New Horizons for CW-EPR Spectroscopy: The Multi-Harmonic Detection Accessory

Electron Paramagnetic Resonance (EPR) spectroscopy is the paramount technique for detecting unpaired electrons spin species in a variety of sample types, ranging from free radicals to transition metal or rare-earth ions in organic compounds, biology, and materials. While EPR is intrinsically very sensitive, achieving good signal-to-noise can still be a lengthy process at times, particularly when the unpaired electron spin species is unknown. Detection of multiple harmonics provides researchers with a means to improve signal-to-noise in CW-EPR with reduced acquisition times. In collaboration with Novilet, a Multi-Harmonic Detection (MHD) accessory is now part of the Bruker CW-EPR portfolio.

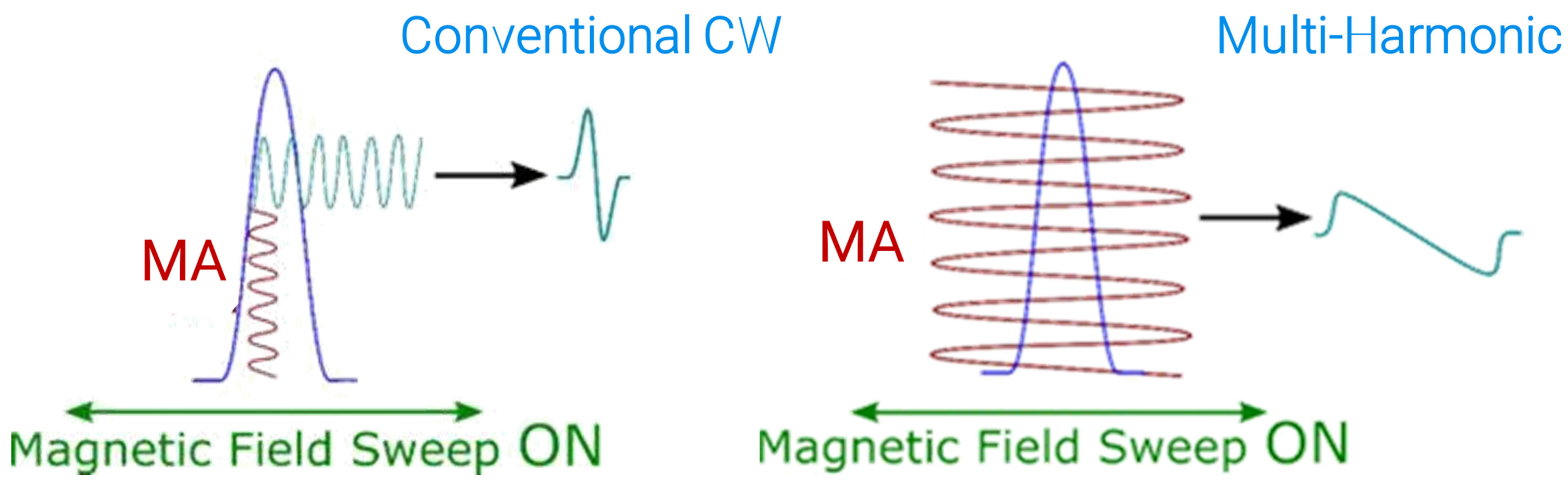

Fig. 1 Conventional CW-EPR Detection vs Multi-Harmonic Detection.

Multi-Harmonic Detection (MHD)

- Conventional CW-EPR typically measures the 1st harmonic of a field-modulated signal. Setting the modulation amplitude is often a compromise between sensitivity and field resolution (Figure 1).
- By detecting multiple-harmonics of an over-modulated EPR signal, it is possible to reconstruct the 1st derivative CW-EPR signal with increased signal-to-noise and desired modulation amplitude (Figure 2).
- The number of harmonics depends on the overmodulation factor. For higher overmodulation factors, more harmonics are detected and incorporated into the reconstruction of the first derivative spectrum.

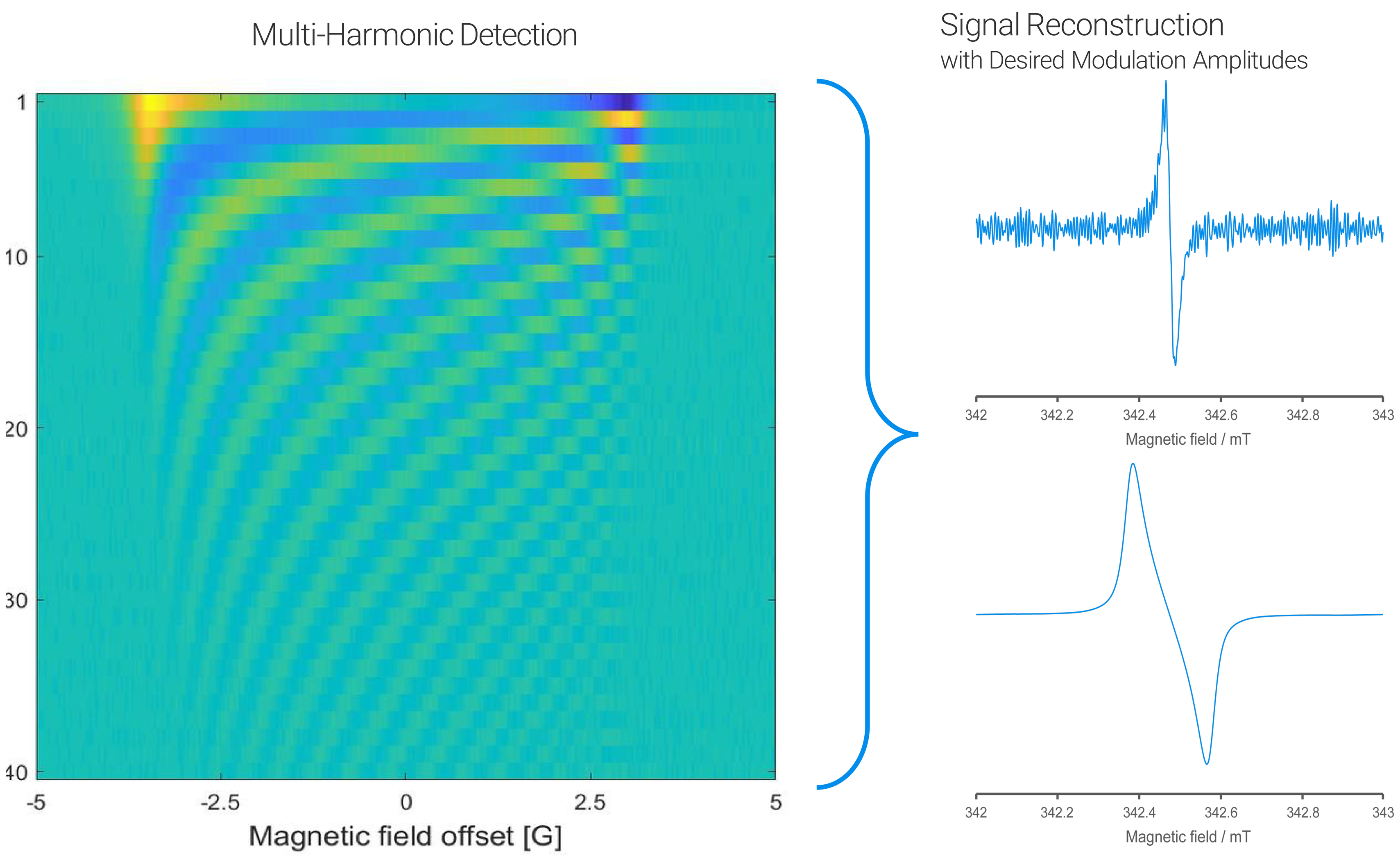

Fig. 2 Multi-harmonic detection of a single EPR line (left). Reconstruction of 1st derivative CW-EPR signal with desired modulation amplitudes with MHD (right).

Signal-to-Noise Enhancement

Using the MHD, it is possible to rapidly obtain information of unpaired species without prior knowledge of the EPR lineshape. In a single scan, the EPR spectrum of solid Mn(II) shows little information and results in a signal-to-noise ratio (SNR) of 10 (Figure 3, left). Overmodulation and harmonics reconstruction of the same single scan results in a fully resolved EPR spectrum with an unprecedented factor of 350-fold increase in the SNR (Figure 3, right), displaying the capability of Multi-Harmonic Detection and reconstruction in EPR signal deconvolution and acquisition times.

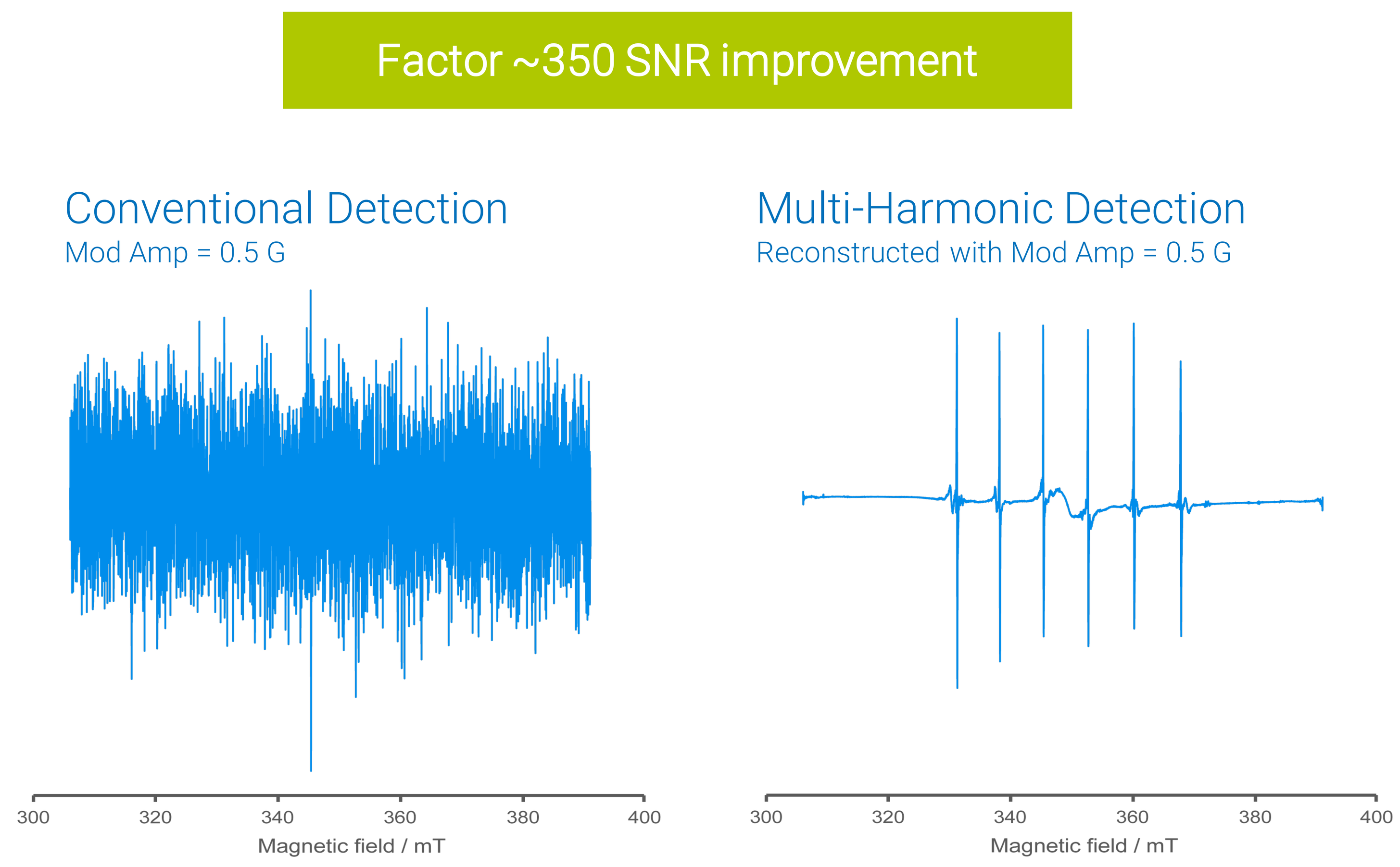

Fig. 3 Reconstruction of 1st derivative CW-EPR signal with Multi-Harmonic Detection of a Mn(II) solid sample provides large signal-to-noise enhancement.

See the Unseen

The MHD also enables increasingly challenging EPR experiments. Zebrafish embryo are used as models for aging, cancer and neurodegenerative diseases. The organism must have low microwave exposure and be kept in an aqueous solution, already making CW-EPR a tedious task. With the MHD, an EPR spectrum was reliably detected in a single zebrafish embryo in a flat cell in the ESR5000 (Figure 4). The signal in this study arises from melanin radicals as a response to anti-thyroid drug. The possibility of multiple measurements on the same organism allows for investigation of response to various stimuli.

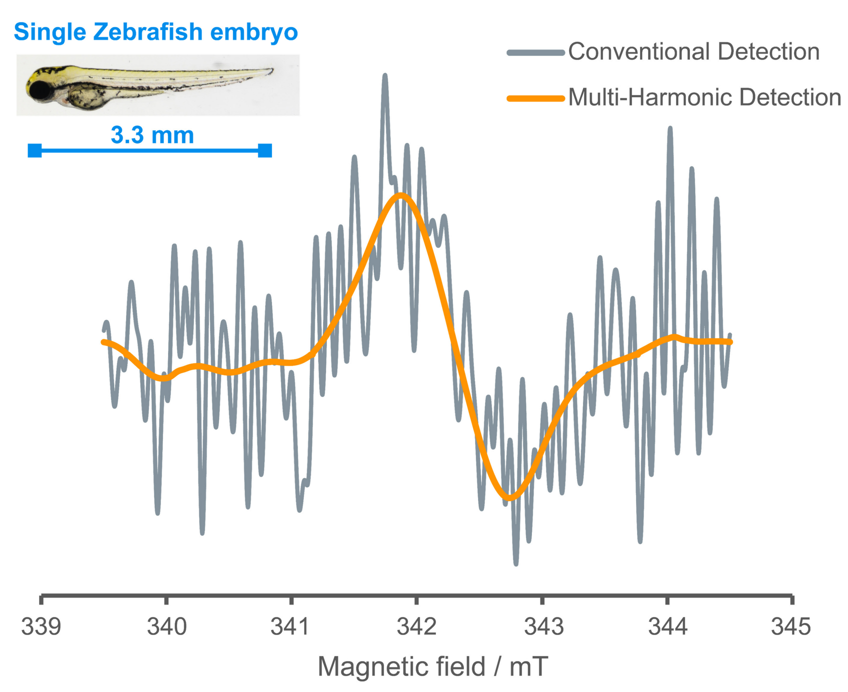

Fig. 4 CW-EPR for in vivo study of free radicals in zebrafish embryo. Weak microwave power and aqueous samples resulting in weak signals that were improved through MHD.

Package Content and Compatibility

The MHD is compatible with all CW-EPR spectrometers available in our catalog. It also comes in two flavors. The SD10 that can detect up to 10 harmonics works for ESR5000 and ESR5000 only. The HD100, which can detect up to 100 harmonics, is compatible for both ESR5000 and floor-standing EPR units! Both systems come with a computer and the acquisition/analyzing software.

SD10

HD100

Fig. 5 Multi-Harmonic Detection Accessory units with the ESR5000 (left) and the floor-standing EPR units (right).

Conclusion

- Excellent tool for enhancing SNR in EPR experiments
- Advantageous when no prior knowledge of EPR lineshape is known
- Reduces experimental acquisition times
- Allows for in-vivo experiments
- Compatible with our entire CW-EPR portfolio