

Frequency Mixing Magnetic Detection for Viscosity Sensitive Biosensing Using Magnetic Nanoparticle Relaxation

Ali Mohammad Pourshahidi¹, Timur Bikulov¹, Amirreza Gholivand², Abdalhalim Abuawad¹, Ulrich M. Engelmann³ and Hans-Joachim Krause^{1,3}

¹ Institute of Biological Information Processing, Bioelectronics (IBI-3), Forschungszentrum Jülich, 52425 Jülich, Germany,

² Institute of Epileptology and Cognition Research, Sensory Dynamics and Behavior Lab, University of Bonn, Faculty of Medicine, 53127 Bonn,

³ Medical Engineering and Applied Mathematics, FH Aachen University of Applied Sciences, 52428 Jülich, Germany
a.pourshahidi@fz-jeulich.de

Summary:

Frequency Mixing Magnetic Detection (FMMD) enables magnetic biosensing by probing the nonlinear dynamic response of magnetic nanoparticles (MNPs) under dual-frequency (f_1 , f_2) excitation. Known Brownian-relaxation dominated MNPs BNF-Dextran 80 nm were studied in media with varying viscosities. The complex signal components at the mixing harmonics $f_1 + 2 \cdot f_2$ and $f_1 + 4 \cdot f_2$ were analyzed as a function of the low frequency excitation field, and the harmonic ratio $R = A_{(f_1+2f_2)}/A_{(f_1+4f_2)}$ was evaluated as a sensitive metric. The measured ratios show a pronounced dependency on viscosity and field amplitude. The complex-plane trajectories additionally reveal viscosity-dependent changes in both amplitude and phase. These findings show that harmonic-ratio analysis in FMMD can provide environment-sensitive readout beyond conventional single-harmonic amplitude detection. The approach is therefore promising for viscosity-sensitive biosensing, where target binding or matrix changes modify the local hydrodynamic environment of the MNPs.

Keywords: Magnetic Nanoparticles, Magnetic Biosensor, Viscosity sensing, Frequency mixing magnetic detection

Introduction

Frequency Mixing Magnetic Detection (FMMD) is a sensitive magnetic readout technique based on the nonlinear response of magnetic nanoparticles (MNPs) under dual-frequency excitation [1]. While FMMD is commonly used for MNP characterization [2] and concentration-dependent detection in biosensing applications [3], its mixing harmonics also encode information on nanoparticle relaxation processes [4]. Since these magnetic relaxation processes depend on the local hydrodynamic environment, FMMD can provide viscosity-sensitive biosensing, particularly for particles with larger hydrodynamic size.

This aspect is highly relevant for bioassays in which target recognition does not only change the quantity of magnetic markers, but also affects their mobility, aggregation state, and rotational freedom. Such changes are expressed in Brownian relaxation and can therefore modify the amplitude, phase, and relative spectral content of the FMMD signal. Relaxation-sensitive analysis may thus provide an additional

biosensing dimension beyond pure particle quantification. Here, we investigate the viscosity dependence of FMMD relaxation signatures using commercially available MNPs across a physiologically relevant viscosity range. The complex amplitudes of selected mixing harmonics were analyzed under varying driving field conditions.

Method description

In this study, BNF-Dextran 80 nm (1.5 mg/mL) magnetic nanoparticles (from Micromod GmbH Rostock Germany) were investigated in glycerol water mixture media covering a viscosity range from 0.8 to 612 mPa·s. The particles were excited by two sinusoidal magnetic fields of a form

$$B(t) = B_1 \sin(2\pi f_1 t) + B_2 \sin(2\pi f_2 t). \quad (\text{Eq.1})$$

The excitation settings are presented in Tab.1.

The complex FMMD response was evaluated at the mixing harmonics $f_1 + 2 \cdot f_2$ and $f_1 + 4 \cdot f_2$. Furthermore, the harmonic ratio $R = A_{(f_1+2f_2)}/A_{(f_1+4f_2)}$ defined here as the amplitude of

$(f_1 + 2 \cdot f_2)/(f_1 + 4 \cdot f_2)$, was obtained as a viscosity sensitive metric.

Tab. 1: Measurement excitation settings

Excitation field	Frequency [Hz]	Magnetic field [mT]
Low frequency magnetic field B_2	62.95	Varied from 1.0 to 18.7
High frequency magnetic field B_1	40500	1.2

Results

The complex FMMD trajectories show smooth, non-monotonous and reproducible viscosity-dependent evolution in both magnitude and phase (Fig. 1). The $A_{(f_1+2f_2)}$ component remains dominant, while the $A_{(f_1+4f_2)}$ component shows lower amplitude response as expected.

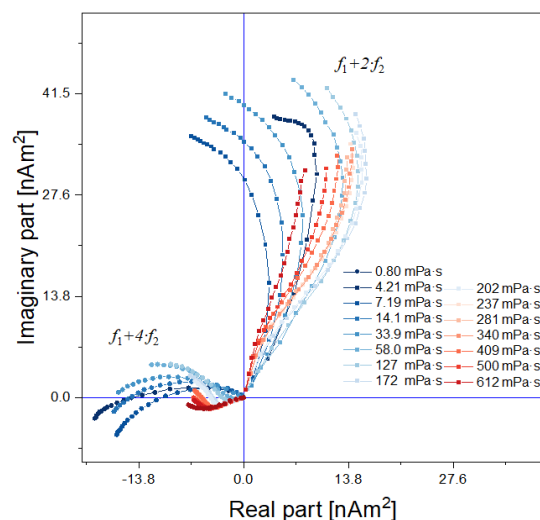


Fig. 1. Complex representation of measurement signals $f_1+2 \cdot f_2$ and $f_1+4 \cdot f_2$ under varying B_2 magnetic field. Colors represent media viscosity.

The ratio R shown in Fig. 2 depends on both viscosity and excitation field. It can be seen that for the lower viscosity regime, it decreases with increasing B_2 and the ratio approaches 2 at the highest fields. At intermediate viscosities, the ratio is larger, while at higher viscosities the ratio slightly increases, compared to the lower viscosity levels after the initial drop. Over the measured field range, the ratio shows the most separation between viscosities in the intermediate to high B_2 region, approximately above 10 mT. At lower fields, the spread is larger and less stable, while at higher fields, the curves move closer together.

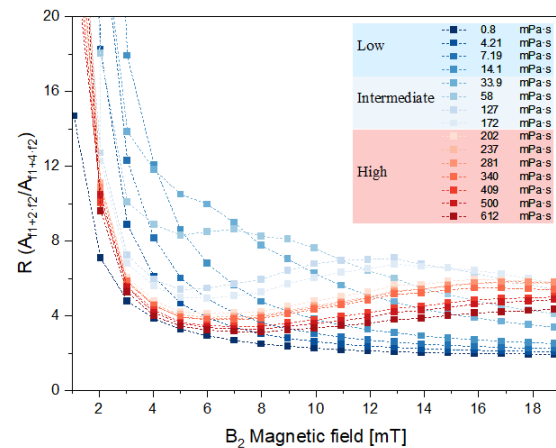


Fig. 2. The obtained ratio R for varying viscosities as a function B_2 magnetic field.

Conclusion

The results demonstrate that harmonic-ratio analysis in FMMD can serve as viscosity-sensitive signature beyond single-harmonic amplitude readout. In particular, the ratio R captures changes in the dynamic state of Brownian relaxation-dominated particles, in this case BNF-Dextran 80 nm, over a wide viscosity range, and therefore represents a metric feature for bio-sensing tasks involving binding, gelation, aggregation, or microenvironmental changes. The presented approach supports the development of FMMD toward relaxation-based and environment-sensitive magnetic biosensing.

References

1. Krause, H.-J.; Engelmann, U.M. Fundamentals and Applications of Dual-Frequency Magnetic Particle Spectroscopy: Review for Biomedicine and Materials Characterization. *Adv. Sci.* **2025**, *12*, 2416838, doi:10.1002/advs.202416838.
2. Pourshahidi, A.M.; Jean, N.; Kaulen, C.; Jakobi, S.; Krause, H.-J. Impact of Particle Size on the Nonlinear Magnetic Response of Iron Oxide Nanoparticles during Frequency Mixing Magnetic Detection. *Sensors* **2024**, *24*, 4223, doi:10.3390/s24134223.
3. Achtsnicht, S.; Neuendorf, C.; Faßbender, T.; Nölke, G.; Offenhäusser, A.; Krause, H.-J.; Schröper, F. Sensitive and Rapid Detection of Cholera Toxin Subunit B Using Magnetic Frequency Mixing Detection. *PLOS ONE* **2019**, *14*, e0219356, doi:10.1371/journal.pone.0219356.
4. Bikulov, T.; Eivazi, F.; Offenhäusser, A.; Krause, H.-J. Multicontrasting MPS by Dual-Tone Nonlinearity Probing. *Int. J. Magn. Part. Imaging IJMPI* **2024**, *10*, doi:10.18416/IJMPI.2024.2403014.

Acknowledgements

Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Project number 574089568.

The authors thank Oumaima Chig and Ibrahim Nasr Allah for conducting parts of the measurements.