Introduction 1

This document describes the basic operation of a Bruker Elexsys E 500 EPR (Electron Paramagnetic Resonance) spectrometer. No assumptions have been made about the background of the readers except that they have a general scientific or technical background. Many of the elementary principles necessary for following the chapters are presented in a concise form.

This manual is one of three manuals supplied with your spectrometer. In addition, there is also a second volume of this manual (Elexsys E 500 User's Manual: Advanced Operations) that describes more advanced operations and procedures of the spectrometer. Finally, there is an Xepr User's Manual describing many details of the Xepr application.

We start with a list of EPR applications. A brief description of the spectrometer and its capabilities follows. The chapter concludes with an explanation of how to use this manual.

EPR Applications 1.1

EPR has matured into a powerful, versatile, nondestructive, and nonintrusive analytical method. Unlike many other techniques, EPR yields meaningful structural and dynamical information, even from ongoing chemical or physical processes without influencing the process itself. Therefore, it is an ideal complementary technique for other methods in a wide range of studies and application areas. Here is a list of some of the EPR applications which are commonly used.

Chemistry   1.1.1

- Kinetics of radical reactions
- Polymerization reactions
- Spin trapping
- Organo-metallic compounds
- Catalysis
- Petroleum research
- Oxidation and reduction processes
- Biradicals and triplet states of molecules

Physics   1.1.2

- Measurement of magnetic susceptibility
- Transition metal, lanthanide, and actinide ions
- Conduction electrons in conductors and semiconductors
- Defects in crystals (e.g. color centers in alkali-halides)
- Optical detection of magnetic resonance, excited states of molecules
- Crystal fields in single crystals
- Recombination at low temperatures

Materials Research   1.1.3

- Degradation of paints and polymers by light
- Polymer properties
- Defects in diamond
- Defects in optical fibers
- Laser materials
- Organic conductors
- Influence of impurities and defects in semiconductors
- Properties of novel magnetic materials
- High T_C superconductors
- C_{60} compounds
- Behavior of free radicals in corrosion

Ionizing Radiation   1.1.4

- Alanine radiation dosimetry
- Control of irradiated foods
- Archaeological dating
- Short-time behavior of organic free radicals produced by radiation
- Radiation effects and damage
- Radiation effects on biological compounds

Biology and Medicine 1.1.5

- Spin label and spin probe techniques

- Spin trapping

- Dynamics of biomolecules using saturation transfer techniques

- Free radicals in living tissues and fluids

- Antioxidants, radical scavengers

- Contrast agents

- Oximetry

- Drug detection, metabolism, and toxicity

- Enzyme reactions

- Photosynthesis

- Structure and identification of metal-binding sites

- Photochemical and radiolytic generation of radicals

- Oxygen based radicals

- NO in biological systems

- Carcinogenic reactions

The Spectrometer 1.2

The Bruker Elexsys E 500 EPR spectrometer is a research grade scientific instrument. It is capable of routine measurements, as well as sophisticated and advanced experiments when equipped with the proper accessories. The modular design makes the spectrometer easy to upgrade or expand. For information and assistance in choosing and ordering accessories for your specific application, contact your local Bruker EPR sales representative.

Using this Manual   1.3

This manual is one of three manuals supplied with your Bruker Elexsys E 500 spectrometer. It describes basic operations. In addition, there is also a second volume of this manual (Elexsys E 500 User's Manual: Advanced Operations) that describes many procedures and operations in greater depth than this manual. Finally, there is the Xepr User's Manual describing many details of the Xepr application.

How to Find Things   1.3.1

Preface   First, you should read the safety guide in the preface of the manual. Microwaves can be dangerous, particularly to your eyes. With normal precautions, the risk for injury can be minimized.

Chapter 2   Users who are not familiar with EPR should start by reading Chapter 2, which is a concise introduction to the theory and practice of EPR spectroscopy. It is by no means exhaustive; it gives the necessary information to follow the other chapters of the manual. A short list of references is given at the end of the chapter for more information.

Chapter 3   This chapter is a simple "how to" section describing how to acquire the spectrum of a sample. It covers turning the spectrometer on, tuning the microwave cavity and bridge, and acquiring spectra. The step by step instructions lead you through the acquisition of a strong pitch (a standard sample) EPR signal.

Chapter 4   This chapter introduces you to the essential concepts of Xepr. It describes the basic features and how to use those Xepr features.

Chapter 5   This tutorial guides you through many common data acquisition tasks. It gives you detailed examples of how to use many of the convenient and commonly used features of the spectrometer. The chapter covers both 1D and 2D spectra.

Chapter 6   This chapter gives you a tutorial on the basic operations of data processing such as data handling, baseline correction, peak picking, integration, and curve fitting.

Chapter 7   The most basic operations of the Bruker EPR spectrometer are covered in Chapter 3. This chapter introduces you to additional techniques such as manually tuning the bridge, changing cavities, and adjusting the AFC.

Chapter 8   General helpful hints for acquiring EPR spectra are presented in this chapter. Before consulting this chapter, you should be familiar with the material in Chapters 2 and 3. It gives tips on where to find EPR signals as well as how to optimize the sensitivity of the spectrometer for your particular sample.

Chapter 9   Sometimes, things go wrong. Chapter 9 gives some possible solutions to problems you may be having. Many times, problems appear to be the fault of the instrument; however, with the proper choice of operating conditions, these problems often disappear.

Chapter 10   An extensive bibliography of EPR references is given in this chapter. It includes many different EPR applications as well as educational texts. This is a good place to start a literature search.

This manual consists of two volumes. This volume is for basic operations. If you have more advanced questions please refer to volume II, Advanced Operations. All the answers to all EPR questions can not possibly fit in one manual. This manual is only part of the Elexsys E 500 documentation. It provides instructions to the essential operations of Elexsys E 500 systems. For help with software questions, consult the Xepr User's Manual. It can be accessed on-line from the Xepr program by clicking on Manual in the Help menu. If you cannot find the answer to your question, contact your nearest Bruker EPR representative. We have a team of skilled application scientists with diverse expertises. One of us will probably come up with an answer.

Fonts   1.3.2

Special fonts are used in the text to differentiate between normal manual text and the text displayed in the program.

Times   This is the font used for the normal text in the manual.

Helvetica   This is the font used for text that is displayed by the program or must be entered into the program by you.

# Special notes   1.3.3

Some special notation is employed in this manual to simplify the descriptions.

< ... >   The content between the brackets needs to be substituted with proper entries by the user.

>   The right bracket indicates sequential selection of the menu entries. For example, Processing > Filtering > Smoothing means clicking the Processing button in the menu bar, followed by clicking Filtering in the sub-menu, and then clicking Smoothing.

Warning box.

You will see a warning box sometimes in the lefthand margin. These are meant to point out critical information. In particular, it warns you about any procedures or operations that may be dangerous to the spectrometer or you. Always read and follow this advice.
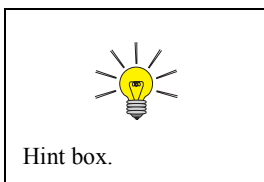
Hint box.

In addition, there are also hint boxes in the lefthand margin These are meant to be helpful hints and point out important information.

An EPR Primer 2

This chapter is an introduction to the basic theory and practice of EPR spectroscopy. It gives you sufficient background to understand the following chapters. In addition, we strongly encourage the new user to explore some of the texts and articles at the end of this chapter. You can then fully benefit from your particular EPR application or think of new ones.

Basic EPR Theory 2.1

Introduction to Spectroscopy 2.1.1

During the early part of this century, when scientists began to apply the principles of quantum mechanics to describe atoms or molecules, they found that a molecule or atom has discrete (or separate) states, each with a corresponding energy. Spectroscopy is the measurement and interpretation of the energy differences between the atomic or molecular states. With knowledge of these energy differences, you gain insight into the identity, structure, and dynamics of the sample under study.

We can measure these energy differences, ΔE, because of an important relationship between ΔE and the absorption of electromagnetic radiation. According to Planck's law, electromagnetic radiation will be absorbed if:

$$\Delta E = h\nu , \qquad\qquad [2\text{-}1]$$

where h is Planck's constant and ν is the frequency of the radiation.
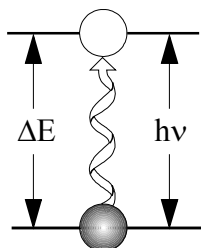
Figure 2-1   Transition associated with the absorption of
electromagnetic energy.

The absorption of energy causes a transition from the lower
energy state to the higher energy state. (See Figure 2-1.) In con-
ventional spectroscopy, ν is varied or swept and the frequencies
at which absorption occurs correspond to the energy differences
of the states. (We shall see later that EPR differs slightly.) This
record is called a spectrum. (See Figure 2-2.) Typically, the fre-
quencies vary from the megahertz range for NMR (Nuclear
Magnetic Resonance) (AM, FM, and TV transmissions use elec-
tromagnetic radiation at these frequencies), through visible light,
to ultraviolet light. Radiation in the gigahertz range (the same as
in your microwave oven) is used for EPR experiments.
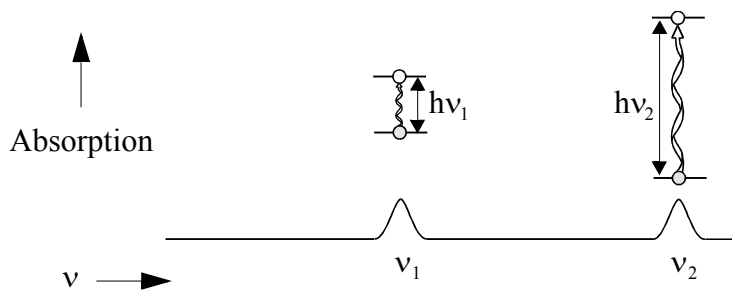
Figure 2-2   A spectrum.

The Zeeman Effect 2.1.2

The energy differences we study in EPR spectroscopy are predominately due to the interaction of unpaired electrons in the sample with a magnetic field produced by a magnet in the laboratory. This effect is called the Zeeman effect. Because the electron has a magnetic moment, it acts like a compass or a bar magnet when you place it in a magnetic field, B_0. It will have a state of lowest energy when the moment of the electron, μ, is aligned with the magnetic field and a state of highest energy when μ is aligned against the magnetic field. (See Figure 2-3.) The two states are labelled by the projection of the electron spin, M_s, on the direction of the magnetic field. Because the electron is a spin 1/2 particle, the parallel state is designated as $M_s = -1/2$ and the antiparallel state is $M_s = +1/2$.
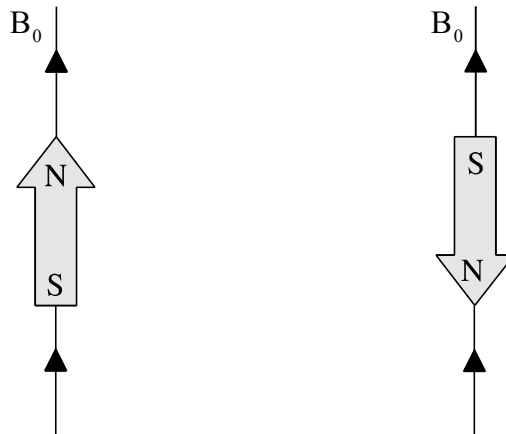
Figure 2-3   Minimum and maximum energy orientations of μ with respect to the magnetic field B_0.

From quantum mechanics, we obtain the most basic equations of EPR:

$$E = g\,\mu_B\,B_0\,M_s = \pm\frac{1}{2}\,g\,\mu_B\,B_0 \qquad\qquad [2\text{-}2]$$

and

$$\Delta E = h\nu = g\,\mu_B B_0. \qquad\qquad [2\text{-}3]$$

g is the g-factor, which is a proportionality constant approximately equal to 2 for most samples, but varies depending on the electronic configuration of the radical or ion. μ_B is the Bohr magneton, which is the natural unit of electronic magnetic moment.

Two facts are apparent from equations Equation [2-2] and Equation [2-3] and its graph in Figure 2-4.

- The two spin states have the same energy in the absence of a magnetic field.

- The energies of the spin states diverge linearly as the magnetic field increases.

These two facts have important consequences for spectroscopy.

- Without a magnetic field, there is no energy difference to measure.

- The measured energy difference depends linearly on the magnetic field.

Because we can change the energy differences between the two spin states by varying the magnetic field strength, we have an alternative means to obtain spectra. We could apply a constant magnetic field and scan the frequency of the electromagnetic radiation as in conventional spectroscopy. Alternatively, we could keep the electromagnetic radiation frequency constant and scan the magnetic field. (See Figure 2-4.) A peak in the absorption will occur when the magnetic field "tunes" the two spin states so that their energy difference matches the energy of the radiation. This field is called the "field for resonance". Owing to the limitations of microwave electronics, the latter method offers superior performance. This technique is used in all Bruker EPR spectrometers.
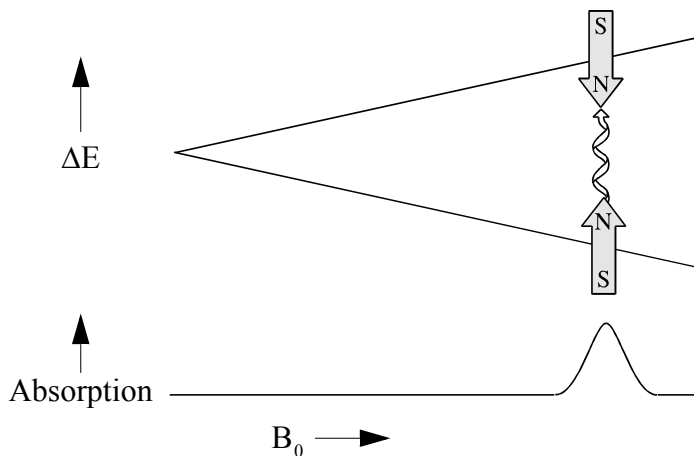
Figure 2-4   Variation of the spin state energies as a function of the applied magnetic field.

The field for resonance is not a unique "fingerprint" for identification of a compound because spectra can be acquired at several different frequencies. The g-factor,

$$g = \frac{h\nu}{\mu_B B_0} \quad ,$$ [2-4]

being independent of the microwave frequency, is much better for that purpose. Notice that high values of g occur at low magnetic fields and vice versa. A list of fields for resonance for a $g = 2$ signal at microwave frequencies commonly available in EPR spectrometers is presented in Table 2-1.

Microwave Band	Frequency (GHz)	B_{res} (G)
L	1.1	390
S	4.0	1430
X	9.75	3480
Q	34.0	12100
W	94.0	33500

Table 2-1 Field for resonance, B_{res}, for a $g = 2$ signal at selected microwave frequencies.

Hyperfine Interactions   2.1.3

Measurement of g-factors can give us some useful information; however, it does not tell us much about the molecular structure of our sample. Fortunately, the unpaired electron, which gives us the EPR spectrum, is very sensitive to its local surroundings. The nuclei of the atoms in a molecule or complex often have a magnetic moment, which produces a local magnetic field at the electron. The interaction between the electron and the nuclei is called the hyperfine interaction. It gives us a wealth of information about our sample such as the identity and number of atoms which make up a molecule or complex as well as their distances from the unpaired electron.
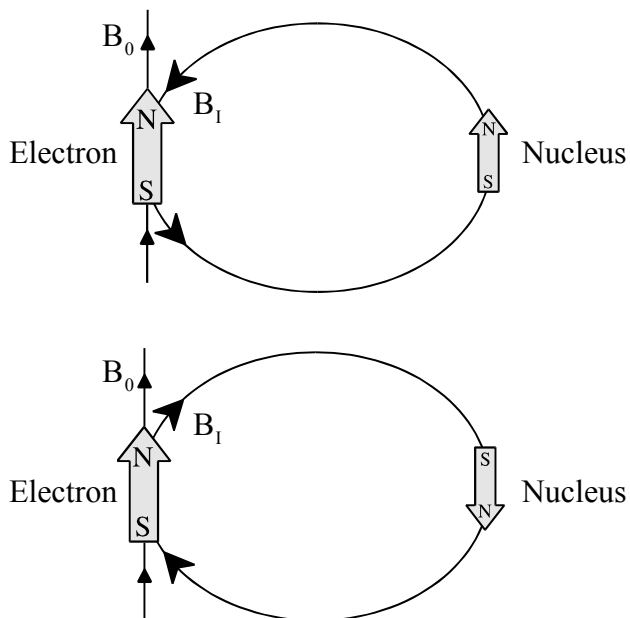
Figure 2-5   Local magnetic field at the electron, B_I, due to a nearby nucleus.

Figure 2-5 depicts the origin of the hyperfine interaction. The magnetic moment of the nucleus acts like a bar magnet (albeit a weaker magnet than the electron) and produces a magnetic field at the electron, B_I. This magnetic field opposes or adds to the magnetic field from the laboratory magnet, depending on the alignment of the moment of the nucleus. When B_I adds to the magnetic field, we need less magnetic field from our laboratory magnet and therefore the field for resonance is lowered by B_I. The opposite is true when B_I opposes the laboratory field.

For a spin 1/2 nucleus such as a hydrogen nucleus, we observe that our single EPR absorption signal splits into two signals which are each B_I away from the original signal. (See Figure 2-6.)
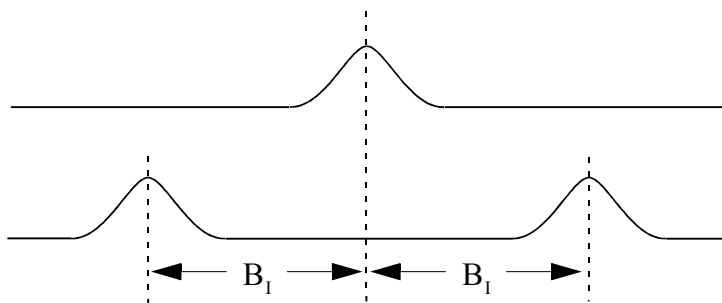
Figure 2-6   Splitting in an EPR signal due to the local magnetic field of a nearby nucleus.

If there is a second nucleus, each of the signals is further split into a pair, resulting in four signals. For N spin 1/2 nuclei, we will generally observe 2^N EPR signals. As the number of nuclei gets larger, the number of signals increases exponentially. Sometimes there are so many signals that they overlap and we only observe one broad signal.

Signal Intensity   2.1.4

So far, we have concerned ourselves with where the EPR signal is, but the size of the EPR signal is also important if we want to measure the concentration of the EPR active species in our sample. In the language of spectroscopy, the size of a signal is defined as the integrated intensity, i.e., the area beneath the absorption curve. (See Figure 2-7.) The integrated intensity of an EPR signal is proportional to the concentration.
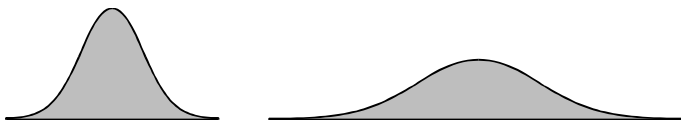
Figure 2-7   Integrated intensity of absorption signals. Both signals have the same intensity.

Signal intensities do not depend solely on concentrations. They also depend on the microwave power. If you do not use too much microwave power, the signal intensity grows as the square root of the power. At higher power levels, the signal diminishes as well as broadens with increasing microwave power levels. This effect is called saturation. If you want to measure accurate linewidths, lineshapes, and closely spaced hyperfine splittings, you should avoid saturation by using low microwave power. A quick means of checking for the absence of saturation is to decrease the microwave power and verify that the signal intensity also decreases by the square root of the microwave power.

Basic EPR Practice   2.2

Introduction to Spectrometers   2.2.1

In the first half of this chapter, we discussed the theory of EPR spectroscopy. Now we need to consider the practical aspects of EPR spectroscopy. Theory and practice have always been strongly interdependent in the development and growth of EPR. A good example of this point is the first detection of an EPR signal by Zavoisky in 1945. The Zeeman effect had been known in optical spectroscopy for many years, but the first direct detection of EPR had to wait until the development of radar during World War II. Only then, did scientists have the necessary components to build sufficiently sensitive spectrometers (scientific instruments designed to acquire spectra). The same is true today with the development of advanced techniques in EPR such as Fourier Transform and high frequency EPR.

The simplest possible spectrometer has three essential components: a source of electromagnetic radiation, a sample, and a detector. (See Figure 2-8.) To acquire a spectrum, we change the frequency of the electromagnetic radiation and measure the amount of radiation which passes through the sample with a detector to observe the spectroscopic absorptions. Despite the apparent complexities of any spectrometer you may encounter, it can always be simplified to the block diagram shown in Figure 2-8.

Source   Sample   Detector
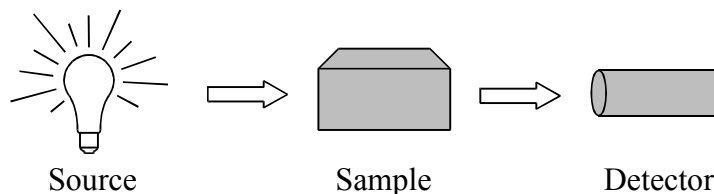
Figure 2-8   The simplest spectrometer.

Figure 2-9 shows the general layout of a Bruker EPR spectrometer. The electromagnetic radiation source and the detector are in a box called the "microwave bridge". The sample is in a microwave cavity, which is a metal box that helps to amplify weak signals from the sample. As mentioned in Section 2.1.2, there is a magnet to "tune" the electronic energy levels. In addition, we have a console, which contains signal processing and control electronics and a computer. The computer is used for analyzing data as well as coordinating all the units for acquiring a spectrum. In the following sections you will become acquainted with how these different parts of the spectrometer function and interact.
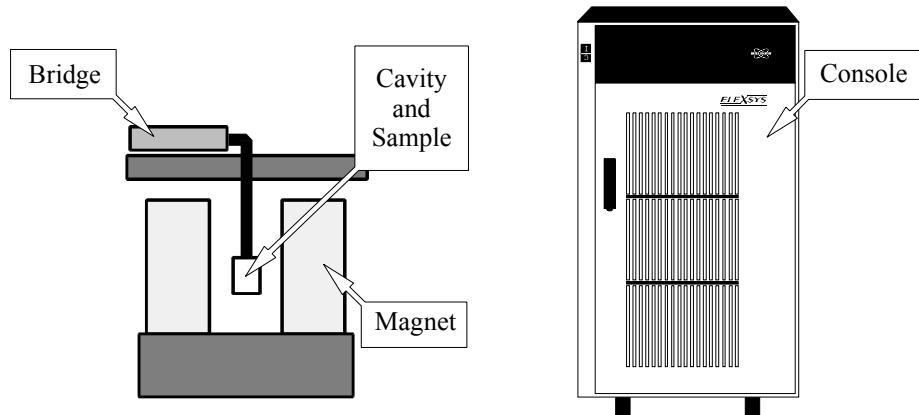
Figure 2-9   The general outlay of an EPR spectrometer.
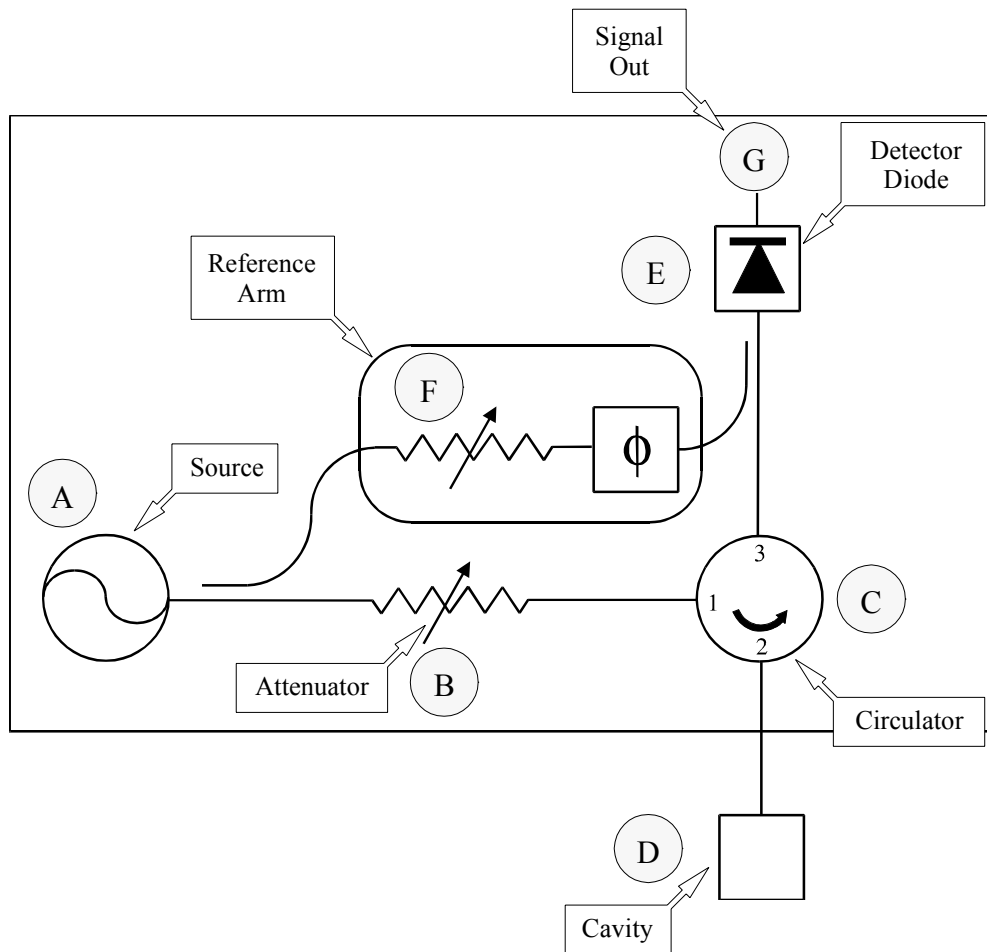
Figure 2-10   Block diagram of a microwave bridge.

The Microwave Bridge   2.2.2

The microwave bridge houses the microwave source and the detector. There are more parts in a bridge than shown in Figure 2-10, but most of them are control, power supply, and security electronics and are not necessary for understanding the basic operation of the bridge. We shall now follow the path of the microwaves from the source to the detector.

We start our tour of the microwave bridge at point A, the microwave source. The output power of the microwave source cannot be varied easily, however in our discussion of signal intensity, we stressed the importance of changing the power level. Therefore, the next component, at point B, after the microwave source is a variable attenuator, a device which blocks the flow of microwave radiation. With the attenuator, we can precisely and accurately control the microwave power which the sample sees.

Bruker EPR spectrometers operate slightly differently than the simple spectrometer shown in the block diagram, Figure 2-8. The diagram depicts a transmission spectrometer (It measures the amount of radiation transmitted through the sample.) and most EPR spectrometers are reflection spectrometers. They measure the changes (due to spectroscopic transitions) in the amount of radiation reflected back from the microwave cavity containing the sample (point D in the figure). We therefore want our detector to see only the microwave radiation coming back from the cavity. The circulator at point C is a microwave device which allows us to do this. Microwaves coming in port 1 of the circulator only go to the cavity through port 2 and not directly to the detector through port 3. Reflected microwaves are directed only to the detector and not back to the microwave source.

We use a Schottky barrier diode to detect the reflected microwaves (point E in the figure). It converts the microwave power to an electrical current. At low power levels, (less than 1 microwatt) the diode current is proportional to the microwave power and the detector is called a square law detector. (Remember that

electrical power is proportional to the square of the voltage or current.) At higher power levels, (greater than 1 milliwatt) the diode current is proportional to the square root of the microwave power and the detector is called a linear detector. The transition between the two regions is very gradual.

For quantitative signal intensity measurements as well as optimal sensitivity, the diode should operate in the linear region. The best results are attained with a detector current of approximately 200 microamperes. To insure that the detector operates at that level, there is a reference arm (point F in the figure) which supplies the detector with some extra microwave power or "bias". Some of the source power is tapped off into the reference arm, where a second attenuator controls the power level (and consequently the diode current) for optimal performance. There is also a phase shifter to insure that the reference arm microwaves are in phase with the reflected signal microwaves when the two signals combine at the detector diode.

The detector diodes are very sensitive to damage from excessive microwave power and will slowly lose their sensitivity. To prevent this from happening, there is protection circuitry in the bridge which monitors the current from the diode. When the current exceeds 400 microamperes, the bridge automatically protects the diode by lowering the microwave power level. This reduces the risk of damage due to accidents or improper operating procedures. However, it is good lab practice to follow correct procedures and not rely on the protection circuitry.

The EPR Cavity 2.2.3

In this section, we shall discuss the properties of microwave (EPR) cavities and how changes in these properties due to absorption result in an EPR signal. We use microwave cavities to amplify weak signals from the sample. A microwave cavity is simply a metal box with a rectangular or cylindrical shape which resonates with microwaves much as an organ pipe resonates with sound waves. Resonance means that the cavity stores the microwave energy; therefore, at the resonance frequency of the cavity, no microwaves will be reflected back, but will remain inside the cavity. (See Figure 2-11.)
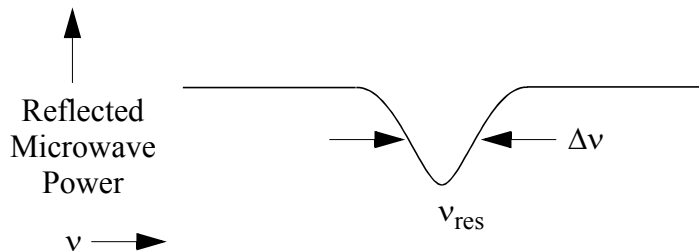
Figure 2-11   Reflected microwave power from a resonant cavity.

Cavities are characterized by their Q or quality factor, which indicates how efficiently the cavity stores microwave energy. As Q increases, the sensitivity of the spectrometer increases. The Q factor is defined as

$$Q = \frac{2\pi \ (\text{energy stored})}{\text{energy dissipated per cycle}} \ ,$$ [2-5]

where the energy dissipated per cycle is the amount of energy lost during one microwave period. Energy can be lost to the side walls of the cavity because the microwaves generate electrical currents in the side walls of the cavity which in turn generates

heat. We can measure Q factors easily because there is another way of expressing Q:

$$Q = \frac{\nu_{res}}{\Delta\nu} \ , \tag{2-6}$$

where ν_{res} is the resonant frequency of the cavity and $\Delta\nu$ is the width at half height of the resonance.

Sample Stack

Sample Stack

Microwave Magnetic Field   Microwave Electric Field
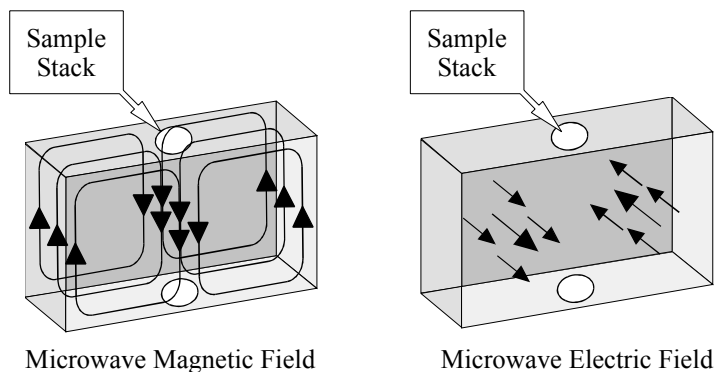
Figure 2-12   Magnetic and electric field patterns in a standard EPR cavity.

A consequence of resonance is that there will be a standing wave inside the cavity. Standing electromagnetic waves have their electric and magnetic field components exactly out of phase, i.e. where the magnetic field is maximum, the electric field is minimum and vice versa. The spatial distribution of the amplitudes of the electric and magnetic fields in the most commonly used EPR cavity is shown in Figure 2-12. We can use the spatial separation of the electric and magnetic fields in a cavity to great advantage. Most samples have non-resonant absorption of the microwaves via the electric field (this is how a microwave oven works) and the Q will be degraded by an increase in the dissipated energy. It is the magnetic field that drives the absorption in

EPR. Therefore, if we place our sample in the electric field min-imum and the magnetic field maximum, we obtain the biggest signals and the highest sensitivity. The cavities are designed for optimal placement of the sample.

We couple the microwaves into the cavity via a hole called an iris. The size of the iris controls the amount of microwaves which will be reflected back from the cavity and how much will enter the cavity. The iris accomplishes this by carefully match-ing or transforming the impedances (the resistance to the waves) of the cavity and the waveguide (a rectangular pipe used to carry microwaves). There is an iris screw in front of the iris which allows us to adjust the "matching". This adjustment can be visu-alized by noting that as the screw moves up and down, it effec-tively changes the size of the iris. (See Figure 2-13.)
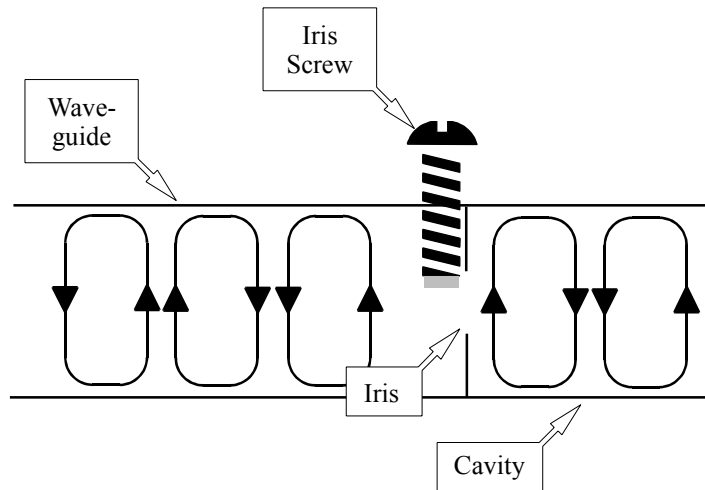
Figure 2-13   The matching of a microwave cavity to waveguide.

How do all of these properties of a cavity give rise to an EPR signal? When the sample absorbs the microwave energy, the Q is lowered because of the increased losses and the coupling

changes because the absorbing sample changes the impedance of the cavity. The cavity is therefore no longer critically coupled and microwave will be reflected back to the bridge, resulting in an EPR signal.

The Signal Channel   2.2.4

EPR spectroscopists use a technique known as phase sensitive detection to enhance the sensitivity of the spectrometer. The advantages include less noise from the detection diode and the elimination of baseline instabilities due to the drift in DC electronics. A further advantage is that it encodes the EPR signals to make it distinguishable from sources of noise or interference which are almost always present in a laboratory. The signal channel, a unit which fits in the spectrometer console, contains the required electronics for the phase sensitive detection.

The detection scheme works as follows. The magnetic field strength which the sample sees is modulated (varied) sinusoidally at the modulation frequency. If there is an EPR signal, the field modulation quickly sweeps through part of the signal and the microwaves reflected from the cavity are amplitude modulated at the same frequency. For an EPR signal which is approximately linear over an interval as wide as the modulation amplitude, the EPR signal is transformed into a sine wave with an amplitude proportional to the slope of the signal (See Figure 2-14.)

First Derivative
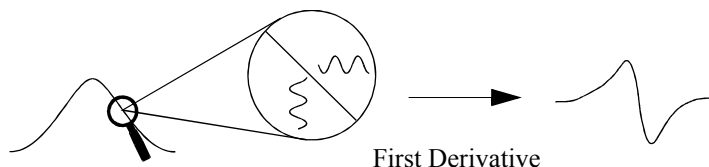
Figure 2-14   Field modulation and phase sensitive detection.

The signal channel (more commonly known as a lock-in amplifier or phase sensitive detector) produces a DC signal proportional to the amplitude of the modulated EPR signal. It compares the modulated signal with a reference signal having the same frequency as the field modulation and it is only sensitive to signals which have the same frequency and phase as the field modulation. Any signals which do not fulfill these requirements (i.e., noise and electrical interference) are suppressed. To further improve the sensitivity, a time constant is used to filter out more of the noise.

Phase sensitive detection with magnetic field modulation can increase our sensitivity by several orders of magnitude; however, we must be careful in choosing the appropriate modulation amplitude, frequency, and time constant. All three variables can distort our EPR signals and make interpretation of our results difficult.
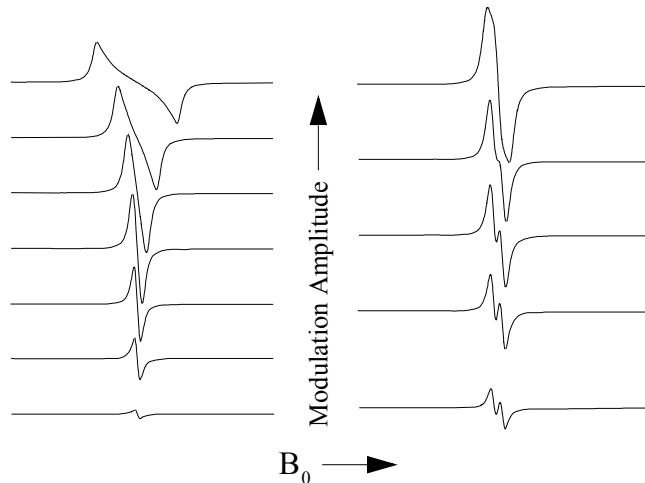
Figure 2-15   Signal distortions due to excessive field modulation.

As we apply more magnetic field modulation, the intensity of the detected EPR signals increases; however, if the modulation amplitude is too large (larger than the linewidths of the EPR signal), the detected EPR signal broadens and becomes distorted. (See Figure 2-15.) A good compromise between signal intensity and signal distortion occurs when the amplitude of the magnetic field modulation is equal to the width of the EPR signal. Also, if we use a modulation amplitude greater than the splitting between two EPR signals, we can no longer resolve the two signals.

Time constants filter out noise by slowing down the response time of the spectrometer. As the time constant is increased, the noise levels will drop. If we choose a time constant which is too long for the rate at which we scan the magnetic field, we can distort or even filter out the very signal which we are trying to extract from the noise. Also, the apparent field for resonance will shift. Figure 2-16 shows the distortion and disappearance of a signal as the time constant is increased. If you need to use a long time constant to see a weak signal, you must use a slower scan rate. A safe rule of thumb is to make sure that the time needed to scan through a single EPR signal should be ten times greater than the length of the time constant.
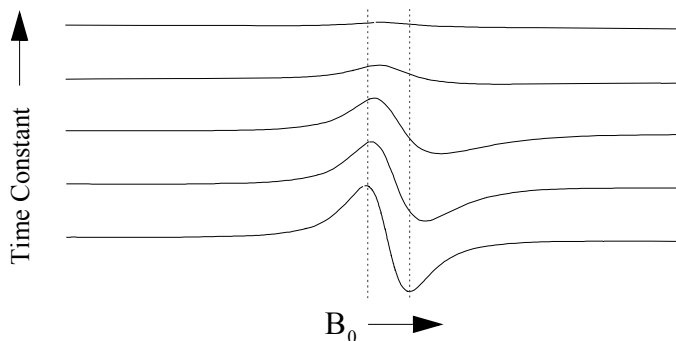
Figure 2-16   Signal distortion and shift due to excessive time constants.

For samples with very narrow or closely spaced EPR signals, (~ 50 milligauss. This usually only happens for organic radicals in dilute solutions.) we can get a broadening of the signals if our modulation frequency is too high (See Figure 2-17.) The broadening is a consequence of the Heisenberg uncertainty principle.
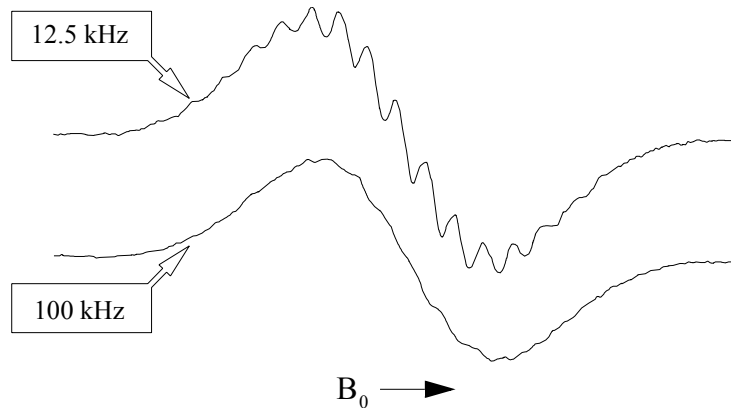
Figure 2-17   Loss of resolution due to high modulation frequency.

The Magnetic Field Controller   2.2.5

The magnetic field controller allows us to sweep the magnetic field in a controlled and precise manner for our EPR experiment. It consists of two parts; a part which sets the field values and the timing of the field sweep and a part which regulates the current in the windings of the magnet to attain the requested magnetic field value.

The magnetic field values and the timing of the magnetic field sweep are controlled by a microprocessor in the controller. A field sweep is divided into a maximum of 4096 discrete steps called sweep addresses. At each step, a reference voltage corresponding to the magnetic field value is sent to the part of the controller that regulates the magnetic field. The sweep rate is

controlled by varying the waiting time between the individual steps.
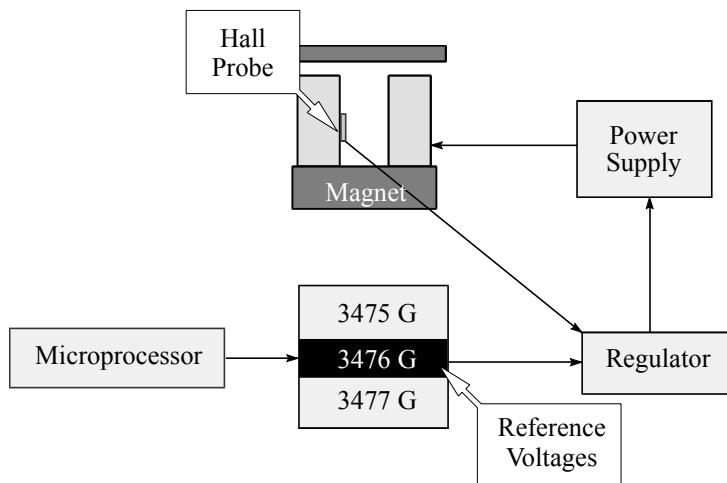
Figure 2-18 A block diagram of the field controller and associated components.

The magnetic field regulation occurs via a Hall probe placed in the gap of the magnet. It produces a voltage which is dependent on the magnetic field perpendicular to the probe. The relationship is not linear and the voltage changes with temperature; however, this is easily compensated for by keeping the probe at a constant temperature slightly above room temperature and characterizing the nonlinearities so that the microprocessor in the controller can make the appropriate corrections. Regulation is accomplished by comparing the voltage from the Hall probe with the reference voltage given by the other part of the controller. When there is a difference between the two voltages, a correction voltage is sent to the magnet power supply which changes the amount of current flowing through the magnet windings and hence the magnetic field. Eventually the error

voltage drops to zero and the field is "stable" or "locked". This occurs at each discrete step of a magnetic field scan.

The Spectrum 2.2.6

We have seen how the individual components of the spectrometer work. Figure 2-19 shows how they work together to produce a spectrum.
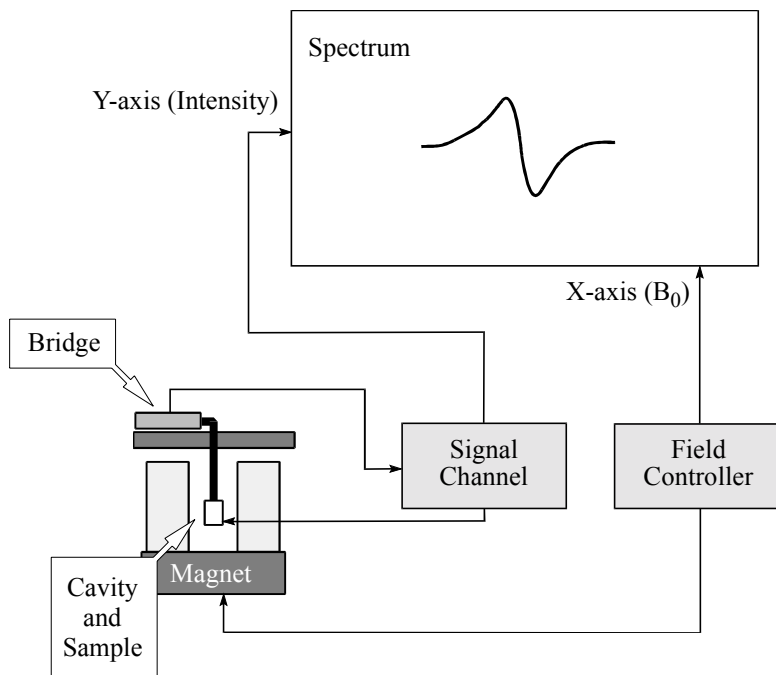
Figure 2-19   Block diagram of an EPR spectrometer.

Suggested Reading   2.3

This chapter is a brief overview of the basic theory and practice of EPR spectroscopy. If you would like to learn more, there are many good books and articles that have been written on these subjects. We recommend the following:

Instrumentation: Poole, C. *Electron Spin Resonance a Comprehensive Treatise on Experimental Techniques, Editions 1,2:* Interscience Publishers, New York, (1967), (1983).

Feher, G. *Sensitivity Considerations in Microwave Paramagnetic Resonance Absorption Techniques:* Bell System Tech. J. <u>36</u>, 449 (1957).

Theory: Knowles, P.F., D. Marsh and H.W.E. Rattle. *Magnetic Resonance of Biomolecules:* J. Wiley, New York, (1976).

Weil, John A., J.R. Bolton, and Wertz, J.E., *Electron Paramagnetic Resonance, Elementary Theory and Practical Applications:* Wiley-Interscience, New York, (1994).

A more extensive bibliography is found in last chapter of this manual.

Getting Started 3

This chapter contains basic operating instructions for first time users of a Bruker Elexsys E 500 spectrometer. It describes basic spectrometer operation with an X-band bridge (9.2-9.9 GHz). This chapter guides you from a completely shut down spectrometer to a hardcopy of your spectrum on a printer. You will learn to acquire an EPR spectrum of a standard sample with the Xepr software. There are also recommended precautions to prevent damage to the instrument. All the components have self-protecting features; however, it is good lab practice to follow correct operating procedures and not rely on the protection circuitry. No in-depth knowledge of EPR is required; however we recommend that you familiarize yourself with some of the material in Chapter 2. To help you in the following sections, Figure 3-1 assists you in identifying the various units which comprise the EPR spectrometer.
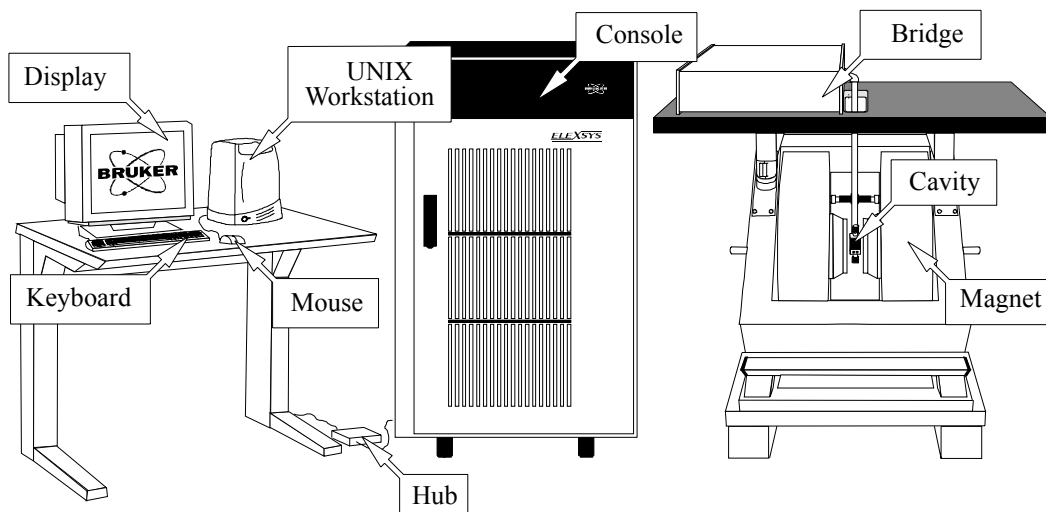
Figure 3-1   The modules and components of the Elexsys E 500 spectrometer.

Turning the Spectrometer On 3.1

If you are not sure how the electric power or water is connected, consult your local instrument or facilities manager.

1. **Turn on the power for the system.** How you do this depends on how the electric power was hooked up when the spectrometer was installed. Most likely you will activate the switch on the breaker box for the spectrometer. Breaker boxes are usually mounted on the wall. Consult the local instrument or facilities manager if you are not sure where the breaker box is.

2. **Turn on the tap water for cooling.** There are usually two valves, one for the supply and one for the return (or drain). Consult the local instrument or facilities manager if you are not sure where the valves are.

3. **Start the Xepr Program.** Log onto the UNIX workstation. If you are unfamiliar with UNIX, please refer to Appendix A or Appendix B for UNIX tips. We recommend using the xuser user account which is set up by the Bruker service engineer when the spectrometer is installed. The initial password for this account is user@xepr. Double-click the Xepr icon on the desktop to launch the program. (See Figure 3-2.)
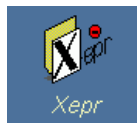
Figure 3-2 The Xepr program desktop icon.

4.   **Turn on the power for the console.** The power switch (green button) for the console is located in the upper left front corner of the console. (See Figure 3-3.) The acquisition server will boot and initialize all the modules of the Elexsys E 500 spectrometer. This process may take 30 seconds or longer: meanwhile you can continue with the next steps.
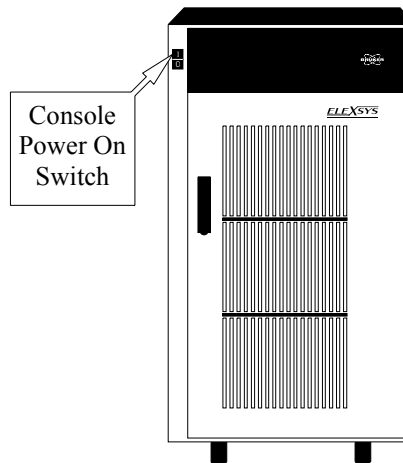
Figure 3-3   The location of the console power switch.

5. **Turn on the heat exchanger and magnet power supply. (Instructions for Small Power Supplies.)** Follow this step if your power supply looks like the power supply in Figure 3-4. You must first turn the heat exchanger on by activating the power switch. The location of the power switch may vary depending on your heat exchanger. To turn the power supply on, push its POWER ON/OFF button. Go to Step 7.

Power Switch

Power On/Off Switch

| The small ER 081 power supply. | The heat exchanger. |
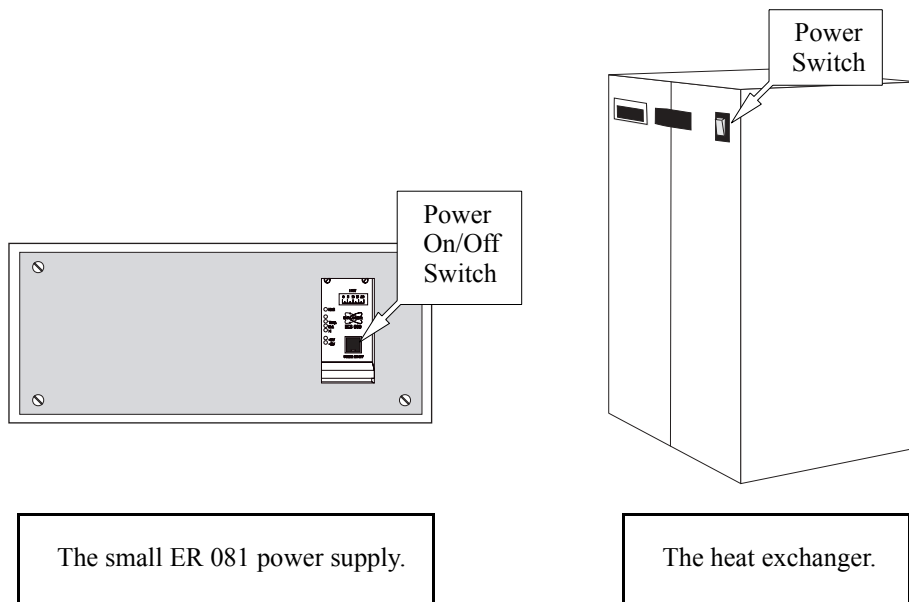
Figure 3-4   The small ER 081 power supply and the heat exchanger.

6. **Turn on the heat exchanger and magnet power supply. (Instructions for Large Power Supplies)** Follow this step if your power supply looks like the power supply in Figure 3-5. On systems with large power supplies, you need to first press the ELECTR. ON button and then the POWER ON button. Pressing the POWER ON button also starts the heat exchanger. If not, make sure that the power switch on the heat exchanger is activated.

Electricity On

Power On

Power Switch

The large ER 082/083/085/086 power supplies.
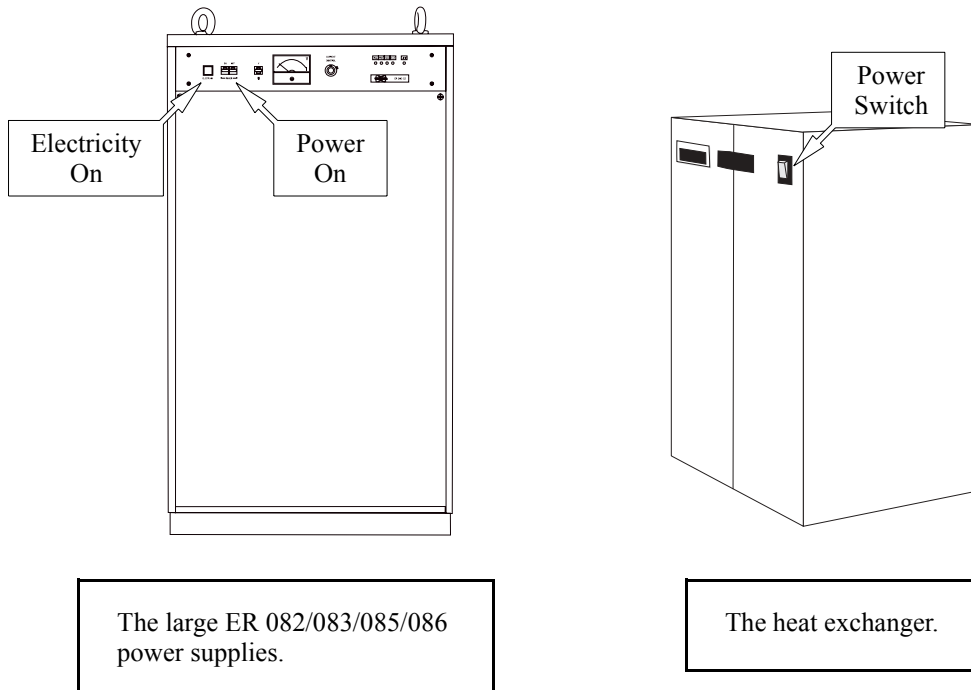
The heat exchanger.

Figure 3-5   The large power supply and the heat exchanger.

7. **Connect to the spectrometer.** To control the spectrometer from the Xepr program you need to connect the workstation to the spectrometer. Click the Acquisition menu bar and then Connect to Spectrometer. A dialog box will appear with the Server Name: click OK. (See Figure 3-6.)
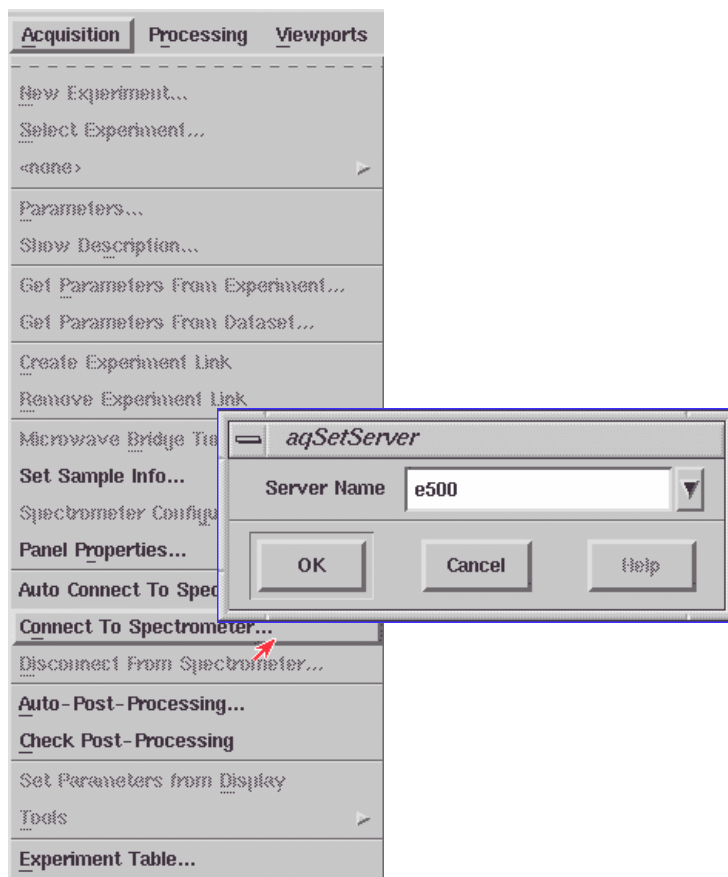
Figure 3-6   Connecting to the spectrometer.

If you were not successful in connecting to the acquisition server, consult Chapter 17 of the Elexsys E 500 User's Manual: Advanced Operations.

When the connection is complete a monitoring panel will appear in the Xepr window. (See Figure 3-7.) The monitoring panel may appear on top of the viewport or at the bottom of the viewport depending on your default settings.
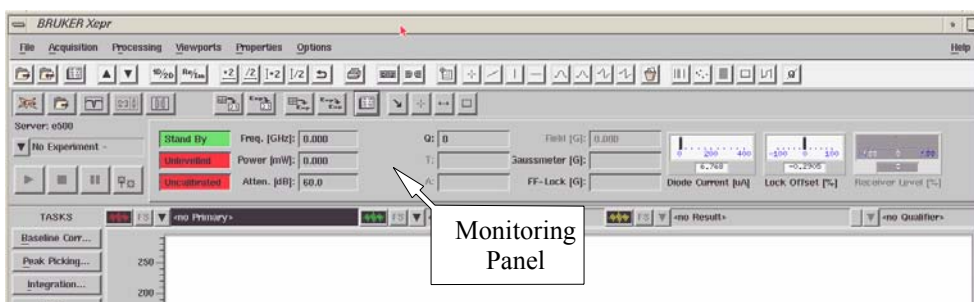
Figure 3-7   The Xepr window after connecting to the spectrometer.

8.   **Install an EPR cavity if there is not one presently installed.** The instructions in this chapter assume you are using a properly installed Bruker ER 4122SHQ or ER 4102ST cavity. If there is no cavity installed or the installed cavity is not one of them, seek the assistance of a knowledgeable EPR colleague or refer to Section 7.2 to learn how to install a cavity.

9.   **Proceed to Section 3.2**.

Tuning the Microwave Cavity and Bridge   3.2

1. **Open the Microwave Bridge Tuning dialog box.** Click the Tuning button in the monitoring panel. The Microwave Bridge Tuning dialog box will then appear. (See Figure 3-8.)
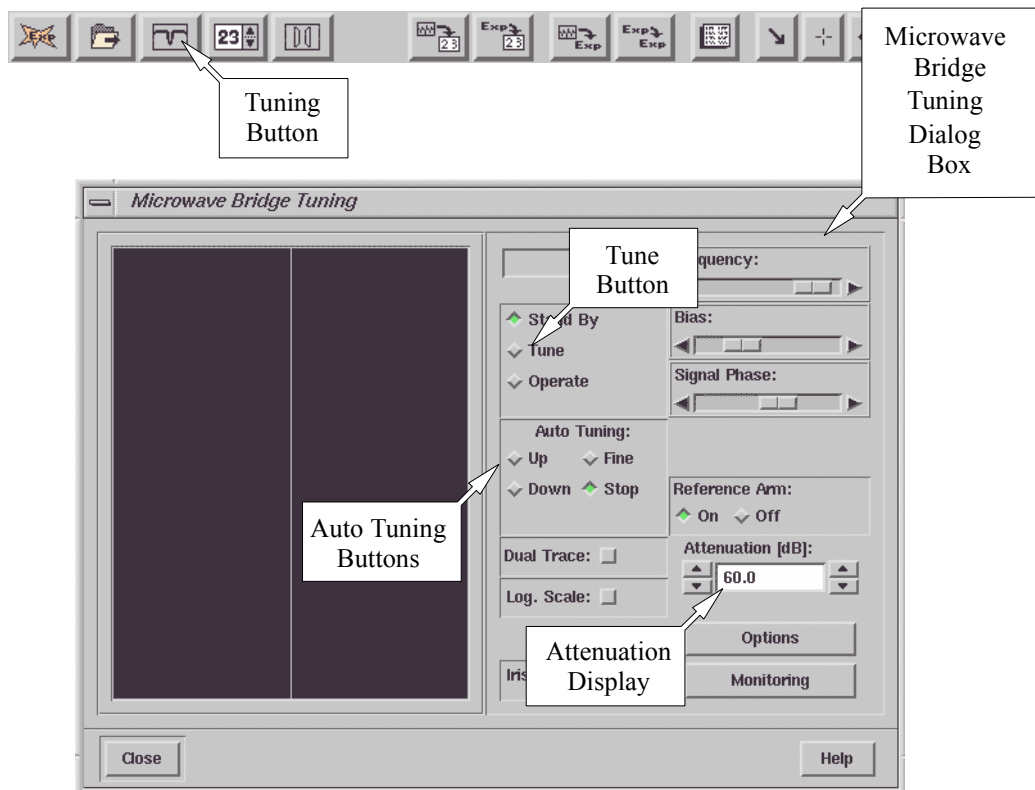
Figure 3-8   The Microwave Bridge Tuning dialog box.

There are three states or modes for the micro-wave bridge: StandBy, Tune, and Operate. When you connect to your spectrometer, the bridge is in Stand By, indicated by a green Stand By button. (See Figure 3-8.) If you have been acquiring spectra already, your bridge will probably be in Operate.

Take care if you are wearing an analog (mechanical) watch. The magnetic field in the air gap of the mag-net is sufficiently strong to magnetize your watch! Therefore, to avoid damage to your watch, remove your watch before put-ting your hands in the magnet air gap.

2. **Switch the microwave bridge to Tune.** Click the Tune button in the dialog box to change to Tune.

3. **Set the microwave attenuator to 30 dB.** The micro-wave attenuation is set by clicking the arrows on either side of the attenuation display in the dialog box. (See Figure 3-8.) The arrows on the left change the attenuator in 10 dB steps; those on the right in 1 dB steps.

4. **Remove the sample.** If there already is a sample in the cavity, remove it. Loosen the top collet nut (You do not need to remove the collet nut.) and carefully remove the sample from the cavity. Pulling the sample tube out as straight as possible prevents you from breaking the sam-ple tube thereby destroying your valuable samples. (See Figure 3-9 and Figure 3-10 for details.) The ER 4122SHQ cavity is shown in the figures; however, the procedure of removing and inserting a sample is the same for ER 4102ST, ER 4122SHQ, and ER 4122SHQE cavi-ties.

Figure 3-9   A Bruker ER 4122SHQ cavity.
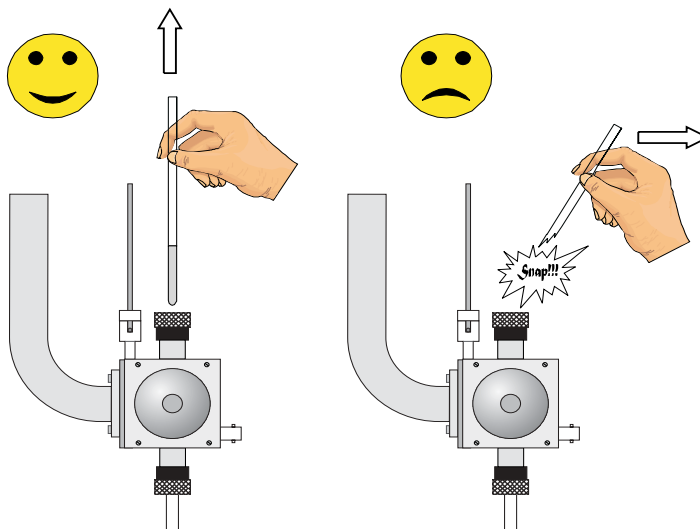
Figure 3-10   The right and wrong technique for removing a sample.

5. **Clean the sample tube to be inserted into the cavity.** It is vital to avoid contaminating the microwave cavity with paramagnetic contaminants that produce spurious EPR signals or distorted base lines. Wiping the outside of the sample tube with tissue paper is usually adequate.

If this is your first time operating an Elexsys E 500 spectrometer, we recommend that you use the strong pitch sample supplied with your instrument. Our instructions in this chapter are based on using this sample.

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Make sure that the pedestal is not in the cavity, as it can give an EPR signal and will also degrade the sensitivity.

6. **Insert the sample tube carefully into the cavity.** (See Figure 3-9 and Figure 3-10.) Make sure you have the appropriate collet size for your sample tube size. The tube should be slightly loose before you tighten the collet nut. The bottom of your sample should rest in the indentation on the pedestal. This ensures that your sample is centered horizontally. The height can be adjusted by loosening the bottom collet nut and moving the pedestal up and down. Tighten the top collet nut to firmly hold the sample tube in place and the bottom collet to firmly hold the pedestal.

7. **Tune the bridge and cavity.** Pressing either the Up button or Down button of Auto Tune starts the automatic tuning procedure. (See Figure 3-8.) The Up button starts by scanning the microwave frequency up in search of the cavity dip (or frequency where the cavity resonates). The Down button starts by scanning the microwave frequency down in search of the cavity dip. If you are not sure if the search should start up or down, do not worry. The frequency will be scanned until its limit is reached and then scan in the other direction until the cavity dip is found. The Auto-Tune routine adjusts the frequency, phase, and bias of the bridge and the coupling (matching) of the cavity. If there is an error message, try manual tuning described in Section 7.1. If you still have difficulty in tuning, it usually means that you have a lossy or conductive sample. Refer to Chapter 9 for trouble shooting.

8. **Close the Microwave Bridge Tuning dialog box.** Click the Close button. The Microwave Bridge Tuning dialog box will then disappear. (See Figure 3-8.)

9. **Proceed to the next section to learn how to acquire spectra.**

Acquiring Spectra   3.3

1. **Follow the instructions of Section 3.1 through Section 3.2.** You should have the spectrometer turned on, a Bruker ER 4102ST standard cavity, ER 4122SHQ or ER 4122SHQE cavity installed, a strong pitch sample inserted in it, and the microwave bridge and cavity tuned.

Figure 3-11   The New Experiment and Parameter buttons.
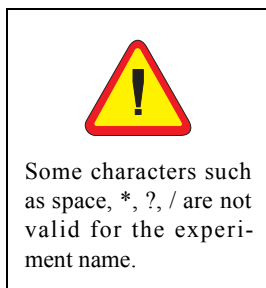
2. **Create a New Experiment.** In order to acquire a spectrum, you need to define an experiment. To create a new experiment, click the New Experiment button in the monitoring panel. (See Figure 3-11.) In the New Experiment dialog box, enter a name for the new experiment. By default the name is Experiment. Click the C.W. tab. Choose Field for Abscissa 1 and None for Abscissa 2. If the Ordinate is not Signal Channel select Signal Channel in the Ordinate drop-down list. If the buttons for Temp Unit (the variable temperature unit), Goniometer, and Gradient Unit are activated (green) click on it to deactivate it. (See Figure 3-12.) Click Create and then the dialog box will close. The spectrometer will now be configured with these settings.

Some characters such as space, *, ?, / are not valid for the experiment name.
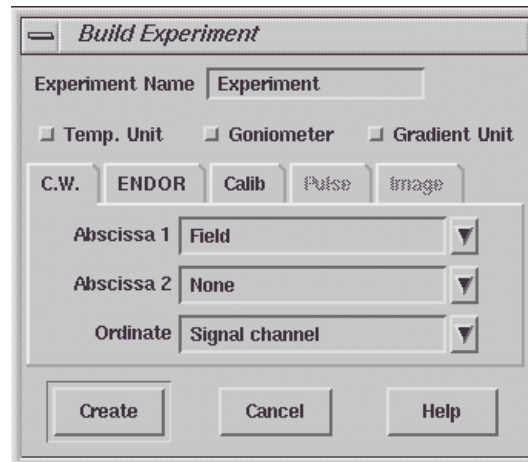
Figure 3-12 The New Experiment configuration.

It is very important to enter the sample information so that you maintain a record of the sample used to acquire the spectrum. Comments are very useful because you can keep track of such things as sample preparation details.

3. **Set Sample Information.** Click Acquisition in the menu bar, and then click Set Sample Info. A sample information dialog box opens in which you can type information regarding your sample. Enter your sample information and then click OK to close the window.
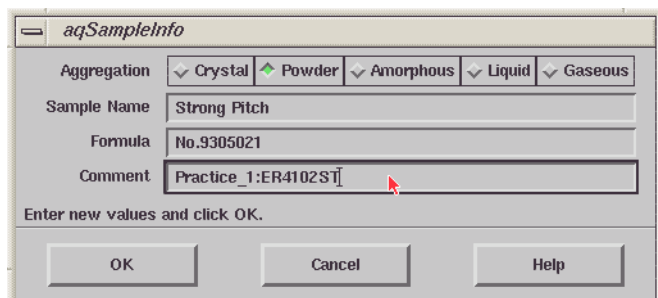
Figure 3-13 The sample information dialog box.

4. **Choose the Interactive Parameter Setting mode.**
    Press the Activate button. (See Figure 3-14.) This button
    toggles between interactive and non-interactive parameter
    setting modes. As you edit parameters in the parameter
    panel in interactive mode, the new parameters are imme-
    diately activated on the spectrometer. In the non-interac-
    tive mode, you can edit instrument parameters in the
    parameter panel without activating them on the instru-
    ment. In this mode, the new parameters are only activated
    when you click the Run button.
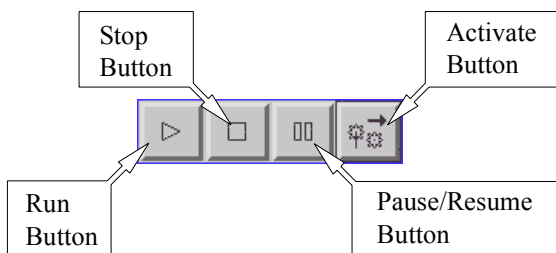
Figure 3-14   The experiment control tools.

5. **Open the Acquisition Parameters dialog box.**
    Click the Parameter button in the tool bar to open the
    dialog box. (See Figure 3-11.)

If the Calibrated button is not green (activated) you need to load a proper calibration file. Consult the local facility manager or refer to Section 7.2 to find out how to load the calibration file.

6. **Check the Signal Channel parameters.** Click the Signal Channel tab. The signal channel parameters are further split into four tabs. Click the Detection tab. Edit the parameters in the dialog box so that they match those shown in Figure 3-15. The other tabs in the Signal Channel tab are for advanced options and their default settings are OK for this experiment.
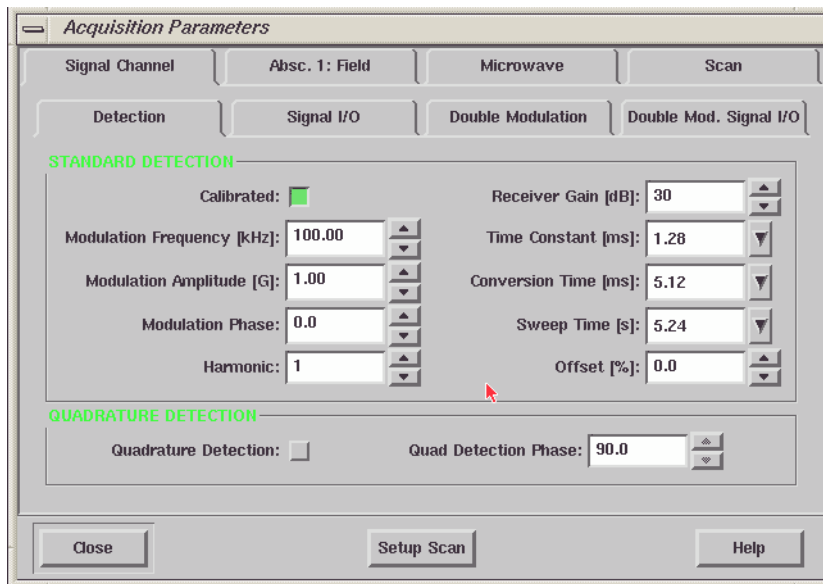
Figure 3-15   The signal channel parameters.

7. **Check the Field parameters.** Click the Absc.1: Field tab. (See Figure 3-16.) Edit the parameters in the dialog box so that they match those shown in Figure 3-16.
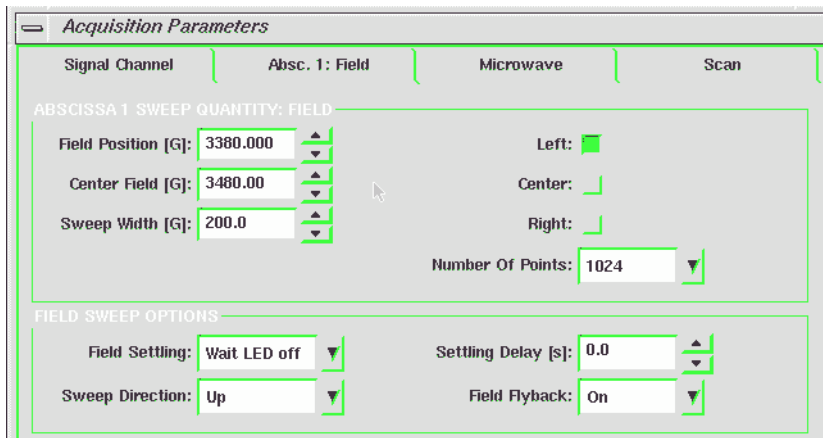
Figure 3-16 The magnetic field parameters.

8. **Check the Microwave parameters.** Click the Microwave tab. (See Figure 3-17.) Set the Attenuation to 30.0 dB.
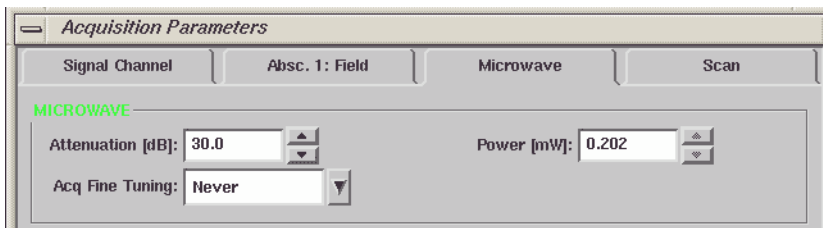
Figure 3-17 The microwave parameters.

9. **Check the Scan parameters.** Click the Scan tab. (See Figure 3-18.) Edit the parameters in the dialog box so that they match those shown in Figure 3-18. After you have finished all the above parameter settings click the Close button.
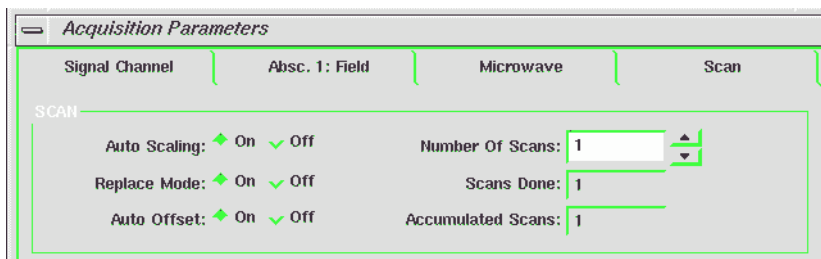
Figure 3-18   The scan parameters.

10. **Acquire a spectrum.** Click the RUN button to start an acquisition. (See Figure 3-19.) If you have a spectrum similar to the one in Figure 3-20, congratulations! You have successfully acquired an EPR spectrum.
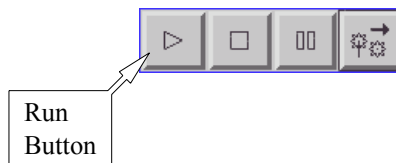
Figure 3-19   The Run button.
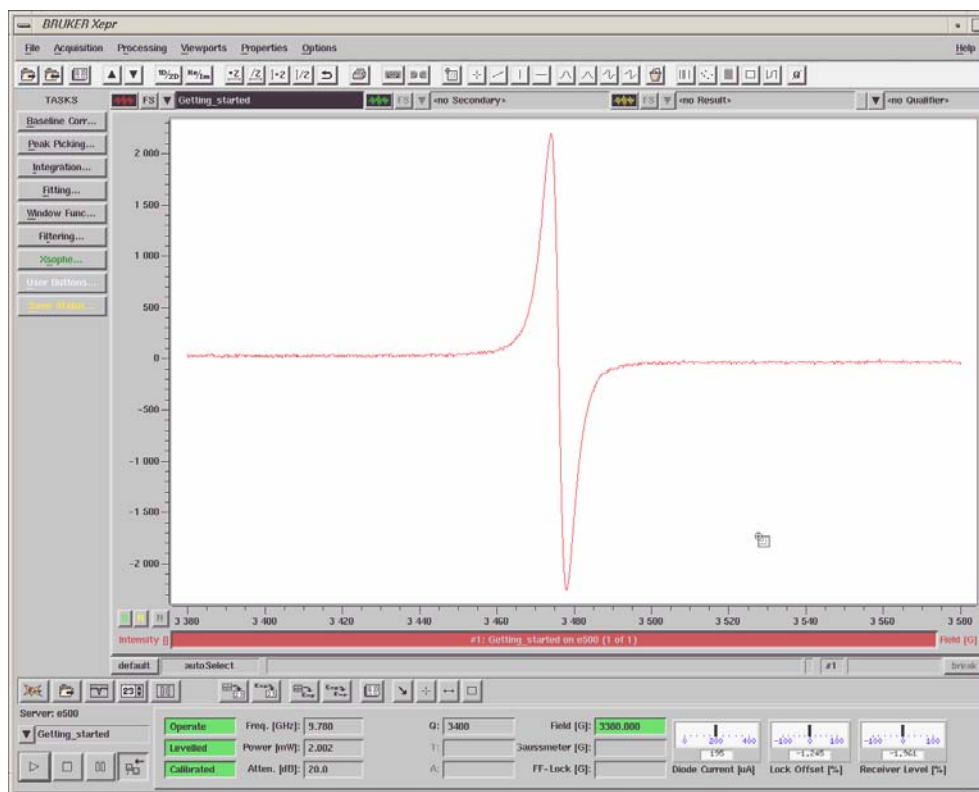
Figure 3-20   An EPR signal of strong pitch.

11.   **Center the spectrum.** You may notice that the EPR line is not nicely centered. The next step will help you center your spectrum. To interactively set the center field, click Acquisition > Tools, and then the Center Tool in the menu bar. (See Figure 3-21.)
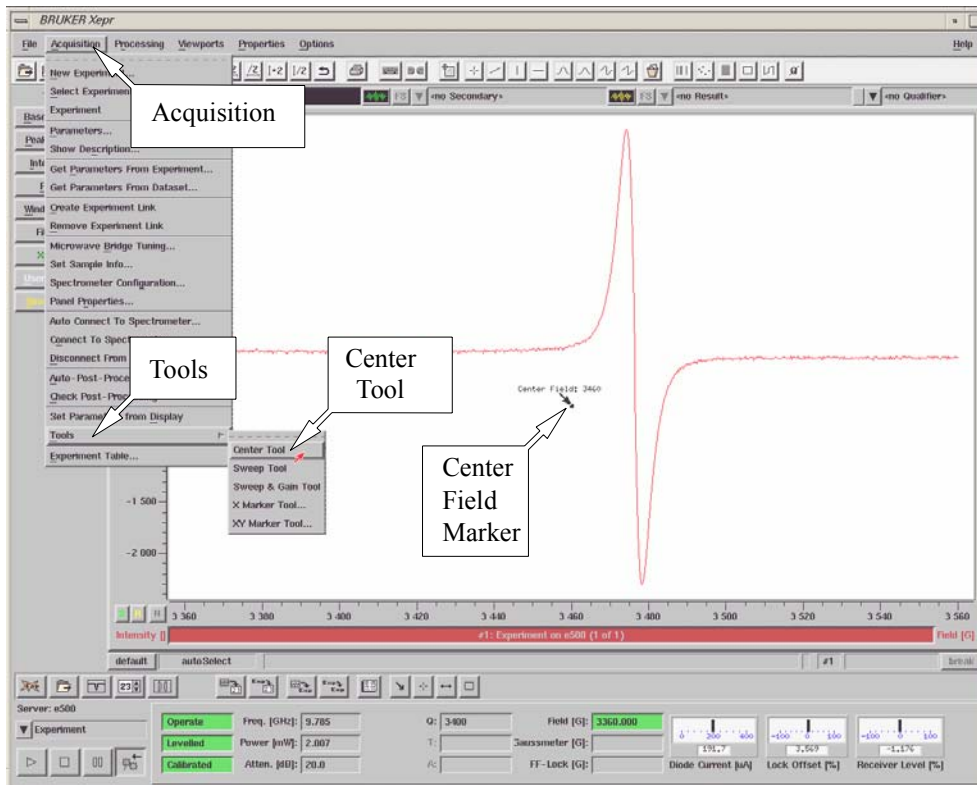
Figure 3-21   The Center Tool.

A center marker in the viewport appears. Drag the marker and place it where you would like the center field to be. (See Figure 3-22.)
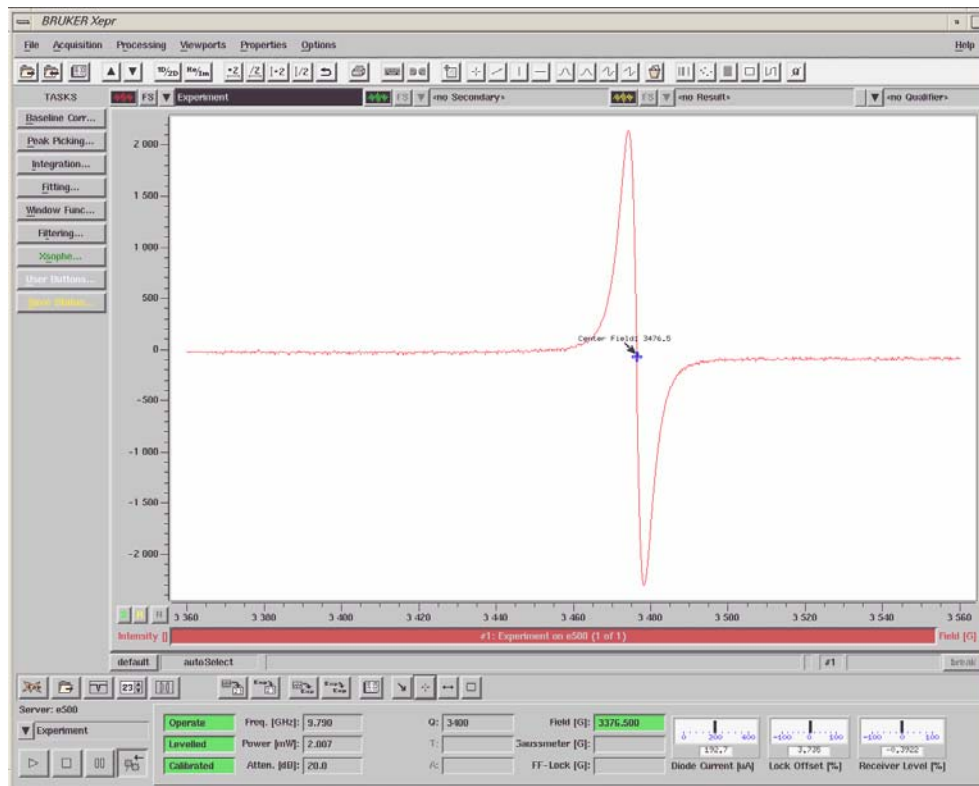

Figure 3-22   Placing the center marker.

This action replaces the center field value with the magnetic field position of the marker. To acquire the spectrum with the new center field, click the RUN button in the monitoring panel. The newly acquired spectrum will then be nicely centered. (See Figure 3-23.)
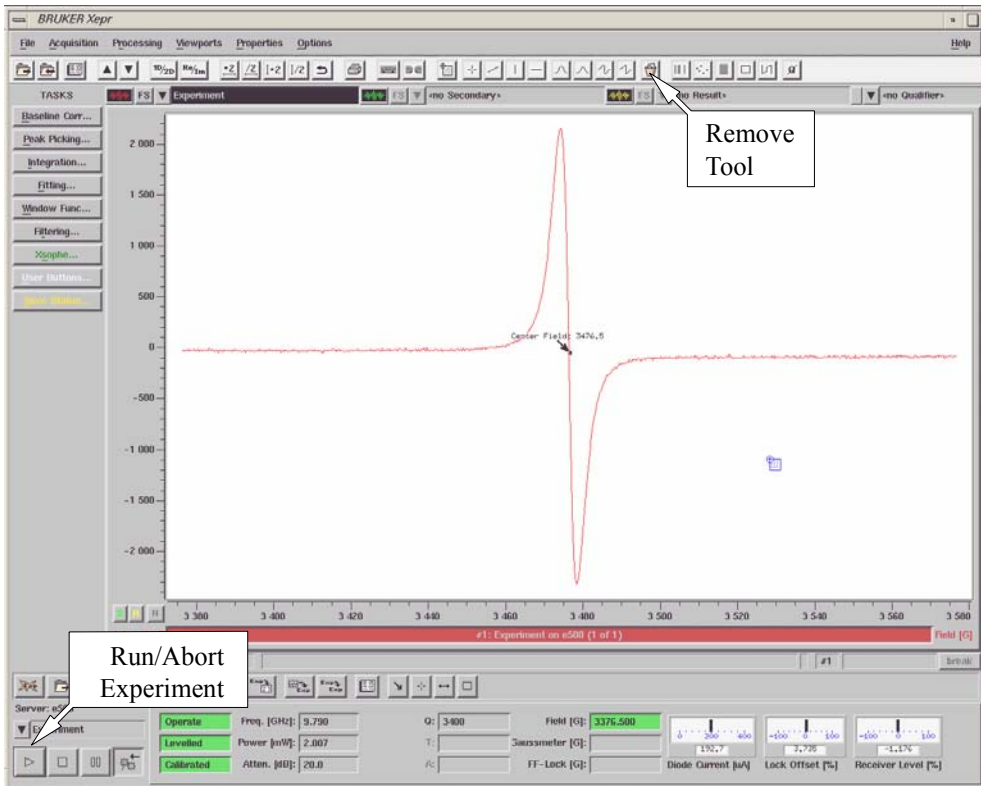

Figure 3-23   The centered spectrum.

If the tool cannot be removed in an active viewport it may be because the tool is inactive. Click the tool to activate it and then click the Remove Tool button to remove it.

12. **Remove the center marker.** You may remove the center marker by clicking the Remove Tool button in the tool bar. (See Figure 3-23.) The Remove Tool function removes the current active tool.

BRUKER

Storing and Saving the Spectrum 3.4

After you have acquired your spectrum, you may wish to save or store it. What is the difference between these two commands? The Store command stores the spectrum temporarily in memory which means it is lost when you exit Xepr. The Save command saves a permanent data file on your hard disk for future reference. Typically, the Store command is used to store intermediate results and the Save command is used to permanently save the results of your data processing.

1. **Store your spectrum.** Click the menu button in the viewport. Click Store in the drop-down menu. (See Figure 3-24.) The Store dialog box allows you to enter a descriptive title for the dataset. The presently active spectrum is stored in memory when you click Store.
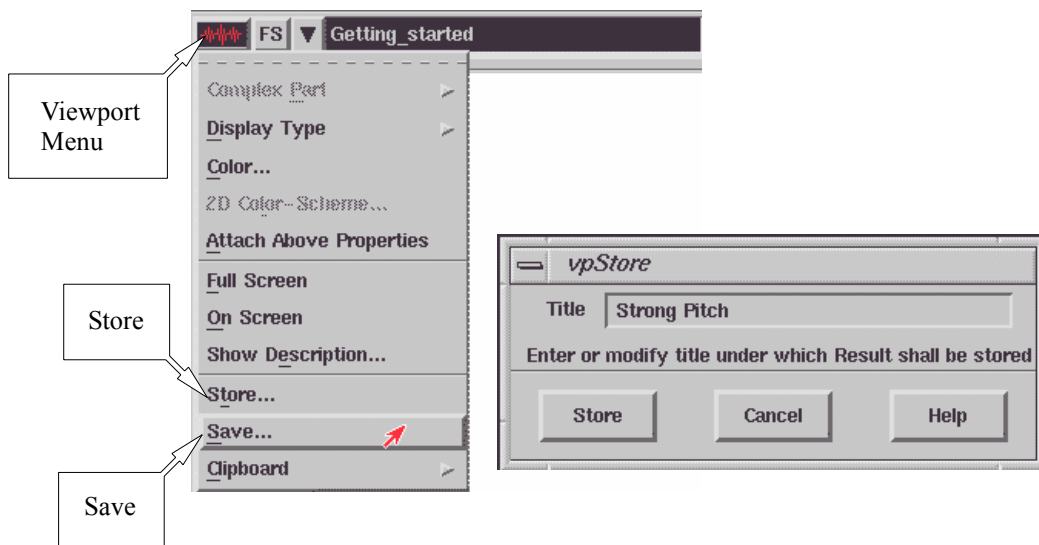

Figure 3-24 The Save, Store buttons and Store dialog box.

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Do not save data in the ../xeprFiles/Data/ sharedData directory. This is a protected directory and your data will not be saved. You will receive an error message.

⚠

Some characters such as space, *, ?, / are not valid for file names.

2. **Save your spectrum.** Click the menu button in the viewport. Click Save in the drop-down menu. (See Figure 3-25.) Enter a descriptive title for the dataset in the Title box. The Save dialog box lets you enter a file-name and destination directory. (See Figure 3-25.) To select the desired directory, click the appropriate paths under Group. Clicking on .. brings you to the parent directory. Enter the file name in the File box. Clicking OK saves the presently active spectrum on the hard disk. Note that the spectrum is also stored in memory when saved.
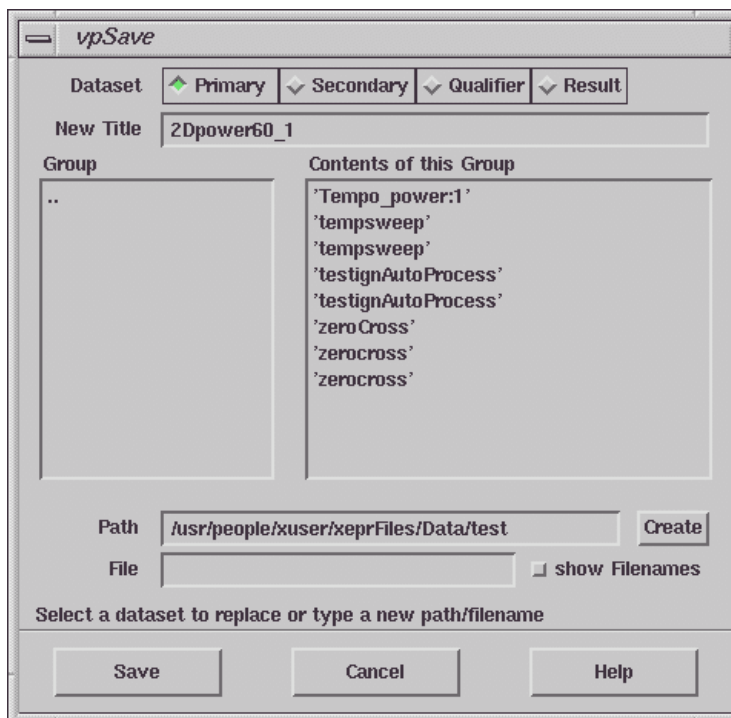

Figure 3-25   The Save dialog box.

If the chosen filename is already used for another file in the same directory, a warning box gives you the opportunity to decide whether to overwrite the existing file with the present spectrum. (See Figure 3-26.) Clicking No cancels the save process and allows you to select another name or directory.
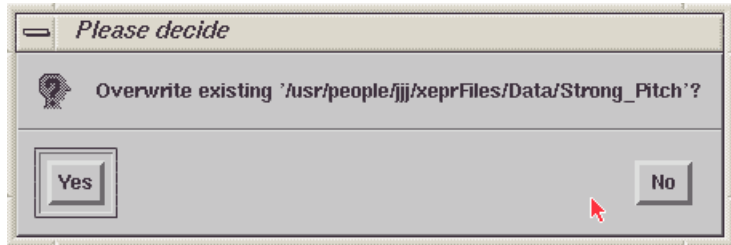

Figure 3-26   The warning dialog box for overwriting files.

Printing the Spectrum 3.5

1. **Prepare to print the spectrum.** Turn the printer on. Make sure that the paper is loaded. Refer to your printer documentation for details. Make sure the printer is properly set up. (See Sections 15.8 or 16.8 of the Elexsys E 500 User's Manual: Advanced Operations.)

2. **Print the spectrum.** Click File in the menu bar and then Print. A dialog box will then appear in which you select desired options and print your spectrum. (See Figure 3-27.)

Is your printer on line?

If you experience problems printing, consult the local facility manager or refer to See Sections 15.8 or 16.8 of the Elexsys E 500 User's Manual: Advanced Operations. to find out how to setup a printer.
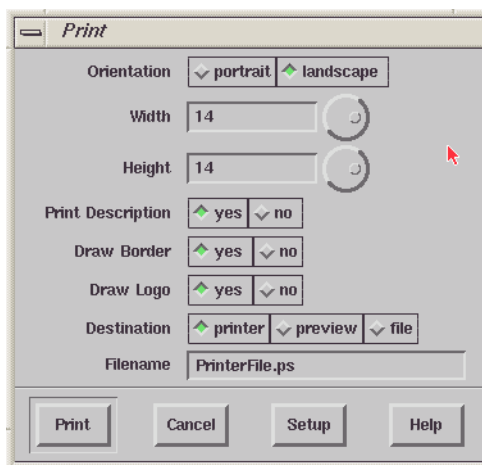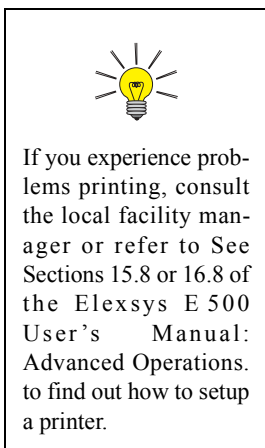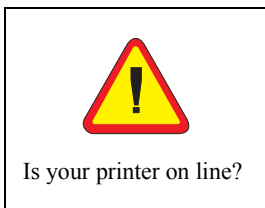

Figure 3-27 The Print dialog box.

Select the options shown in the above figure. Clicking Print starts the document printing and closes the dialog box. The output from the printer will look similar to Figure 3-28.
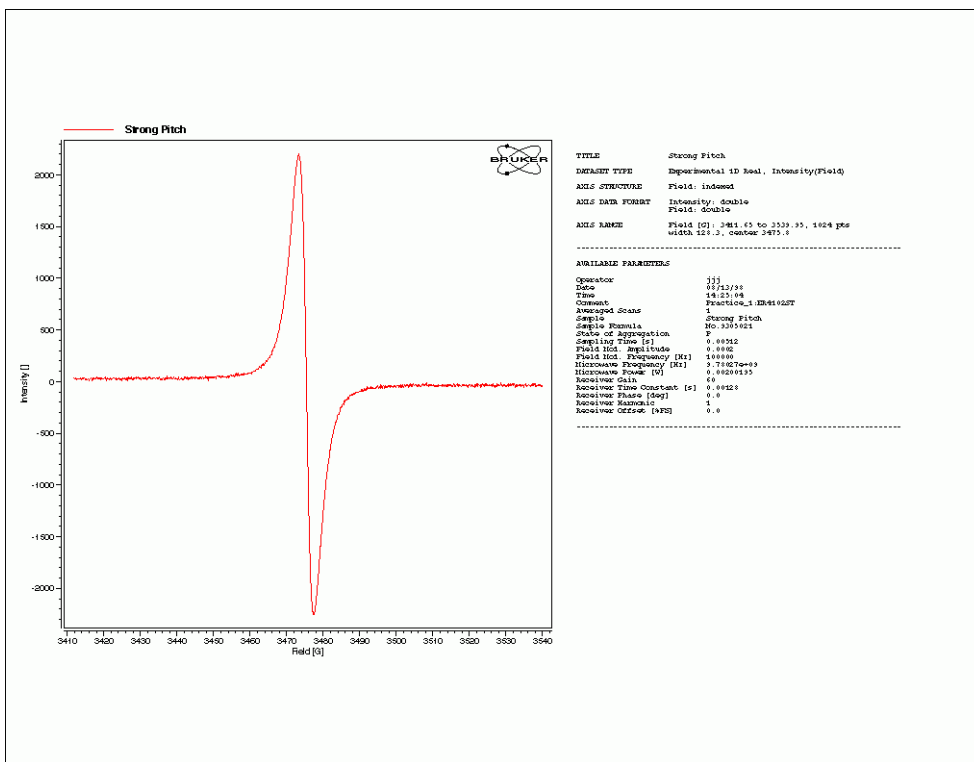
Figure 3-28   Typical output from the printer.

Turning the Spectrometer Off   3.6

1. **Open the Microwave Bridge Tuning dialog box.** If this window is not already open, click its button in the tool bar. The Microwave Bridge Tuning dialog box will then appear. (See Figure 3-29.)
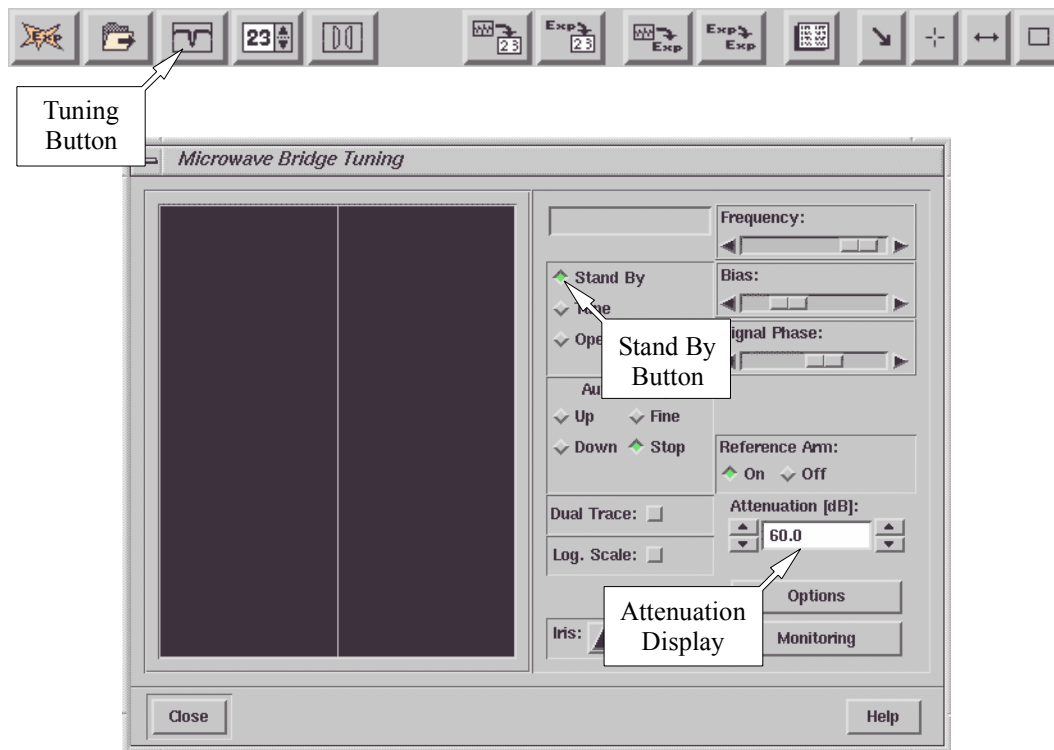

Figure 3-29   The Microwave Bridge Tuning dialog box.

It is important to exit the software in an orderly manner (*i.e.* don't just turn the computer off before disconnecting the instrument and exiting the software) because many instrument parameters are set to specific values for a safe shut-down of the spec-

Exit the software first before logging out.

2. **Switch the microwave bridge to Stand By.** Click the Stand By button in the dialog box to change to Stand By. (See Figure 3-29.) The microwave attenuator will be automatically set to 60 dB.

3. **Close the Microwave Bridge Control dialog box.** Click the Close button in the dialog box. The Microwave Bridge Tuning dialog box will then disappear.

4. **Remove the sample from the cavity.** See Section 3.2 for details on how to do this.

5. **Cover the upper collet or insert a solid collet plug.**

6. **Disconnect from the spectrometer.** Click the Acquisition menu bar and then click Disconnect From Spectrometer. The monitoring panel will disappear.

7. **Exit the Xepr program.** Click the File menu bar and then click Exit. You will be asked if you wish to save the changes.

8. **Log Out**. Click the right mouse button (for SGI O2 workstations) or click on the Gnome icon on the bottom of the screen (for the Linux workstations) and click Log Out in the drop-down menu. You need to confirm this by clicking Yes or select the proper radio button and click OK.

9. **Turn off the heat exchanger and magnet power supply. (Instructions for Small Power Supplies.)** Follow this step if your power supply looks like the power supply in Figure 3-30. To turn the power supply off, push the POWER ON/OFF button. Turn the heat exchanger off by pressing the power switch. The location of the Power ON/OFF button may vary depending on your heat exchanger. Go to Step 11.

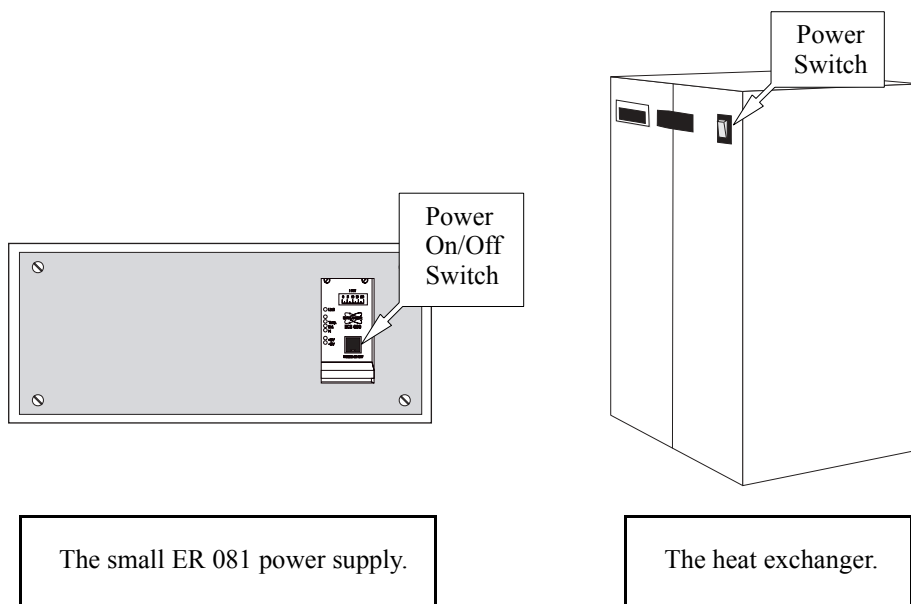| The small ER 081 power supply. | The heat exchanger. |

Figure 3-30   The small ER 081 power supply and the heat exchanger.

10. **Turn off the heat exchanger and magnet power supply. (Instructions for Large Power Supplies)**
     Follow this step if your power supply looks like the power supply in Figure 3-31. On systems with large power supplies, you need to first press the Power OFF button and then the ELECTR. ON button. Pressing the POWER OFF button also turns the heat exchanger off.

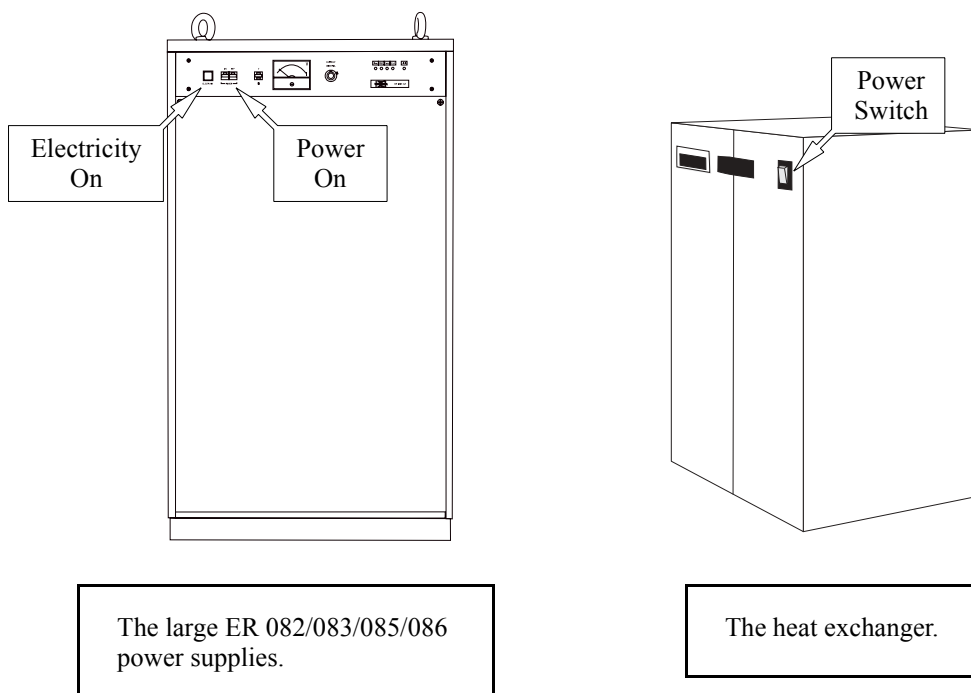The large ER 082/083/085/086 power supplies.

The heat exchanger.

Figure 3-31   The large power supply and the heat exchanger.

11. **Turn off the tap water for cooling.** There are usually two valves, one for the supply and one for the return (or drain). Consult the local instrument or facilities manager if you are not sure where the valves are.

12. **Turn off the power for the console.** The power switch (red button) for the console is located in the upper left front corner of the console. (See Figure 3-32.)

To reduce wear and tear on the hardware it is best to leave the workstation on unless it is not going to be used for a long time or there will be a power outage. Consult the workstation documentation or the SGI manual to learn how to turn off the workstation.
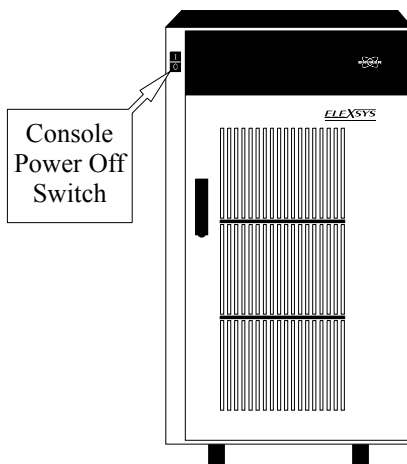

Figure 3-32   The location of the console power switch.

If you have many power outages or electrical storms, it is a very good idea to shut off the power to the spectrometer.

13. **Turn off the power for the system. (Optional)** How you do this depends on how the electric power was hooked up when the spectrometer was installed. Most likely you will deactivate the switch on the breaker box for the spectrometer. Breaker boxes are usually mounted on the wall. Consult the local instrument or facilities manager if you are not sure where the breaker box is.