

Application Notes # MT-72

An EPR Imaging Quick Reference Guide to Data Collection and Processing

The numerous parameters and hardware features associated with EPR imaging can be quite daunting at first. This guide was created to help facilitate image acquisition and processing and provide information relating to the gradient configuration and transformations.

Image Axis Orientation

The orientation of the directions indicated in the *Image Plane* parameter of the *Acquisition Parameters* with respect to the magnet are indicated in Figure 1. The center of the axis is dependent on the gradient coil calibration, typically the origin will be the center of the resonator.

1D Images: Field and Axis Orientation

In 1D Images the angles, Θ and Φ , of the gradient define the orientation of the axis being observed, as indicated in Figure 2. The origin of the axis is determined by measuring the field swept EPR spectrum with the gradient off. Using the negative of the angles, Θ and Φ , from those indicated in Figure 2, or using a negative *Gradient* will invert the spatial axis about the origin and the current will be negative. The field axis of 1D Images is processed so that the x-axis will be in cm with the correct sign of the directions with respect to the origin.

Figure 1

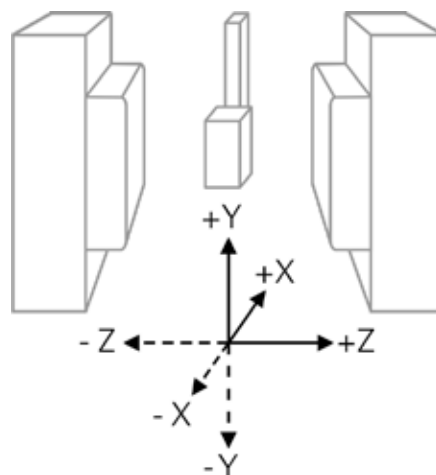
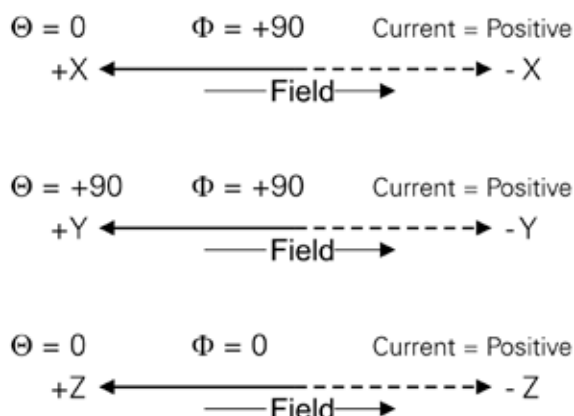


Image directions relative to the EPR Magnet

1D Images: Image Acquisition and Processing

1. Create Experiment: Choose a CW experiment in the *Build Experiment* panel with Abscissa 1 as Field and select the Gradient Unit (small box becomes green).
2. Set EPR Parameters: Choose the appropriate settings for data collection, i.e. modulation amplitude, field range, etc.

Figure 2

Gradient orientation and Axis orientation for a positive Gradient with respect to field sweep direction

3. Set Gradient Direction: Under the *Gradient Unit* tab of the *Acquisition Parameters* window, enter the Φ and Θ according to Figure 2 to select the desired gradient.
4. Set Gradient Strength: In the same tab, enter a value for Gradient (Gauss / cm). For higher resolution a larger value for the gradient is needed.
5. Acquire the 1D Image: Press the *Play* button to acquire the data with the gradient applied and store/save the data after acquisition.
6. Acquire Reference Spectrum: In the *Gradient Unit* tab, set the Gradient to 0 G/cm, press the *Play* button to acquire the data and the store/save the data after acquisition.
7. Spectrum Deconvolution:
 - a. Set the Image data (step 5) as the *Primary Dataset* and the Reference data (step 6) as the *Secondary Dataset*.
 - b. Select the menu *Processing - Transformations - Deconvolution*.
 - c. Adjust the Window and Width parameters for desired image quality. This is a trial-and-error procedure. The Window type affects the shape of the cut-off filter, with the Fermi-Dirac giving sharper images but may produce high frequency noise in the image while the Gaussian is the inverse. The Width is where the cut-off begins: a smaller number gives less noise and a higher number gives more noise. For data with a high signal to noise, a large Width (0.3) with the Fermi-Dirac window is a good starting point. For data with a low signal to noise, a Gaussian Window with a Width of 0.1 is a good starting point.
 - d. Click *Apply*, copy the *Result Dataset* to the *Primary Dataset*, and store/save the data.
8. Transform Field Axis to Distance:
 - a. Deconvolute the reference spectrum with itself using

the same parameters used in step 7c.

- b. From the deconvoluted reference spectrum record the field value for the center of the EPR line.
- c. Set the *Primary Dataset* to the deconvoluted Image data.
- d. Select the menu *Processing - Transformations - Linear*.
- e. Select *Relative to Zero* at the top of the window (diamond will turn green).
- f. Enter the Negative of the value from step 8b into the x-Shift field and select *Apply*.
- g. Determine the reciprocal (1/Gradient) of the gradient strength used (step 4).
- h. Enter the Negative of the value from step 8g into the x-Stretch field and select *Apply*.
- i. The x-axis is now in units of cm with the correct sign for the spatial dimension.
- j. Store/Save the data.

2D & 3D Image Acquisition

The acquisition of 2D and 3D Images are essentially the same in procedure with only one difference in experiment parameters. Similarly the processing of the data is the same and will be covered together.

1. Create Experiments: Two experiments will be used for image acquisition.
 - a. Choose a CW experiment in the *Build Experiment* panel with Abcissa 1 as Field, Abcissa 2 as None, and with the *Gradient Unit* not selected (small box is gray). Set the Experiment Name to Reference. Select *Create*.
 - b. Choose the *Image* experiment tab. Set the Experiment Name to Image. Select *Create*.

Figure 3

2D	Planar spatial image where each pixel is the intensity integral of the spin density along dimension not specified in Image Plane.
3D	Volume spatial image. 2D view of processed data is a series of 2D slices along the non-specified dimension according to the plane specified in Image Plane.
1D + Spectral	
2D + Spectral	
3D + Spectral	Spatial image with added dimension containing the EPR spectrum for each point, pixel or voxel. Used when multiple EPR species are present in the sample and the spatial distribution of each species is desired.

Image types available in Image experiment

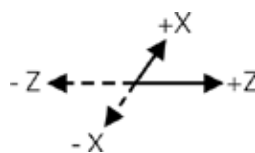
Figure 4

ZX

Top-Down View

Intensity Integral along Y-dimension.

2D-Image view: x-axis is Z-dimension and y-axis is X-dimension.

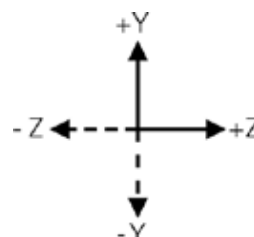


ZY

Front-Back View

Intensity Integral along X-dimension.

2D-Image view: x-axis is Z-dimension and y-axis is Y-dimension.



XY

Right-Left View

Intensity Integral along Z-dimension.

2D-Image view: x-axis is X-dimension and y-axis is Y-dimension.



User Defined

User defined view where dimension perpendicular to the defined plane is the intensity integral dimension in the case of 2D images.

Image planes available in Image experiment where dimensions correspond to those in Figure 1.

2. Reference Experiment: Set the appropriate EPR parameters for the sample being studied. A good parameter set balances acquisition time with the achievable signal-to-noise. For narrow EPR spectra set the Number of Field Points to a lower value (512 or less). Increasing the Conversion Time will increase the overall image experiment time, however it may be necessary if a low signal-to-noise is present. Optimize these parameters at this step in the process to avoid errors in the image experiment. These parameters will be copied to the Image experiment for image acquisition.
3. Copy Reference Parameters to Image Experiment:
 - a. Select the Image experiment as the current experiment and activate the experiment.
 - b. Select the menu *Acquisition - Get Parameters from Experiment*. (There is an icon in the bottom toolbar near the center which does the same as the menu item.)
 - c. Choose the experiment named Reference in the pull down menu.
 - d. Choose *Load*.
4. Set Image Acquisition Parameters:
 - a. Open the *Acquisition Parameters* and select the *Imaging* tab.
 - b. Select the *Image Type* desired. For this guide only 2D and 3D are relevant image types. Figure 3 provides a guide to the types available.
 - c. Select the Image Plane desired. Figure 4 provides an overview of the various image planes. For 3D images the plane selected will be the plane in view after image processing.
 - d. Choose the correct F.O.V. (Field Of View) for the sample in the resonator. The F.O.V. is symmetric with respect to the calibrated origin. It is advisable to align to resonator center to the gradient center before acquiring images. Larger fields of view dramatically increase the total data collection time.
 - e. Choose the desired Pixel Size. Note that the resolution is ultimately determined by the EPR signal's linewidth and the Gradient strength. If the pixel size cannot be decreased to the desired value, set the Gradient (G) under the *Sweep* section to a lower value.
 - f. In the *Sweep* section, set the Gradient to the desired value.
 - g. Set the Spectrum Width to a value larger than the total EPR spectrum width obtained in the Reference spectrum. This parameter defines the Sweep Width which with the Number of Points affects the achieved field resolution.
 - h. Verify the *Signal Channel* parameters.
 - i. Check the Exp. Time. If a shorter experiment time is desired, adjust the Conversion Time, Number of

Points, and F.O.V.

- j. Press *Play*.
- k. After data acquisition Store/Save the data.
5. Copy Image Parameters to Reference Experiment: Follow the process in step 3 swapping "Reference" for "Image" and vice-versa to set the Reference experiment parameters to those of the Image experiment.
6. Acquire Reference Spectrum: Activate the Reference experiment after copying the Image experiment parameters. Press *Play* to acquire the Reference spectrum. Store/Save the data after acquisition.
7. Proceed to Data Processing.

2D & 3D Image Processing

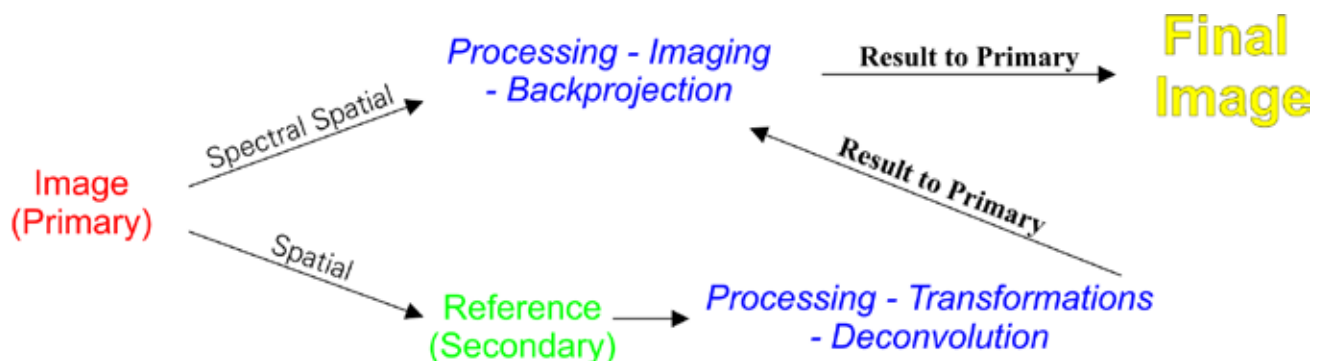
Image processing for 2D & 3D image data are the same.

1. Spectrum Deconvolution:
 - a. Set the Image data as the *Primary Dataset* and the Reference data as the *Secondary Dataset*.
 - b. Select the menu *Processing - Transformations - Deconvolution*.
 - c. Set Slice to All (diamond becomes green).
 - d. Adjust the Window and Width parameters for desired image quality. This is a trial-and-error procedure. Check the various slices within the data set to verify optimum parameter selection. The Window type affects the shape of the cut-off filter, with the Fermi-Dirac giving sharper images but may produce high frequency noise in the image while the Gaussian is the inverse. The Width is where the cut-off begins: a smaller number gives less noise and a higher number gives more noise. For data with a high signal to noise, a large Width (0.3) with the Fermi-Dirac window is a good starting point. For data with a low signal to noise, a Gaussian Window with a Width of 0.1 is a

good starting point.

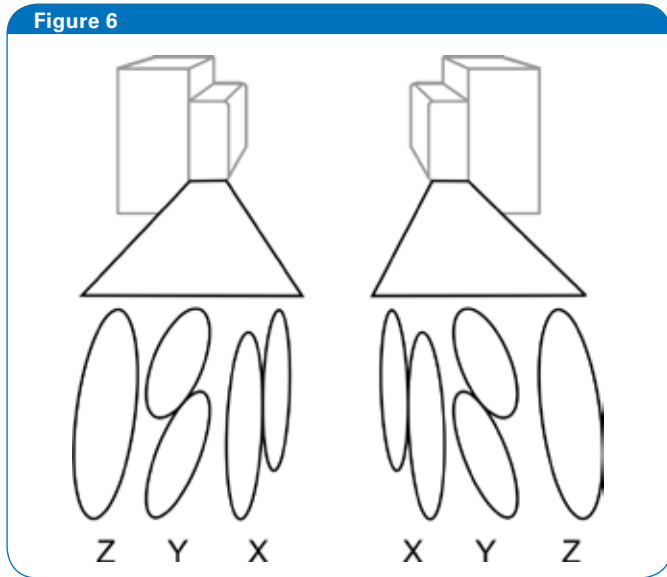
- e. Click *Apply*, copy the *Result Dataset* to the *Primary Dataset*, and store/save the data.
2. Image Backprojection:
 - a. Select the menu *Processing - Imaging - Backprojection*.
 - b. The default parameters are usually sufficient. The Reduce parameter indicates how many points from each field scan to use in the final backprojection. Increasing this value may add artifacts into the reconstructed image, however a good starting point is to set Reduce to twice the number of pixels or Image Size. Adjusting the Image Size will change the final reconstructed image by interpolating the data to the specified size.
 - c. Choose *Apply* then *Close*.
 - d. Choose *Yes* to move the resulting image to the *Primary Dataset*.
3. Store/Save the backprojected image.
4. Refer to figure 4 for interpretation of axes with respect to spatial dimensions.

Figure 5



Gradient Coil Orientations

The physical construction and orientation of the gradient coils is depicted in Figures 6 and 7. Figure 6 shows a perspective view highlighting the orientation of the coils from the magnet pole face. Figure 7 shows a flat view of the gradient coils on the magnet pole face. The space between the gradient coils are occupied by the water cooling system.

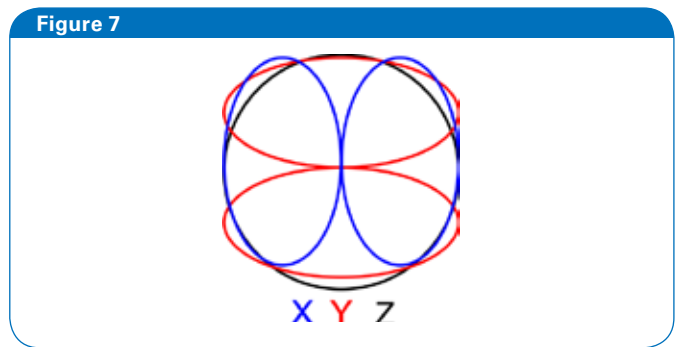


Perspective View of Gradient Coil Orientations

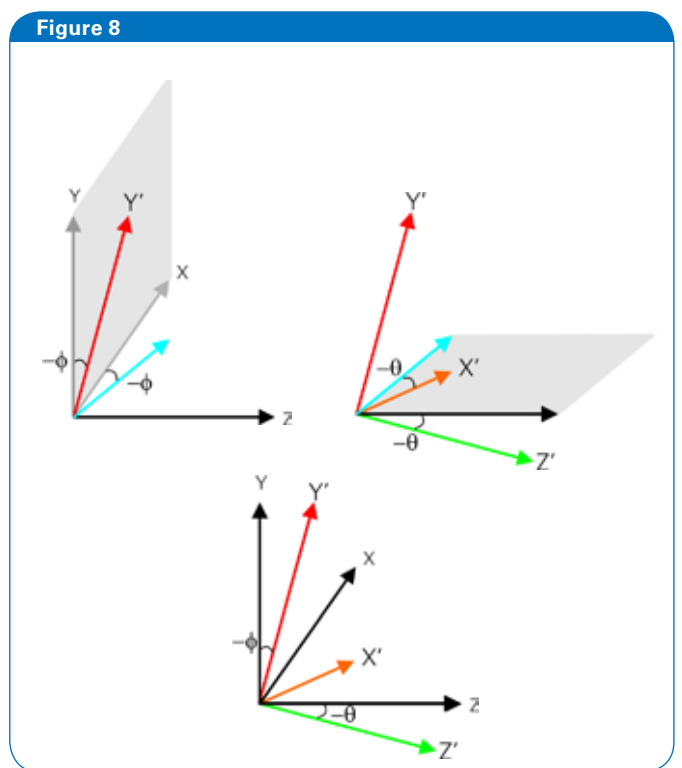
Image Plane Definition by Θ and Φ

The imaging frame of axes ($X'Y'Z'$) relates to the gradient coil frame of axes (XYZ) in the following way: The Experiment Parameters Φ and Θ define the normal to the imaging plane, which is the Z' axis. The X' axis lies in the plane formed by the projection of Z' onto the XY plane. The transformation from XYZ to $X'Y'Z'$ is accomplished by, first, a rotation around Z over Φ and, second, a rotation around Y' over Θ .

These transformations are depicted in Figure 8. The first rotation around Z over Φ moves the Y and X axes in the XY plane (shaded area) to the red and blue positions, respectively, resulting in the new Y' axis. Rotation about the Y' axis by Θ results in the new X' and Z' axes, orange and green, respectively. Finally, the full transformation from XYZ to $X'Y'Z'$ with Φ and Θ is indicated. The 2D plane imaged will then be the plane perpendicular to Z' , specifically the $X'Y'$ plane.



Flat View of Gradient Coil



Relation of θ and ϕ to the imaging plane. Rotations are based on the right-hand-rule so the depicted rotations are negative

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