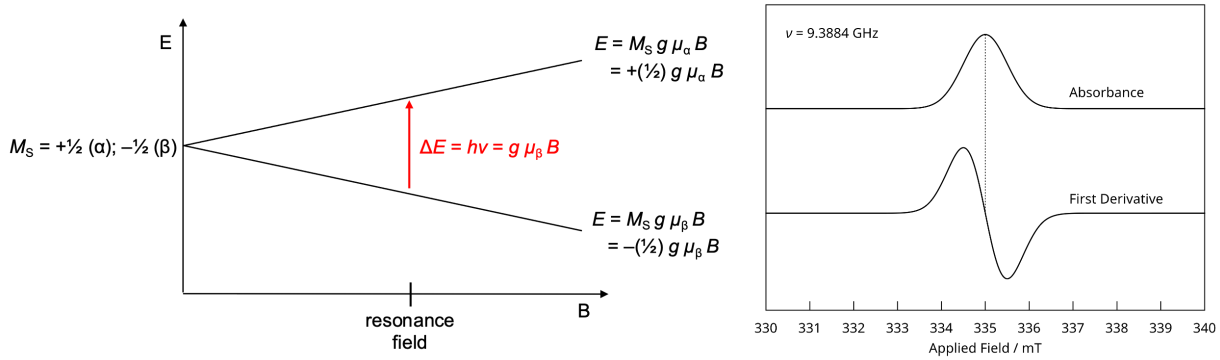
A Beginner's Guide to EasySpin and CW EPR

A Brief Overview of How EPR Works

How the measurement works

In the presence of an external magnetic field, an unpaired electron's spin can either be aligned parallel (M_S = ½) or antiparallel (M_S = –½) with the field. With increasing field strength, the splitting in energy between these two states also increases (Zeeman effect). The rate at which the energy splitting changes is proportional to the *g* factor. In continuous wave (CW) electron paramagnetic resonance (EPR) spectroscopy, the magnetic field strength is slowly increased, while the sample is irradiated by microwaves of a fixed frequency (in CW, this irradiation is continuous, in pulse EPR, it is pulsed). When the gap in energy between the M_S sublevels matches the energy of the microwaves, there will be an absorbance of energy, as electrons can now travel from the lower energy spin state into the higher energy spin state. The field strength at which this resonance energy is reached gives information about the *g* factor.



Typically, the CW EPR spectrum is reported as a derivative of the absorbance spectrum, because the measurement is performed using field modulation, which improves the signal-to-noise ratio. There are multiple microwave frequences at which one can measure CW EPR, but the most commonly used techniques are X-band (10 GHz) and Q-band (35 GHz).

EPR samples usually must be prepared with the spin center diluted. When the spin centers are too close together, spin-spin interactions can lead to line broadening and loss of signal. Often, this is done by dissolving the compound of interest in a glassing solvent for measurement in frozen solution, or by crystallizing the compound with a diamagnetic analog to form a diluted crystal for measurement as a powder or single crystal sample.

When you measure your spectrum, you should also record the temperature at which the data is collected (often 77 K in liquid nitrogen), the sample matrix, the frequency, and the modulation amplitude. You will need to include this information when you report your data.

Interpreting the spectrum

The *g* factor of a free electron is 2.0023. The chemical environment of an unpaired electron will cause the *g* factor to deviate from this value. Structural features of the paramagnetic molecule can lead to more complex structural features, through hyperfine coupling and anisotropy, for example. The coupling of the electron spin to nearby nuclear spins leads to **hyperfine coupling**, which is a further splitting of the Zeeman sublevels. This means that multiple spectral signals will appear at a fixed distance apart (proportional to *A*, the hyperfine coupling constant). The number of signals can be predicted based on multiplicity rules that take into account the number of nearby nuclei and their nuclear spins.

When a compound is measured in the solid state and the sample is disordered (frozen solution or powder), the molecules are in various different orientations relative to the direction of the magnetic field. These orientations, which consist of a parallel component (aligned in the direction of the magnetic field) and a perpendicular component (orthogonal to the magnetic field), can have different *g* factors and hyperfine coupling. The orientation dependence of these parameters is called **anisotropy**. If there is no anisotropy (or if the compound is rapidly tumbling, in solution at room temperature, for example), then the *g* factor is equivalent for all orientations and is isotropic.

Analyzing CW EPR Data Using EasySpin
(even if you know nothing about how EPR works)

1. Download EasySpin following the instructions from: https://easyspin.org/download.html
   Open MATLAB. First we will need to tell MATLAB how to find relevant files. Click Home Set Path. Then click "Add Folder" or "Add Folder with Subfolders" and add the folders that contain EasySpin and the folders that contain your EPR data. To avoid file path issues, save the EasySpin folder and your EPR data and analysis folders in the same parent folder.

2. *Setting up the MATLAB script*
   You will need a separate MATLAB script for each spectrum that you want to fit, saved in the same folder as your EPR data file. Below, there is an annotated sample MATLAB script (written by NPK) that simulates and plots an X-band frozen solution CW spectrum. Create a new MATLAB script and copy and paste the sample script as a starting point. Start by editing the information about the sample in the comment at the top, and then **change the filename** in line 8 to the name of your EPR data file (without the extension).
   Next, **change the microwave frequency** in line 40. This is important, otherwise the values that you fit will be wrong. The microwave frequency can be found in the .DSC file of your EPR data. Make sure the units are correct.
   Check whether you are running EasySpin 5 or EasySpin 6. The syntax for esfit changed significantly between these two versions. In lines 47 and 48, the syntax for each version is given. The sample script is set up for EasySpin 5. If you are using EasySpin 6, add a "%" to the start of line 47, and delete the "%" at the start of line 48. Make sure the one you want does not have a "%" at the start of the line, and the one you don't want does and appears green.

   Sample MATLAB Script for EasySpin; a matlab script file is included in the google drive folder associated with the tutorial in the "Files" hyperlink

   EPR:

   MATLAB + EasySpin Installation Guide

   EasySpin Guide and Files for the tutorial

```
1   % Comment information about the sample here, e.g. "KTX-Qu-025-3 Cu(tbaa)2 in 2:1 toluene:DCM"
2
3   close all
4   clear
5   clc
6
7   % change the file name here to the name of your EPR data file
8   fn = 'filename';
9
10  [B,SPC] = eprload(fn);
11  figure;
12  plot(B,SPC);
13  title(fn,'Interpreter','none');
```

```
% this part pulls the file name, and plots the initial graph of the data

limvals = [2000,4000]; % change this range to match the relevant data range, trimming excess baseline
goodinds = B < limvals(2) & B > limvals(1);
Buse = B(goodinds);
SPCuse = SPC(goodinds);
Buse = Buse/10;

% Start by running to here to use Curve Fitter to add a baseline correction
BASELINE = true;
if BASELINE
    eqn = @(x) -0.0423*x + 11.4378; % Write curve fit values here
    SPCuse = SPCuse - eqn(Buse);
    SPCuse = SPCuse./max(SPCuse);
end

% These are the parameters for the spectrum simulation and fitting
Sys.g = [2.052,2.054,2.271];
Sys.Nucs = 'Cu';  % Can list multiple nuclei here
Sys.A = [83,52,560]; % hyperfine values in MHz
Sys.gStrain = [0.01,0.01,0.01];
Vary.g = [0.1,0.1,0]; % bounds on the least squares fitting, set to zero to maintain manual fit values
Vary.A = [50,50,0];
Vary.gStrain = [0.01,0.01,0.01];

% Manually set the microwave frequency from DSC file, convert Hz to GHz by 10^9
Exp.mwFreq = 9.400677; % interpreted in Ghz for the full scan
Exp.Range = [min(Buse),max(Buse)]; % make sure B is divided by 10 (line 20)
Exp.nRange = numel(Buse); % number of points
Exp.Harmonic = 1; % 1 for CW (derivative) spectra; 0 for EDFS (integral) spectra

FIT = false; % This part runs the least squares fitting program; first set to false and fit manually, then set
to true to run the fitting program
if FIT
    esfit(@pepper,SPCuse,Sys,Vary,Exp); % for easyspin 5
    %esfit(SPCuse,@pepper,{Sys,Exp},{Vary}) % for easyspin 6
end

% This part generates the plot with the experimental and simulated spectra overlaid
PLOT_BEST = true;
if PLOT_BEST

    [simB,simspec] = pepper(Sys,Exp);
    simspec_atBuse = interp1(simB,simspec,Buse);

    figure;
    plot(Buse,SPCuse,'k','LineWidth',1.5);
    norm_factor = SPCuse\simspec_atBuse;
    % norm_factor=20 % Manually change the scale of the simulated spectrum
    hold on
    plot(simB,simspec/norm_factor,'r--','LineWidth',1.5);
    legend('Experiment','Simulation');
    xlabel('B (mT)');
    set(gca,'ytick',[])
```

```
67        set(gca,'FontSize',12);
68        xlim([min(Buse),max(Buse)]);
69        f = gcf;
70        f.Position = [[129.8000 125 964 690.4000]];
71        f.Color = 'w';
72    end
```

3. *Baseline correction*
   First, we will fit and correct the baseline. Run the script up to line 23. You can do this by hovering your mouse next to the line number on MATLAB and clicking the little triangle that appears (the triangle will not appear next to empty lines or lines that are commented out). Then stop the run by pressing the big red square at the top of the window (Editor □ Stop). Take a look at the graph and where the features of your spectrum appear. Edit the data range in line 16 to encompass the spectral features with an additional ~500 G to either side. Now run the script to line 23 again.
   Click Apps □ Curve Fitter. (If you do not have Curve Fitter installed, click Apps □ Get More Apps, and then search for the Curve Fitting Toolbox and install it). A new window will open. Click "Select Data," and a small window will pop up. For the X data, select "Buse," and for the Y data, select "SPCuse." Click Close. Select Polynomial Fit from the top of the window. There should be a blue line across the plotted data that aligns with the baseline. On the right side of the window, in the dropdown menu next to "Robust," change the method to Bisquare (or LAR, depending on which looks better). Beneath that, in the "Results" box, there is the formula for the line that the baseline is fit to. Copy the p1 (slope) and p2 (intercept) values into the equation in line 25. Close the Curve Fitter window (you do not need to save).

4. *Choosing starting parameters*
   Lines 31–34 contain the parameters that affect how the spectrum looks. The best way to start is to find reported literature g and hyperfine values for your compound or something similar. In line 31, the g values are in the following order: $[g_x, g_y, g_z]$. If there are only two values, they are: $[g_\perp, g_\parallel]$ (in the case where $g_x = g_y = g_\perp$). Similarly, the hyperfine values in line 33 are: $[A_x, A_y, A_z]$ or $[A_\perp, A_\parallel]$. In line 32, specify the atom where the paramagnetic site is located. Sometimes you may see g_{iso} reported. You can use the following formula to solve for g_x and g_y: $g_{iso} = (g_x + g_y + g_z)/3$. In other words, g_{iso} is the average of the g values.
   If you cannot find literature values to start from, or the paramagnetic species is unknown, see some in the "What to do if…" section below to help you get started.

5. *Choosing the EasySpin fitting method*
   You may have noticed "pepper" in lines 47 and 54. This is the fitting method we have chosen for analyzing a frozen solution CW spectrum. EasySpin has a number of other methods. This table summarizes commonly used methods in our lab and when to use them:

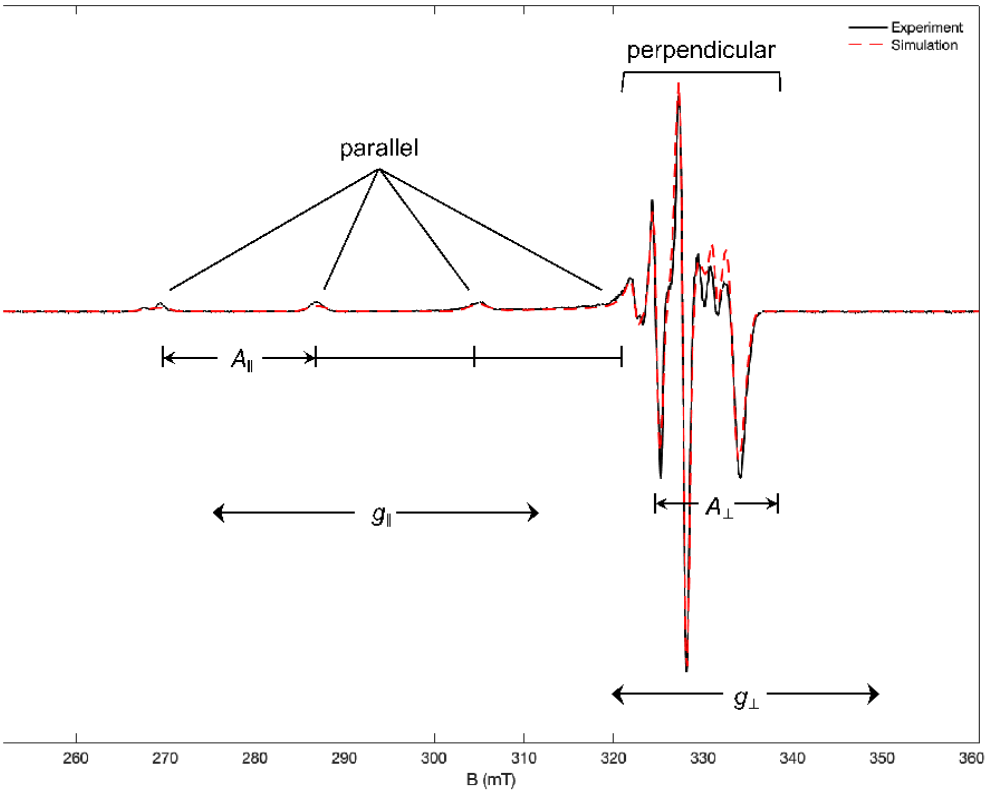
Method	Use cases
pepper	Solid state EPR (e.g. frozen solution, diluted powders/crystals)
garlic	Solution state EPR with fast tumbling (e.g. room temperature liquid solution for small molecules)

chili	Solution state EPR with slow tumbling (e.g. room temperature liquid solution for a large biomolecule)

6. *Manually fitting the spectrum*

Now we are ready to fit the spectrum. Click Run at the top of the MATLAB window to run the whole script. A new plot will appear with your experimental spectrum in black, and the simulated spectrum from the starting parameters you chose in a dashed red line. If the vertical scale of the simulated spectrum is way too big or too small compared to the experimental spectrum, delete the first "%" in line 60 to manually change the normalization factor. The y-values will be divided by this factor, so making the number bigger will make the peak heights smaller. As you iterate to a better fit, you may make this number smaller (closer to 1), or comment it out again by adding the "%" back in.

Here is an example of a fitted spectrum, with the parallel and perpendicular features marked:



We should first try to get as good of a manual fit as possible. In most cases, the parallel features must be fit manually, and in some cases, the fitting must be done completely manually. Start with the parallel features. Changing the $g_{\parallel}$ (a.k.a. g_z) value will shift the spectral features left or right. Changing the parallel hyperfine value ($A_{\parallel}$, a.k.a. A_z) will change the spacing between the parallel features. Changing the parallel g strain will change the broadness of the peaks. It can be helpful to start with a low g strain so that it's more clear to see how the features are changing and aligning with the experimental spectrum (e.g. 0.01 or 0.005). Start by changing one parameter at a time and click Run to see how the plot changes.

The parallel features can often underlie the perpendicular region, so changing the parallel parameters can also change the perpendicular features. However, changing the perpendicular parameters will usually not affect the parallel region, which is why we fit the parallel features first.

Once the parallel features are well aligned, we can start changing the perpendicular features until the positions of the major peaks are aligned. The least square fitting program can usually get the peak heights and widths of the perpendicular features to match up once they are fairly well-positioned.

Here's a table that summarizes ways to change the spectrum:

Changing…	Increase	Decrease
g	Features shift left	Features shift right
A	Features farther apart	Features closer together
g strain	Features broader	Features sharper

Instead of *g* strain, you can also choose to model A strain (Sys.AStrain). While *g* strain increases the uncertainty in *g*, *A* strain increases the uncertainty in *A*. Both result in changes in the width of the peaks.

7. *Using the least squares fitting program*
We can use the fitting program to clean up the perpendicular features. Lines 35–37 specify how much the program is allowed to vary the *g*, *A* and *g* strain parameters. If you have already fit the parallel features well, it's usually a good idea to set the vary values for the parallel *g* and *A* values to 0. The least squares fitting program tries to minimize the difference between the simulated spectrum and the data, and because these peaks are smaller (and therefore the difference is smaller when the fit is bad), the program is bad at fitting these. The vary value for *g* strain should not be larger than the *g* strain value because the program will give an error message if it tries a negative *g* strain value.

Change "false" to "true" in line 45. Now click Run, and a new window will open with the least squares fitting program. On the right you can see the parameters that the program will vary. Beneath that, find the dropdown menu next to "Target" and set it to "data as is." Now click Start and let the program run. This can take a few minutes.

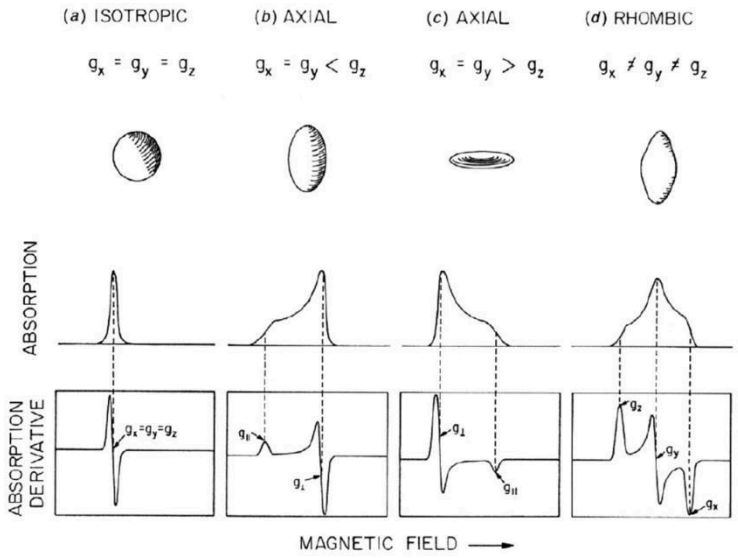
When it is finished and if the fit looks good, copy the values in the "best" column into your MATLAB script. Change "true" to "false" in line 45, and click Run again. If the plot with the experimental and simulated spectra looks good, you can save this, tabulate your *g* and hyperfine values, and you are done!

If the fit looks bad or worse, you may want to try to get a better fit manually and then try running the fitting program again. You can also change the vary values for different parameters if they are causing problems (for example, sometimes the program increases *g* strain too much, and you could set that manually and set the vary to 0).

What to do if you have…

…No literature values to start from

The g value of a free electron is 2.0023. If you can't find any related precedence for your compound, this can be a good starting point. You do need to know the atom where the spin center is likely located so that you can specify the nucleus in line 32 of the MATLAB script. Set all your g values to 2.0023, and from there, make the values larger or smaller so that your simulated spectrum appears in a similar range to your experimental spectral features. Next, look at where the parallel features appear relative to the perpendicular features. This will give you information on whether $g_{\perp}$ is greater or less than $g_{\parallel}$, as summarized in the following diagram:



From here, change $g_{\parallel}$ and $A_{\parallel}$ to align the parallel features, and then move on to fitting the perpendicular features, following the steps explained above.

…Nitrogen hyperfine coupling (or hyperfine coupling from other nuclei)

If there are nitrogens bound to your spin center you will have additional hyperfine splitting from these. EasySpin can model this. You can add the nitrogens to the nuclei in line 32, and then add additional hyperfine values for them. Here is an example of some MATLAB script for the g and hyperfine parameters for a compound with 4 nitrogen-bound ligands:

```
1   Sys.g = [2.046,2.176];
2   Sys.Nucs = 'Cu, N, N, N, N';  % Can list multiple nuclei here
3   Sys.A = [48,625; 50,45; 50,45; 50,45; 50,45]; % hyperfine values in MHz
4   Sys.gStrain = [0.01,0.01,0.01];
5   Vary.g = [0.1,0];
6   Vary.A = [50,0; 0,0; 0,0; 0,0; 0,0];
7   Vary.gStrain = [0.01,0.01,0.01];
```

If you have labeled isotopes, you can specify the isotope by writing 15N or 65Cu for example. Without an isotope specified, the program will simulate the spectrum according to the natural isotopic abundance distribution.

Usually, we also add an optimization method by adding this line after the system parameters:

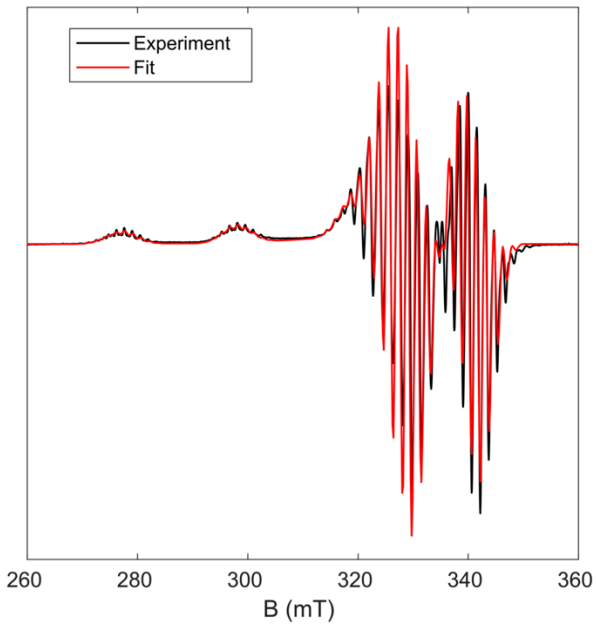
```
Opt.Method = 'perturb2';
```

You must then also add "Opt" to the lines where you use esfit and pepper (lines 47 and 54 in the template script above):

```
47    esfit(@pepper,SPCuse,Sys,Vary,Exp,Opt); % for easyspin 5
48    esfit(SPCuse,@pepper,{Sys,Exp,Opt},{Vary}); % for easyspin 6

55    [simB,simspec] = pepper(Sys,Exp,Opt);
```

Here is an example of a fitted spectrum for copper (II) phthalocyanine, which has 4 nitrogens bound to the copper:

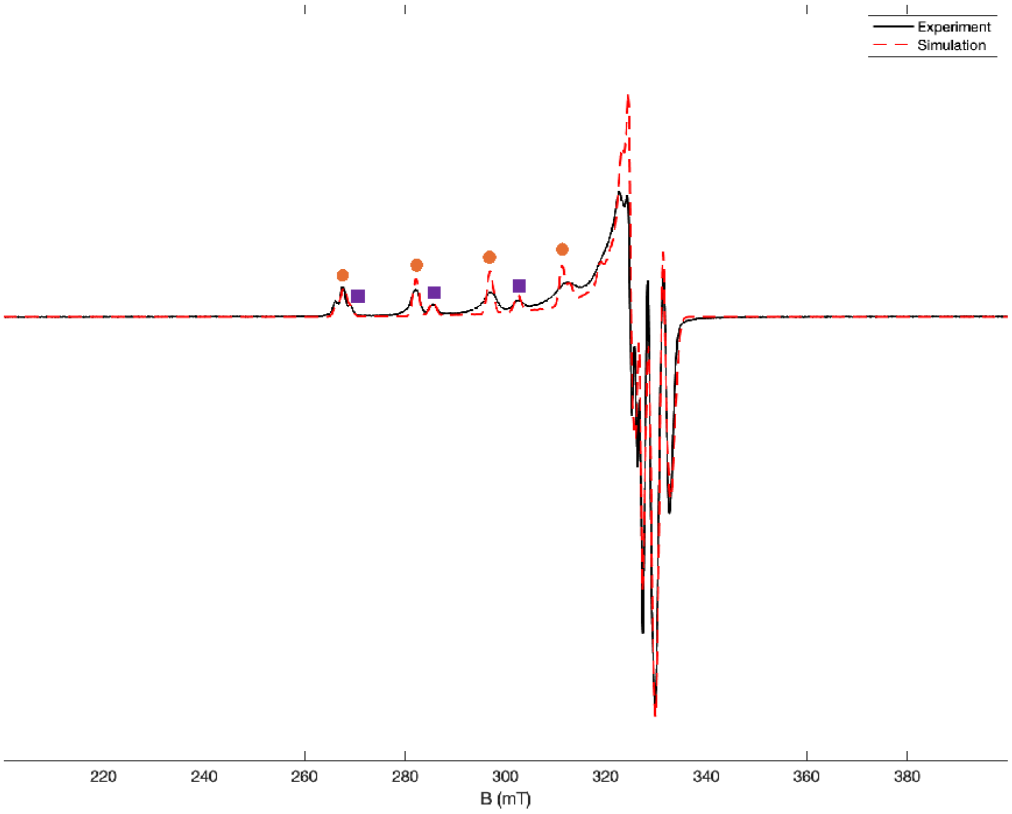


We can see significant hyperfine coupling from the nitrogens, as the larger peaks are each split into many smaller peaks. The nitrogen hyperfine values determine how far apart the small peaks appear. A larger value makes them farther apart, and a smaller value makes them closer together. Again, it's helpful to start with the parallel features first, then move to the perpendicular ones. Because these splitting features are quite small, you will likely need fit them completely manually. If you have symmetric nitrogens, you can usually assume they will all have the same hyperfine values. Also, you can usually assume the two perpendicular hyperfine values are the same. So when you change the value for one nitrogen, change the value for all of them. This leaves you with essentially just two values to fit for the nitrogen hyperfine splitting ($A_x = A_y = A_\perp$ and $A_\parallel$). You can fit the peak positions for the spin center first, and then iterate between changing

the nitrogen hyperfine values and the *g* and hyperfine values for the spin center until you get a good fit.

…Two spin systems

Sometimes, your spectrum may contain more than one spin system. EasySpin can model this too. Here is an example of a fitted spectrum:



In this sample of a square planar $Cu(acac)_2$ derivative, the compound was sometimes axially hydrated and sometimes not. The parallel peaks corresponding to each species are marked in the spectrum with circles and squares respectively.

Here is an example of the MATLAB script for the *g* and hyperfine parameters modelling these two systems:

```
Sys.g = [2.068,2.059,2.320];
Sys.Nucs = 'Cu';
Sys.A = [10,10,470]; %hyperfine values in MHz
Sys.gStrain = [0.015,0.009,0.01];
Vary.g = [0.1,0.1,0];
Vary.A = [20,20,0];
Vary.gStrain = [0.01,0.01,0.01];

Sys2.g = [2.057,2.048,2.285];
Sys2.Nucs = 'Cu';
Sys2.A = [46,51,530]; %hyperfine values in MHz
```

```
12      Sys2.gStrain = [0.004,0.011,0.01];
13      Vary2.g = [0.1,0.1,0];
14      Vary2.A = [20,20,0];
15      Vary2.gStrain = [0.01,0.01,0.01];
16
17      Sys.weight = 2.70
18      Sys2.weight = 1
19      Vary.weight = 2
20      Vary2.weight = 0
```

We also have to add the second system for running the fitting program:

```
esfit(@pepper,SPCuse,{Sys,Sys2},{Vary,Vary2},Exp); % for easyspin 5
esfit (SPCuse,@pepper,{{Sys,Sys2},Exp},{{Vary,Vary2}}); % for easyspin 6
```

And for the plotting at the end:

```
[simB,simspec] = pepper({Sys,Sys2},Exp);
```

In a case like this where the two systems are fairly similar, you can start by modeling just one spin system to fit the major species. Then copy and paste the parameters to create a second spin system, changing "Sys" to "Sys2" and "Vary" to "Vary2." You can add weight parameters to change the relative proportion of the two species. You can then modify the second spin system to fit the parallel features of the minor species. The perpendicular features can get complicated to fit, so you may run the least squares fitting program to finish up the perpendicular fitting. You can also let the program optimize the system weights by keeping the weight of one spin system constant and letting the program optimize the weight of the other.

…Echo detected field sweep (EDFS) data from pulse EPR

This one is easy! Just change the harmonic order in line 43 from 1 to 0 and proceed as usual. This will allow you to fit the absorbance spectrum instead of the usual absorbance derivative spectrum that CW EPR generates.

…A Mac and it won't let you run some EasySpin files

If you have a Mac, the first time you run the esfit function, you will likely run into an error message because EasySpin is downloaded from the internet and your computer will not let you run some of the scripts for safety reasons. To release the two files that esfit needs, locate your easyspin-X.Y.Z folder in Finder. Now click View Show Path Bar. At the bottom of the window, the path to the folder should appear. Right click on this bar and click 'Copy "easyspin-X.Y.Z" as Pathname'. Now open Terminal. Type the two following commands into Terminal, one at a time:

```
sudo xattr -r -d com.apple.quarantine [path/to/easyspin-X.Y.Z]/easyspin/private/cubicsolve.mexmaci64
```

```
sudo xattr -r -d com.apple.quarantine [path/to/easyspin-X.Y.Z]/easyspin/private/lisum1i.mexmaci64
```

You will be prompted to enter your account password (to your computer). After this, try running esfit again and it should work.

In general, to release files from quarantine, type this command into the Terminal:

```
sudo xattr -r -d com.apple.quarantine [path/to/file]
```

Still having trouble? Or want to know more?
The EasySpin website has more information: https://easyspin.org/easyspin/documentation/
You can also post questions in the forum: https://easyspin.org/forum/
Talk to Paul Oyala (phoyala@caltech.edu) if you are running a more complex experiment or have more advanced questions that others in our group can't answer.

Written by KTX, NPK, and CAT, Nov 2024