coda signal, left-click there and hold (a horizontal green line marks the maximum height), then move the mouse to the bottom (i.e. the minimum height) of the 2nd pulse coda signal and release the mouse there (a horizontal green line marks the minimum height). The value in the "**Point #**" cell increases one.
- Increase the "**P-Interval**" to different values, e.g. 15 ms, 20 ms, 25 ms, … repeat the above procedure to acquire their amplitudes of the 2nd pulse coda signal under different pulse intervals. (it is recommend to at least 6 points).

Note 2: when the pulse interval is small, the coda amplitude takes a negative value. So, when acquiring the amplitude by mouse clicking, we should move the mouse from top to bottom. As the pulse interval increases, the coda amplitude decreases to zero. After passing zero, the coda amplitude takes a positive value and gradually increases again. So, at this time, we should move the mouse from coda bottom to coda top for acquiring coda amplitude.

c) Then click "**T_1 Fit**" to get the transverse relaxation time T_1.





Principle for the measurement of T_1 with inversion recovery method

The inversion recovery method for the measurement of T_1 requires a two pulse sequence. The 1st pulse is the 180° pulse, which rotates the net magnetization vector by 180° to the –z direction. The magnetization state then gradually returns to its original state, +z direction (due to constant magnetic field B_0).

The 2nd pulse is the 90° pulse, which rotates the net magnetization vector by 90° from –Z direction to the x-y plane, where it can be detected by the probe as the FID signal.

After immediate of the 1st pulse (i.e. at the end point of the 1st pulse), there is no net magnetization in the x-y plane.

The maximum amplitude of the Free Induction decay (FID) signal which follows the 2nd pulse is directly proportional to the magnitude of net magnetization at the time the 2nd pulse occurs.

When the interval (delay time) between the 1st pulse and the 2nd pulse is set to 0 (i.e. the 2nd pulse follows the 1st pulse immediately), the FID signal would be at a maximum. By changing the interval between the 1st and 2nd pulses, the rate at which the net magnetization returns to alignment with the constant magnetic field can be investigated. When the delay time results in a 0 (minimum) FID signal, it means that the net magnetization along the z-axis is zero (minimum). When the signal again reaches a maximum, the spins have relaxed back to alignment along the z-axis.

The overall process indicates that the spins rotate from –z to +z direction. In this process, the magnitude of the net magnetization is decreased following an exponential function. This is also the function of magnitude changes of the FID signal as a function of time interval between the two pulses.

This experiment is to acquire the magnitude values of FID signal at different pulse intervals, then use exponential fitting method to calculate the transverse relaxation time T_1.

6) Measurement of chemical shift of xylene sample by Fourier transform

a) The measurement of chemical shift requires high magnetic field homogeneity, that is to say, the FID signal coda should be adjusted to the maximum. The copper sulfate solution sample should

be used firstly in order to achieve relatively stronger 90° pulse signal (FID signal).

b) Under "**Continuous"** acquisition mode, set the width of the second pulse to zero (i.e. off), adjust the "**P-Interval**" to the maximum, and adjust the homogeneity field, to maximize the 90° pulse coda.

c) Replace the copper sulfate solution sample with the xylene sample. Click the "**Stop**" button and wait for a while.

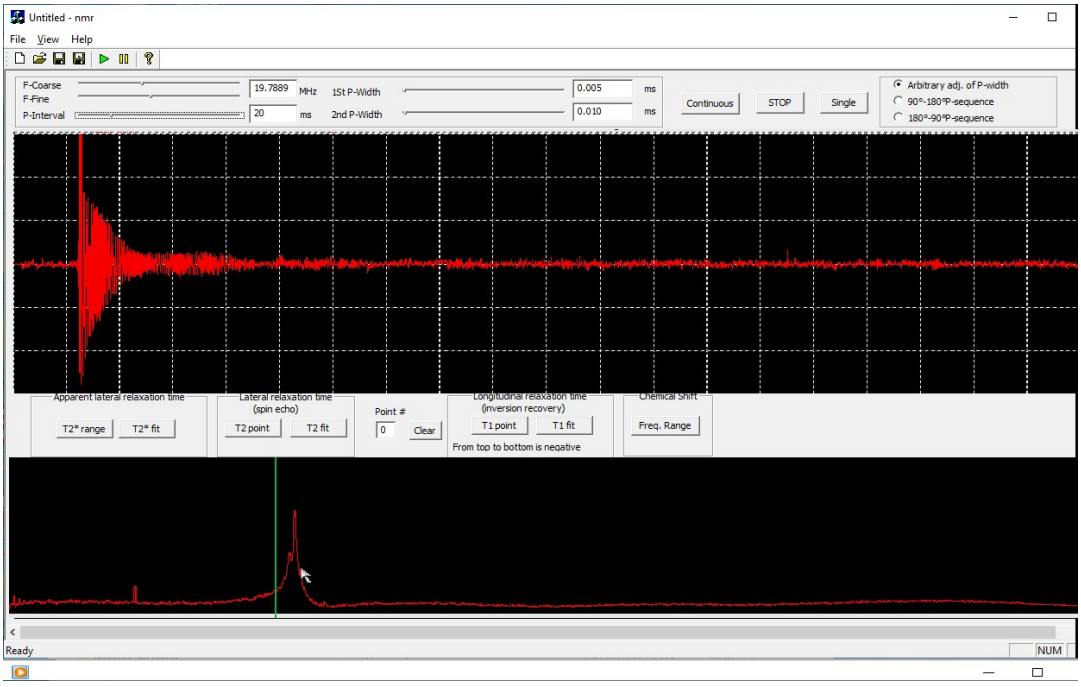
d) Click the "**Single"** acquisition button. At the bottom of the software panel, the Fourier transform spectrum of the FID signal is displayed.

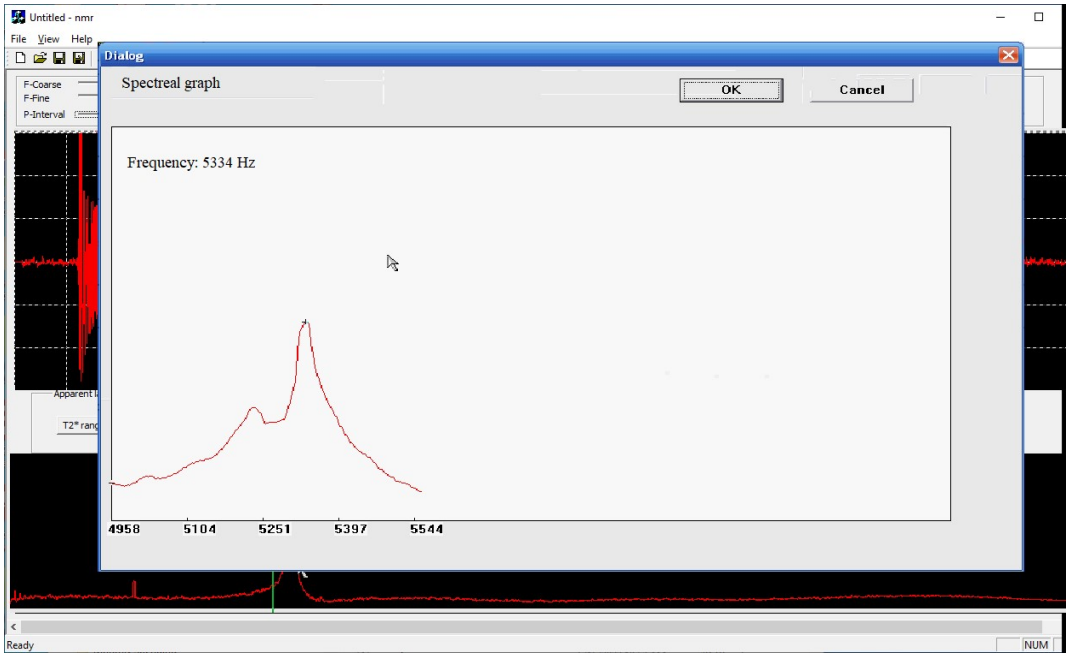
e) In the "**Chemical Shift**" frame, click "**Freq. Range**" button.

f) To select a spectral segment containing the interested peaks, move the mouse to the left side of the desired spectral segment, left-click there and hold, then move the mouse to the right side of the desired spectral segment and release mouse there, the locally enlarged spectral segment will pop-up. It has a double-peak structure.

g) On the enlarged spectral graph, the frequency value of current position of the cursor (+) is displayed. Hit the left shift key (←) or the right shift key (→) on the keyboard to move the cursor (+) on the spectral graph to the desired position of the spectral graph (e.g. the 1st spectral peak). Click "OK", the current frequency values is displayed as "*Frequency 1"*, and a new cursor (+) is generated at the same position. Again, hit the left shift key (←) or the right shift key (→) on the keyboard to move the new cursor (+) on the spectral graph to the desired position of the spectral graph (e.g. the 2nd spectral peak). Click "OK", the current frequency values is displayed as "*Frequency 2"*, also, the frequency interval between *Frequency 1* and *Frequency 2* are displayed.

h) So the relative chemical shift of xylene can be measured by acquiring the frequency values at the two peaks, it is generally around 110 Hz.

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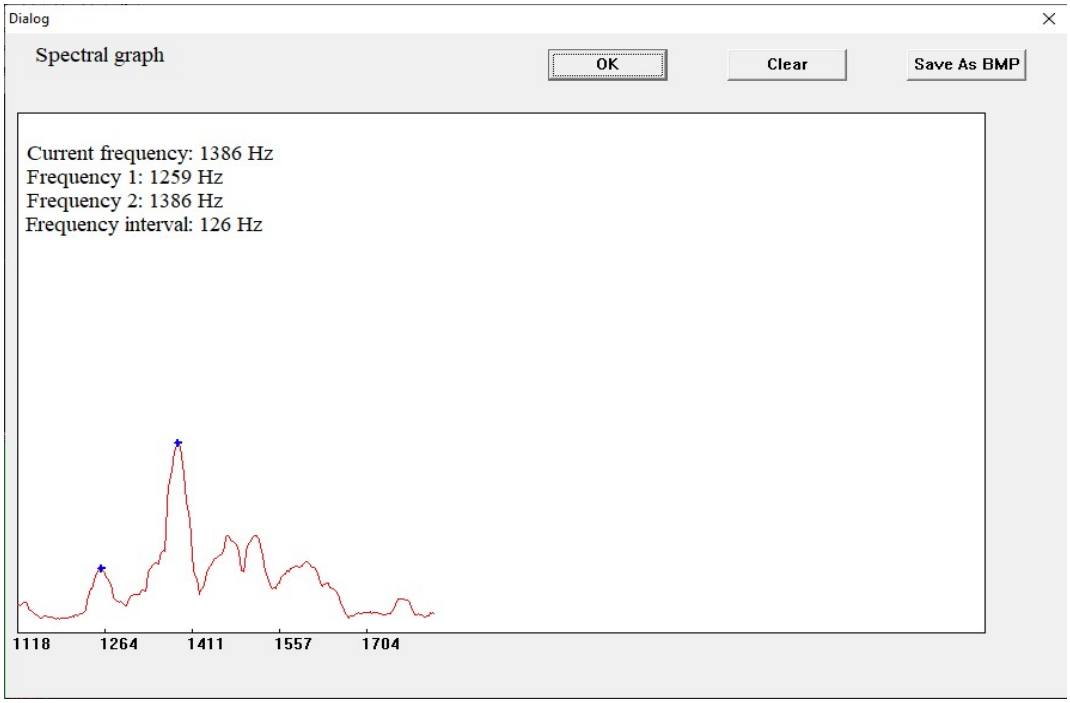
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1557
1704