Design and Implementation of a Two-Cell Sample Holder for High Field Electron Paramagnetic Resonance Spectrometers

Sherwin Group
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Research Mentorship Program

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Abstract

- Quasi-optical sample holders for Electron Paramagnetic Resonance (EPR) spectrometers currently measure **one sample**
- Coupling between sample and microwave varies with each sample exchange and decreases efficiency of measurements
- We design, 3D print, and troubleshoot two-cell SH to exchange samples inside the probe
- Our sample holder integrated into spectrometer under temperatures of 1.6-300 K, frequency of 240 GHz, and magnetic fields up to 12 T
- **Quantitative measurements** made comparing two liquid TEMPO samples

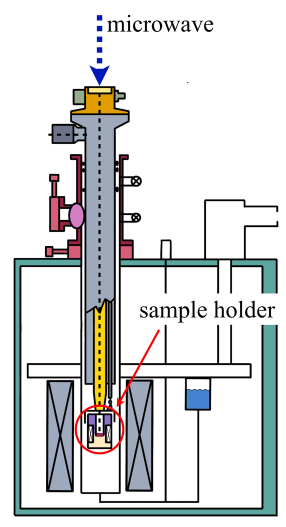

FIG. 1. Diagram of EPR probe inside the superconducting cryogen-free magnet (adapted from [4])

EPR Spectroscopy

Zeeman Effect

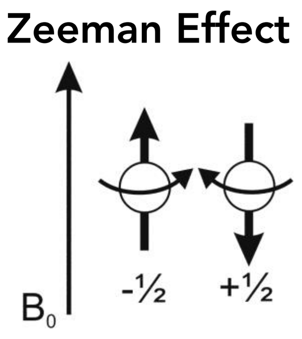

FIG. 2. Alignment of electron's magnetic moment to the field [1]

- **Energy differences** created by interaction between unpaired electron spins in external magnetic field
- For single unpaired electron spin (S = 1/2), m_s= -½ or m_s= +½
- lowest energy when aligned parallel (m_s= -½)
- highest energy when aligned antiparallel (m_s= +½) [1]

g-Factor

- Characterizes **magnetic moment** and **angular momentum** of structures with unpaired electrons
- Remains constant regardless of microwave frequency, making it a **reliable fingerprint** for each measured system
- To calculate *g*-factor for quantitative EPR, the resonance condition is used as given by equation 1 [2]：

$$\Delta E = E_1 - E_2 = hf = g\mu_B B_{Res} \quad (1)$$

Derivative Spectra

- Modulation coil generates oscillating magnetic field, B_{mod}, at a few kHz to improve Signal-to-Noise-Ratio (SNR)
- Converts weak signal into easily detectable modulated signal
- At B_0 = 0, the energy difference between spin states is degenerate
- At $B_0 \neq 0$, two energy states E_1 and E_2 are separated by Δ*E*
- At $hf = \Delta E$, absorption signal detected [3]
- B_{mod} modulates the x-axis which results in the **first derivative** of the absorption EPR signal [4]

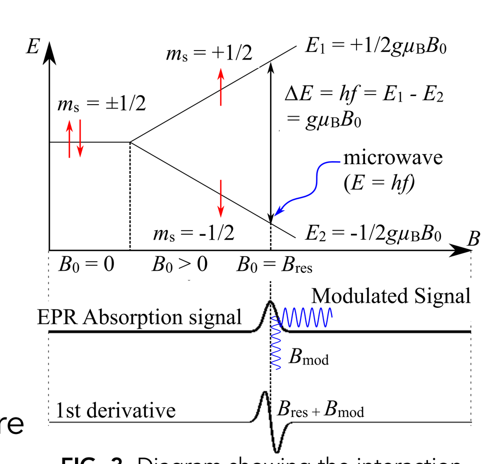

FIG. 3. Diagram showing the interaction between an electron spin (S = ½) with an external magnetic field, B_0, and the frequency (*f*) of an oscillating electromagnetic irradiation. When the microwave radiation matches the gap at the absorption signal, the lower energy levels flip to a higher energy level [4]

High-Frequency EPR

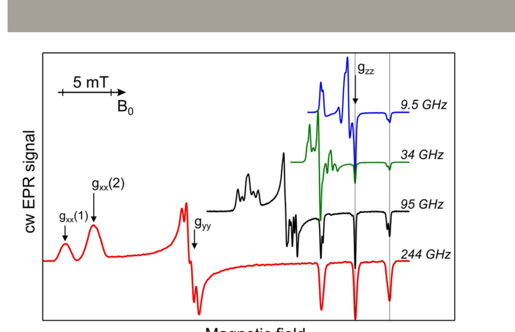

FIG. 4. Enhanced signal detection via HF-EPR [5]

- High-Frequency EPR (HF-EPR) increases **spectrometer resolution** and **sensitivity** to enable the study of high spin systems [5]
- Operates at high frequencies above 100 GHz and magnetic fields above 3.5 T [6]

Quasi-Optical Sample Holder

- Focusing quasi-optics keep microwave beam within specified path
- Enhanced field modulation amplitude and homogeneity on the sample
- Rooftop and parabolic mirror **improve SNR by 6** to detect signal
- Functions in continuous-wave, pulsed, and rapid-scan EPR modes [7]
- 3D printed with exception of rooftop mirror, parabolic mirror, and coil
- Limitations due to ability to only measure **one sample** at a time

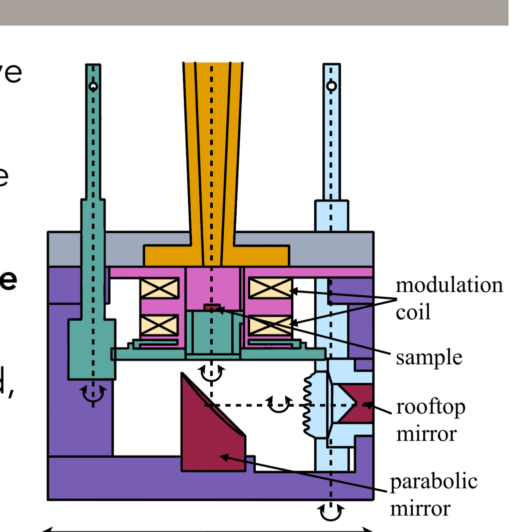

FIG. 5. Diagram of the quasi-optical sample holder (adapted from [7])

Research Objectives

- Design a sample holder that can switch between two samples within the probe in Onshape
- 3D print and troubleshoot using fused deposition modeling printer
- Implement 3D printed two-cell sample holder into HF-EPR
- Make **quantitative comparisons** between two liquid TEMPO samples with different concentrations

Two-Cell Sample Holder

- Two spaces 5 mm in diameter for each sample
- Attached to end of 1 m long probe (microwave waveguide)
- Mechanical feedthrough to switch samples
- Sample located 9 mm under a corrugated waveguide
- Magnet bore diameter of 60 mm
- Mirror with diameter of 7.01 mm and height of 2.13 mm placed 2.478 mm under the sample
  - Distance between sample and mirror must be a multiple of the **wavelength number** (1.239 mm for 240 GHz)
  - Ensures that sample sits on maximum oscillating magnetic component of microwave radiation [8]

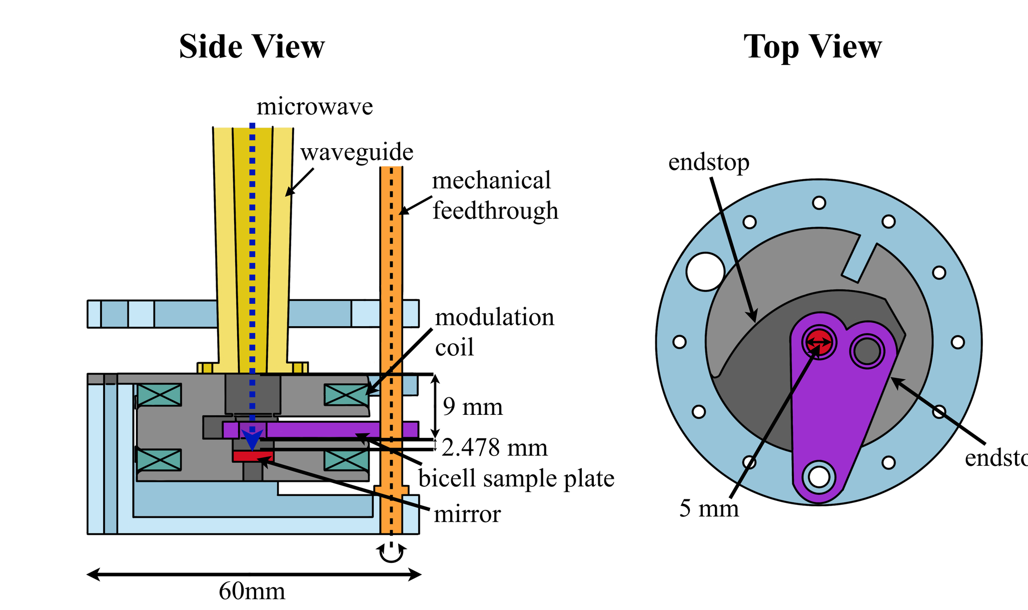

FIG. 6. Proposed design for the 3D printed two-cell sample holder and modulation coil

Calibration using LiPc Crystal

- Modulation coil calibrated using lithium phthalocyanine (LiPc) crystal
- Coil wound from Cu32 American Wire Gauge using 200 revolutions
- Measured at 40 mW and 240 GHz
- Amplitude normalized by dividing measurements by largest value
- Measured *g*-factor was **2.005** compared to actual 2.0024 [9]
  - Typical error is 18 G due to variation in main field
- Modulation coil operated at **0.3 G/mA**

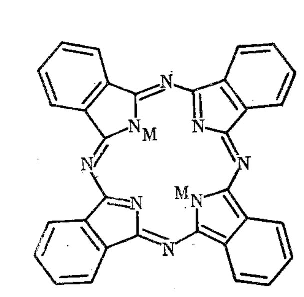

FIG. 7. LiPc crystal [9]

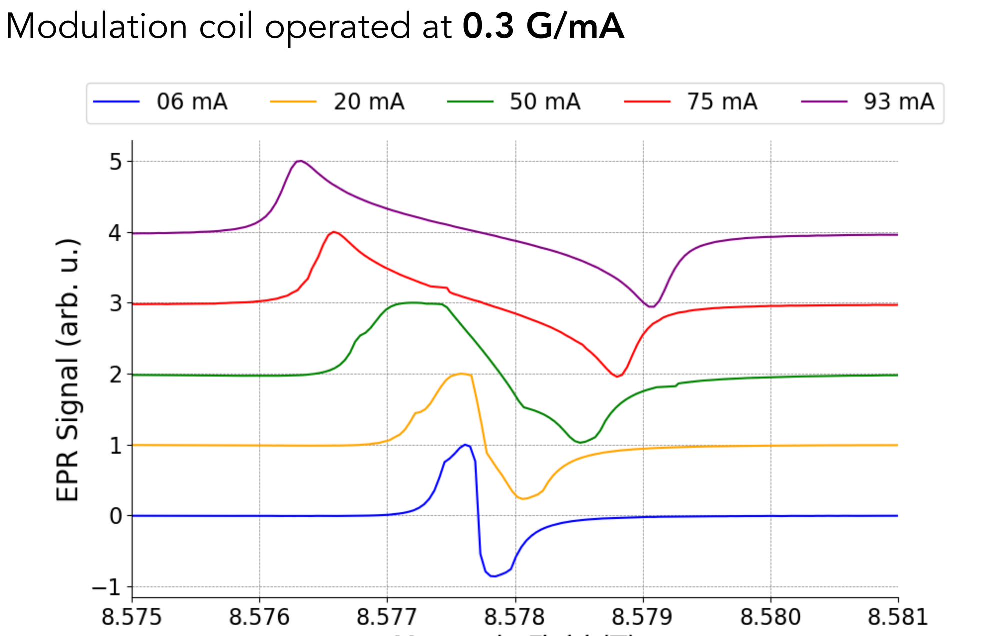

FIG. 8. The difference in linewidths of the LiPc crystal at five currents: 6, 20, 50, 75, and 93 mA

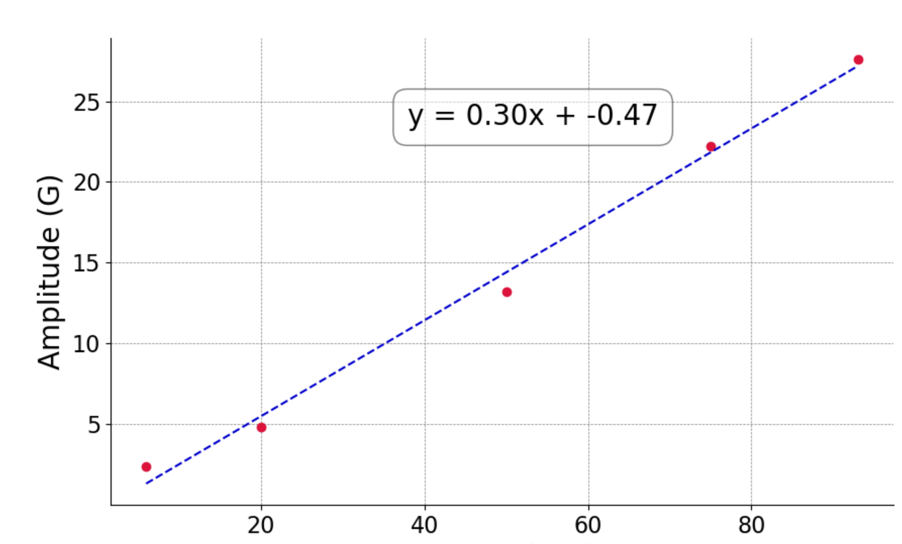

FIG. 9. The modulation coil amplitude as a function of the current. The dashed line represents the linear fit

Liquid TEMPO Samples

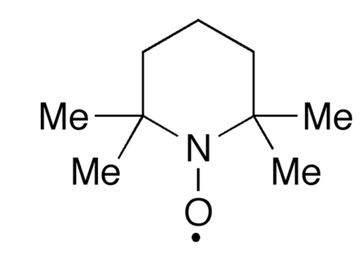

FIG. 10. TEMPO molecule [10]

- First sample is 10 mg TEMPO dissolved in 1 mL toluene, **2.5 mM**
- Second sample is 10 mg TEMPO dissolved in 10 mL toluene, **0.25 mM**
- 1.6 μL of each solution inserted into 3 mm long flat cell

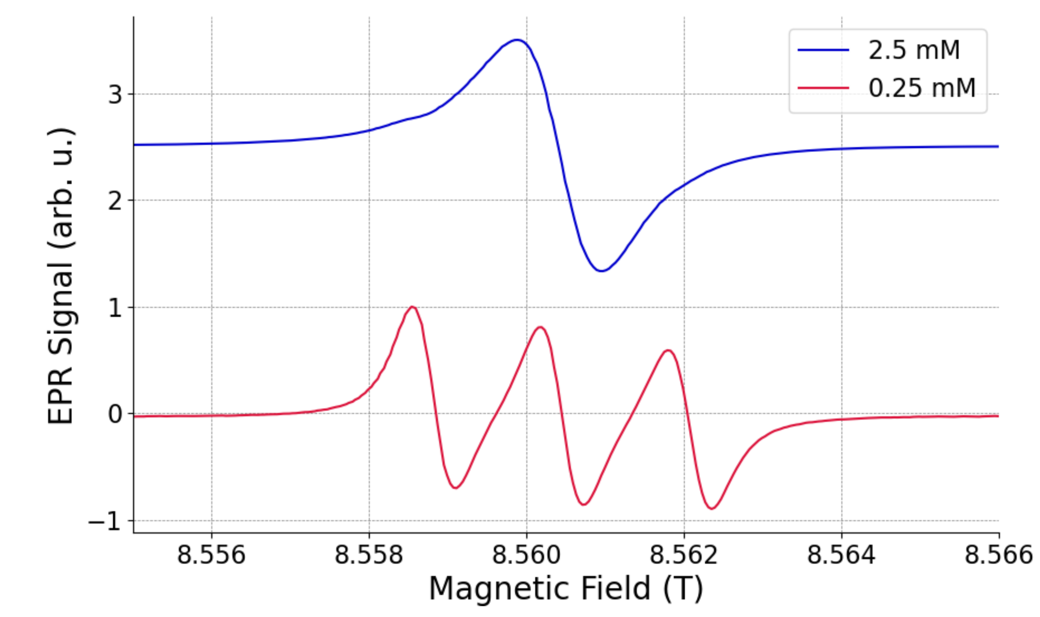

FIG. 11. EPR signal of 2.5 mM and 0.25 mM of TEMPO dissolved in toluene

- Hyperfine interactions cause **three peaks** for 0.25 mM sample
- Linewidth of 2.5 mM sample broader due to **spin-spin relaxation** time and **hyperfine interactions**
- Average distance (r) between two spins from the concentration (n) calculated using equation 2 [11]:

$$\langle r\rangle = \int_0^\infty r w(r) dr = \Gamma(4/3)/(4\pi/3)^{1/3} = 0.55396 n^{-1/3} \quad (2)$$

	Linewidth	Spin-spin distance	Signal-to-Noise-Ratio
2.5 mM	1.1 mT	4 nm	2500
0.25 mM	0.549 mT	10 nm	430

TABLE. 1. The observed linewidth, spin-spin distance, and SNR for the 2.5 mM and 0.25 mM samples

- Difference in SNR is **6** times compared to expected 10 times because concentration is 10 times smaller, errors may result mostly from line shape but also from concentration and position variations
- Resonance condition changes due to splitting of energy levels
- Spin states split by exchange interaction, which alters position of EPR signal
- When $S_1 = S_2$ = 1/2, energy levels split into singlet (S = 0) and triplet (S = 1) states
- At low concentration (0.25 mM), TEMPO molecules spaced far apart
  - Hyperfine interactions occur between **unpaired electron** and **nitrogen nucleus** (S = 1)
  - Nucleus has three spin states (-1, 0, +1)
  - Splits EPR signal into three peaks [12]
- At high concentration (2.5 mM), TEMPO molecules are spaced closer together
  - **Electron-electron interactions** occur
  - Electron has two spin states (-1/2, +1/2)
  - Three hyperfine peaks average out and broaden into **single peak**, called exchange narrowing [13]

Findings and Impact

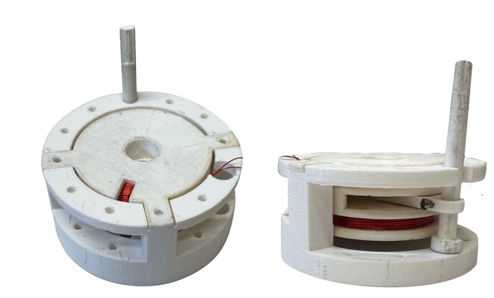

FIG. 12. Two-cell sample holder printed from polylactic acid using the Original Prusa i3 MK3S+ 3D printer

- Three peaks observed for 0.25 mM TEMPO compared to 2.5 mM TEMPO due to Heisenberg interaction
- Reduced downtime between measurements by **five hours**
- Improved SNR for higher accuracy measurements
- Enables quantitative measurements

Future Work

- Incorporate the two-cell sample holder into the quasi-optical sample holder
- Conduct quantitative AsLOV2 protein measurements
- Enable sample loading within the probe

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