

Number of Spins Calculation

Usage Notes

The Spin Calculation procedure allows the direct determination of the number of unpaired spins in the sample from the acquired EPR spectrum without the additional steps of preparing and measuring a standard.

Equation 1 shows the parameters used for the determination of the number of spins in a sample. The calculation involves acquisition parameters, the physical dimensions of the sample, and the characteristic properties of the resonator. The acquisition parameters are stored together with the experimental spectrum along with the resonator properties which are set by a factory calibration of the resonator with a standard of known concentration.

The physical dimensions of the sample are left to be determined by the user and are the input in the processing dialog along with the double integral of the EPR spectrum. Two requirements are implicit in Equation 1. The double integral is determined from a normalized EPR signal, making the signal intensity independent of receiver gain, conversion time, and number of scans. The sample temperature is constant and known. The Boltzmann factor is calculated from the microwave frequency and sample temperature (from Variable Temperature Unit) stored with the EPR spectrum. If the temperature is not available from the VT unit, a default temperature of 298 K will be used. The following outlines the specific procedure to quantify the number of spins in an EPR sample. Details covering the Resonator Type definition, Resonator Properties, Q-factor determination, and Sample Positioning are presented after the overall procedure.

Equation 1

$$N_S = \frac{DI \cdot V}{P_{1/2} \cdot B_m \cdot Q \cdot c \cdot S(S+1) \cdot n_B \cdot f(B_1, B_m)}$$

DI = Double Integral
V = Sample Volume
P = Microwave Power
B_m = Modulation Amplitude
Q = Resonator Q-factor
c = Resonator Calibration Factor
S = Electron Spin
n_B = Boltzmann Factor
f(B₁, B_m) = Resonator Field Profile

Calculation of number of spins in sample.

Figure 1



Navigation of Processing task bar to the Spin Calculation processing routine available in Xenon .

Spin Calculation Procedure

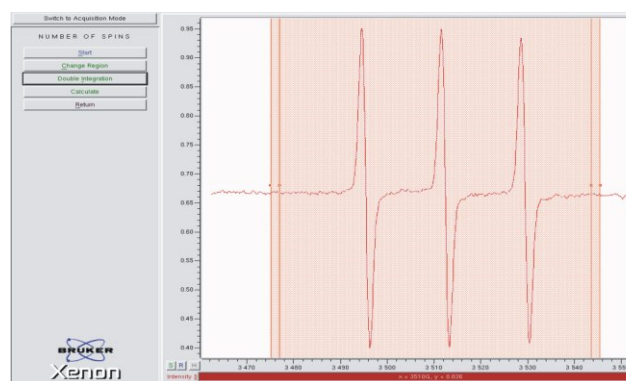
The following outlines how to measure and process the EPR spectrum with the Spin Calculation processing commands. Some aspects of the following are explained in further detail throughout this document.

1. Measure the inner **Diameter** of the sample tube to be used.
2. Measure the **Length** of the sample in the sample tube.
3. Determine the **Center** of the sample by measuring from the middle of the sample to the collet.
4. Place the sample in the resonator.
5. Verify that the correct **Calibration Data Set** and **Resonator Type** are present in the **Signal Channel** tab of the **Spectrometer Configuration**.
6. Critically couple the resonator.
7. Measure the Q-factor and verify that the **Q-value** field is green.
8. Set the appropriate attenuation value for spectrum acquisition.

9. Acquire the EPR spectrum. A higher signal to noise will improve the Double Integral therefore more scans or a longer acquisition time may be necessary.
10. Store/Save the EPR Spectrum.
11. On the task bar, select the **Quantitative EPR** task, then the **Absolute Number of Spins** task as illustrated in Figure 1.
12. On the **Number of Spins** task bar presented press the **Start** button. This clears all previous Qualifiers.
13. Use the **Define Region** button to activate the Integral Region Qualifier.
14. Left click and drag on the spectrum to highlight the area of the spectrum which is to be doubly integrated. The Integral Region Qualifier is composed of two regions: the baseline region and the integral region. The center region is the area which will be doubly integrated while the outer two regions are used to define the baseline. The baseline region is used to fit a first-order polynomial for background subtraction, therefore a larger area will improve the background subtraction.
15. Select the **Change Region** button (previously the **Define Region** button) to modify the qualifier. The baseline region should be resized to cover enough of the baseline for subtraction and the integral region should cover the entire EPR spectrum of the species to be quantified.
16. With the desired qualifier in place, perform the double integration with the **Double Integration** button. The double integral will appear in the Result data. The Integral Region Qualifier can be modified and the double integration performed again until a satisfactory result is achieved.
17. Once the desired double integral is obtained, use the **Calculate** button to start the Spin Calculation dialog. If any of the required parameters are missing from the experimental spectrum, a dialog will appear stating which parameter is missing and the calculation will fail. Note: if the sample temperature was not acquired from the Variable Temperature unit, a default temperature of 298 K will be used for determining the Boltzmann factor in Equation 1. All other parameters must be present for the calculation to proceed.
18. The Spin Calculation dialog requests the parameters determined in steps 1-3 as well as the electron spin of the EPR species in the spectrum. An error will be generated if the **Length** of the sample exceeds the **Resonator Length** specified in the **Resonator** calibration file.
19. Selecting **OK** results in the final report being generated. The Report window indicates which magnetic field range was analyzed through the double integral and the resulting number of spins per unit volume (in micro liters) and the total number of spins in the resonator. These results can be saved as ASCII text.

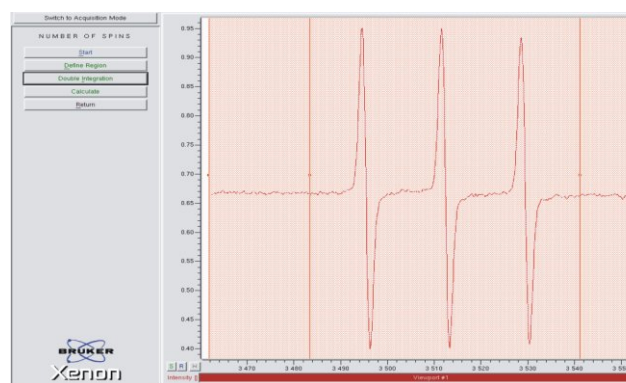
Following the above procedure can result in spin determinations with a minimum error of approximately 10%.

Figure 2



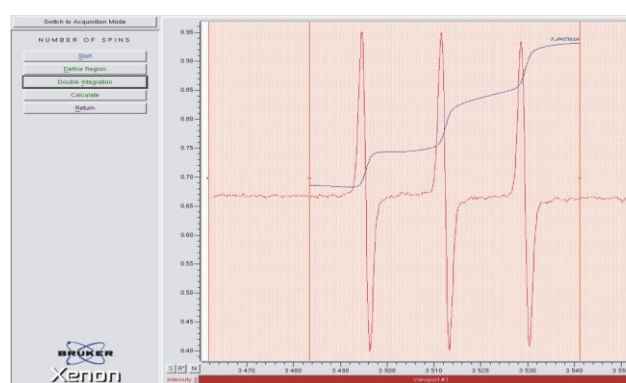
Initial Region Selection with the Integral Region Qualifier

Figure 3



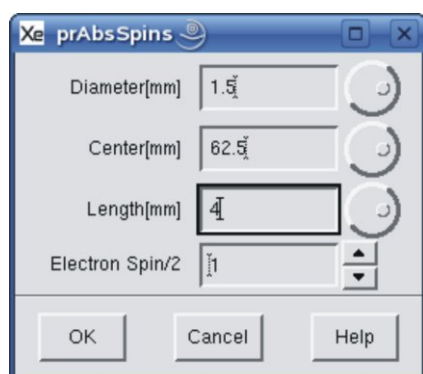
Modification of qualified area for baseline definition and integrated area.

Figure 4



Double Integration result using defined qualifier.

Figure 5



Spin Calculation dialog.

Figure 6

The 'Absolute Spins Report' window displays the following data:

REPORT	xmin	xmax	spins/mm ³	spins
END_REPORT	3.481e+03	3.545e+03	4.158e+15	1.633e+16

Buttons at the bottom: Close, Save, Help On Selection.

Final results of Spin Calculation showing number of spins per unit volume and total number of spin in the resonator.

Resonator Type Definition

There are two calibration files which must be set properly before the EPR measurement: the Calibration Data Set and the Resonator Type. The Calibration Data set is the calibration of the signal channel and modulation. The Resonator Type is a factory generated file containing the resonator properties.

The Resonator Type file contains the physical characteristics of the resonator (distance to center and length) as well as the Resonator Calibration Factor (c) and the Resonator Field Profile ($f(B_1, B_m)$) values. Selection of the Resonator Type is made via the **Spectrometer Configuration** menu item. It is important to have the correct **Resonator Type** selected, otherwise the spin calculation will be incorrect.

To set the Resonator Type, select the **Spectrometer Configuration** menu item and choose the **Signal Channel** tab (see Figure 1). Select the correct **Calibration**

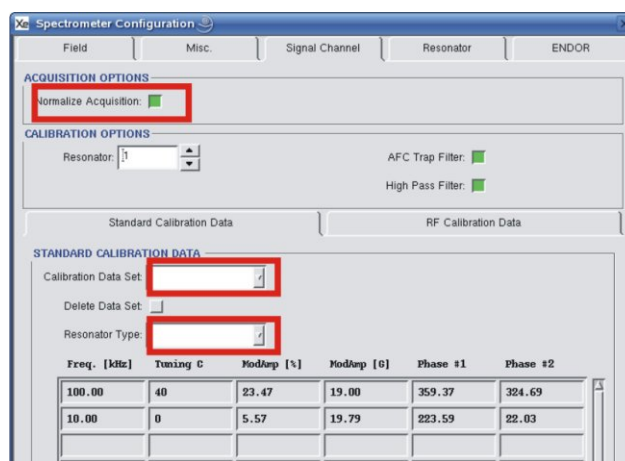
Data Set for the resonator in use, then choose the appropriate **Resonator Type**. To activate the changes press the **Apply** button. Note that any changes in the **Resonator Type** will affect all spectra acquired after the selection and cannot be applied to spectra acquired with a different **Resonator Type**.

Ensure that **Normalized Acquisition** is selected. If this is turned off, the calculated number of spins will be incorrect.

Resonator Properties

The properties of the resonator are accessible via the **Spectrometer Configuration** menu item under the **Resonator** tab. The values from saved calibration files can be viewed by selecting the file in the **From Resonator** field and then pressing the **Load Values From Resonator** button. Similarly the values can be saved to a new file by typing or selecting a file name in the **To Resonator** field and pressing the **Save Values To Resonator** button.

Figure 7



Selection of Resonator Type in the Spectrometer Configuration - Signal Channel

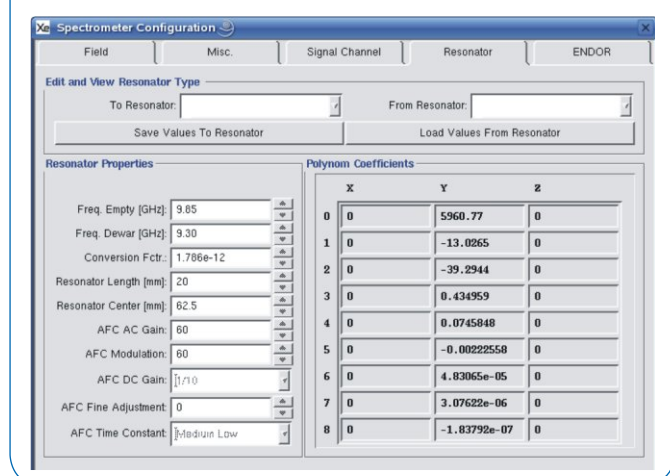
For the Spin Calculation the important parameters are **Conversion Fctr.**, **Resonator Length**, **Resonator Center**, and **Polynom Coefficients**.

- Polynom Coefficients define the Resonator Field Profile in Equation 1 and should not be changed.
- Conversion Fctr. is determined using a standard sample of known concentration under specific experimental conditions. This factor accounts for all of the non-specified influences of the resonator on the EPR spectrum.
- Resonator Length and Resonator Center parameters should be considered when preparing the sample to be measured. The sample size should not exceed the Resonator Length since the Resonator Field Profile is not applied outside of this limit. Similarly the sample should be positioned so that the center of the sample coincides with the Resonator Center. For example, the ER 4119 HS resonator has a Resonator Center of 62.5 mm and a Resonator Length of 40 mm, therefore the

total length of the sample should be less than 40 mm and be centered 62.5 mm from the top of the resonator collet.

- Measurement of the Resonator Center depends on the type of resonator. For resonators where the sample collet is attached to the resonator, the resonator center is measured from the top of the collet. For resonators where the sample collet is attached to the sample tube, the resonator center is measured from the bottom of the collet.

Figure 8



Resonator properties available under Spectrometer Configuration - Resonator. Values shown are typical for the ER 4119 HS resonator.

Sample Positioning and Measurements

The placement of the sample in the resonator as well as the dimensions of the sample tube are critical parameters for accurate determination of the number of spins in the sample. The Spin Calculation procedure requires the input of the sample tube **Diameter**, sample **Center**, and the sample **Length** in millimeters. These measurements are made as follows:

Diameter: measure the inner diameter of the sample tube to be used. For improved accuracy, high precision EPR sample tubes are available.

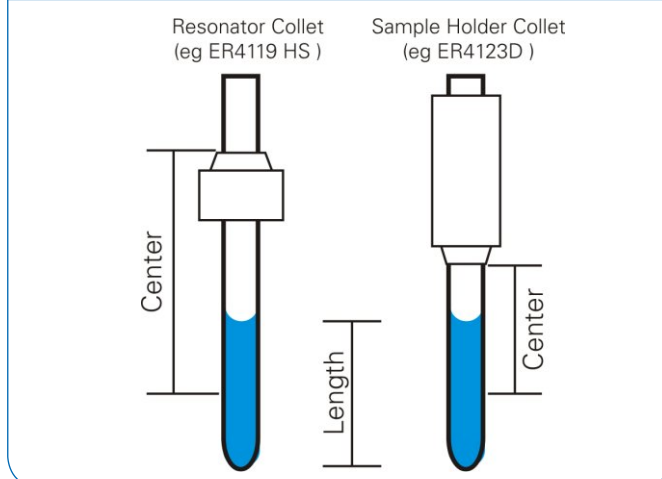
Center: measure from the center of the sample volume to the collet. Two different collet measurements are possible and are illustrated in Figure 3.

Length: measure from the bottom of the meniscus to the bottom of the sample in the sample tube as illustrated in Figure 3.

Q-Factor Determination

Measurement of the resonator Q-factor is performed by the microwave bridge under a defined set of conditions. To accurately measure the resonator Q-factor:

Figure 9

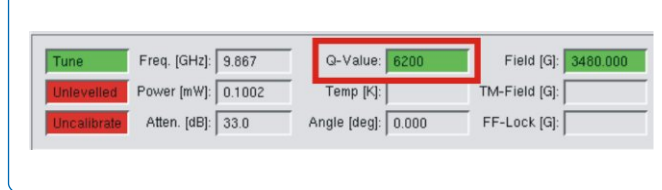


Measurement of sample dimensions for input into Spin Calculation dialog. Note that two types of collets are possible.

1. Critically couple the resonator (see Spectrometer Manual)
2. Switch to **Tune** mode in the **Microwave Bridge Tuning** panel
3. Set the **Attenuation** to 33 dB
4. Wait for the **Q-value** field in the **Spectrometer Monitoring** panel to stabilize and turn green.
5. Switch to **Operate** mode.
6. Set the **Attenuator** to the desired value for spectrum acquisition.

The **Q-value** field will remain green until the microwave bridge is switched to **Tune** mode at an attenuation other than 33 dB. When the **Q-value** field is green, the Q-factor will be stored with the experimental spectrum. Whenever the resonator coupling changes, it is best to measure the Q-factor again.

Figure 10



Resonator Q-value indicated in the Spectrometer Monitoring panel.

Notes on Quantitative Measurements

The following notes are intended to point out a few of the most common concerns when measuring EPR spectra with the goal of quantifying the EPR species present. No order of importance is implied in the following collection.

- The positioning of the sample in the resonator is often the largest source of error in quantitative measurements. The sample center should be carefully measured and positioned to coincide with the center of the resonator. Similarly care should be taken to insure that the sample remains axially centered in the resonator.
- Ideally the length of the sample would be small with respect to the length of the resonator so that the sample experiences a uniform field along its entire length. Significant errors in the number of spins calculated will be introduced if the sample is larger than the resonator area due to the field present but not account for by the Resonator Field Profile in Equation 1.
- The result of double integration is heavily influenced by the amount of noise in the EPR spectrum. Increasing the signal to noise through longer acquisition times or signal averaging will improve the reliability of the number of spins determined.
- The physical state of the EPR sample does not change the results of the quantitative measurement. As long as the entire EPR signal is included in the double integral, the result from Equation 1 will be the same for the same sample in a liquid state or a frozen state. Implicit in Equation 1 is the sample temperature which is included in the Boltzmann factor. This temperature is stored with the EPR spectrum if the Variable temperature unit is used, otherwise a temperature of 298 K is assumed.
- In Normalized Acquisition mode (required for Equation 1 to be valid), only the microwave power directly impacts the spin calculation through saturation effects while the modulation amplitude affects the spectrum signal to noise.
 - Since the value for the double integral scales linearly with the modulation amplitude, any modulation amplitude can be used. If the modulation amplitude is less than the line width, a significant decrease in signal to noise will result. To compensate, the total acquisition time should be increased to achieve reliable values for the double integral.
 - The choice of microwave power is very important since for Equation 1 to be used, the EPR signal being observed must be acquired under non-saturating conditions. The saturation curve of the EPR species should be measured for each resonator where the EPR species will be quantified. Changes in the microwave field for different resonators will affect the onset of saturation. Once the saturation curve is known, a microwave power well below the saturation point in the linear region should be used for all subsequent quantitative experiments. A quick check of the saturation is to determine the number of spins at one microwave power and then determine the number of spins at either 6 dB more or less power ($6\text{dB} = 4$). The resulting number of spins should be independent of microwave power within the errors of the measurement (typically 10% error).

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