Reviewer 1 (Frédéric Mentink-Vigier):

Intermolecular contributions, filtration effects and composition of the SIFTER signal

SIFTER is a pulse sequence that can be used to extract the dipolar coupling, and thus the distance, between two unpaired electrons. The pulse sequence uses a single frequency, unlike PELDOR. The article describes a model to explain the SIFTER experimental data to better understand how the presence of inter molecular dipolar couplings affects the evolution of the signal and its decay/background. The work is very interesting and thorough. In general, the article describes in detail the strategy to tackle the problem and I have not found major issues. However, I have found at times the text to be quite dense or unclear, and below are my suggestions to make the reading easier.

We thank the reviewer, Frédéric Mentink-Vigier, for his positive evaluation of our work and for the constructive criticism and suggestions provided. We address point-by-point below and describe our changes.

Section 2: When starting the description of SIFTER, it would be good to have an example of a SIFTER trace can and how the pulses are changed. This would help the reader get into the pulse sequence without going back to previous articles. Could you also add the Hamiltonians you consider in each section

In order to clarify the pulse sequences in Fig. 1, we extended the figure by adding the time incrementation schemes to the pulse sequences. As time traces from these pulse sequences are shown in Figures 3-7 for different cases of mono and biradicals, we would here refrain from picking a single condition as an example. We also added the respective Hamiltonian terms to each section.

L105: "In the second parentheses the first term appears due to the time evolution of the first term in the first parentheses and vice versa." The sentence is a little convoluted. I suggest rephrasing it.

The sentence is extended and rephrased to be more explicit (now L113-115): "The term $S_{A,y}$ in the first parentheses and the term 2 $S_{A,x}$ $S_{B,z}$ in the second parentheses result from the evolution of the term $S_{A,y}$ in the equation (3). Accordingly, the term $S_{B,y}$ in the first parentheses and the term 2 $S_{A,z}$ $S_{B,x}$ in the second parentheses result from the evolution of the term $S_{B,y}$ in the equation (3)."

L140 "Such monoradical like signal" is not clear. While you discuss, monoradical before, the "like" is not clear. May be just say: Monoradical-like signal or invert the order of the paragraph describing what was observed first in Doll's work

We dropped the word "such" as suggested (now L147).

L159: wl and wlm are confusing. My understanding is that there is some redundancy here though I do not think it matters. wl refers to the intramolecular dipolar coupling of the biradical l but not the spins themselves, while lm is for the coupling between two spins l and m belonging to two different molecules. I would use upper case Wlm for intramolecular, and lower case wlm for intermolecular. This would ease understanding where intermolecular couplings contribute.

In fact, there is no real redundancy here, yet to clarify we now illustrate the different inter- and intramolecular dipolar frequencies in a new Figure 2. The frequency w_l is written with one index (this is the spin index, not the molecule index) because there are only N/2 different intramolecular frequencies. Summing over all spins (only single index summation is needed here) would just count all these frequencies exactly twice, which is convenient enough to handle. The intermolecular frequencies need to be summed in some cases over two indices, and there are N(N-2)/2 different frequencies w_{lm}, i.e. here two indices are really necessary. Based also on the preliminary clarification and agreement with the reviewer (see open discussion comments), we would prefer to stay here with the originally chosen abbreviations. The new Figure 2 was added to illustrate graphically the differences in these coupling frequencies. In addition, in the text we comment now on the number of different frequencies of each type (L164 to 167).

For Eq 9, Since you use the same approach as in 2.1, I would suggest, for consistency, to use sigma(2tau_1-deltat) should be used not V.

We would like to discriminate between the two-spin density matrix in the section 2.1. and the multi-spin density matrix in the section 2.2. To do so, we changed for the operators to σ_V to still distinguish it from σ_S in the first section and kept for the detected signals the symbol 'V'. Time points were added to the equations to allow the readers to follow more easily. (See also below a similar comment from the editor.)

L170 $\tan(\omega n\tau 1)\cdot\cos(\omega n\tau 1)\approx\omega n\tau 1\cdot\cos(\omega n\tau 1)$ is a disturbing approximation. It is far worse than a simple Taylor expansion of sin. I understand why you do this to have a product over all N, I would do it first, then apply the approximation of tan(x) as ~x . Actually you explain this later, L258 "In equation (21) we can add the missing factor cos"

*We explain this now in the text in two steps (L182 to 184). First, we use the exact relation sin(x)=tan(x)*cos(x), and explain the reason to do so. In the next step we write the first Taylor term for the tan(x) while keeping the whole cos(x) function unchanged. This is correct up to the linear terms on x, but keeps some terms ~x^3, which is not important within our approximation level. In fact, in the final equation for V we get the most significant term ~x^2. The next order term, which we partially neglect and partially keep in the cos(x), would be ~x^4. This was also clarified with the reviewer in the open discussion.*

Eq(14), it would be easy to have some numerical values for those terms using a random distribution of radicals and have the corresponding decay. Also in the weak coupling approximation the equation can be further simplified using a Taylor expansion of the cos. Note: for simulations, a good random distribution could be made with packmol.

These decays are actually the same functions that appear e.g. in DEER background description (with formally setting the modulation depth to 100%). We added a comment on this after the equation (now eq. 15, L205-208), and a relevant DEER reference for the reader to look up the characteristic shapes and decay times/spin concentration relations.

L195 and subsequent, these sentences could be represented on a figure to simplify the writing.

Please see next answer.

L215, a graphical representation would help as well.

Regarding both these reviewer comments to L195 and L215: We thank the reviewer for the suggestion and we now added a detailed figure (new Figure 3), which schematically explains a number of key steps in the coherence transfer and intermolecular evolution, relation to auxiliary experiments and the shapes of some important functions present in the SIFTER signal as background or coherence transfer factors, thus illustrating the different contributions.

Eq 15. If B corresponds to decay, then should not we see operators in this equation?

The electron nuclear evolution cannot be computed analytically. Therefore, we avoid writing these signals in operator form. Instead, we formally write the functions B_S(tau1, tau2) and B_t(tau) assuming, they represent the corresponding signals after computing the trace with the detection operator and averaging over the ensemble of electron spins. Some explanations of these points are also added in the text and we note that these signals can be measured experimentally and hence used in a numerical background correction, see e.g. L241-244.

L 243 what do you mean by all the A-B coupling terms? This would explain the introduction of Q.

This is rephrased to (L300) "In order to describe the density matrix evolution upon all dipolar couplings between A-spin and B-spins we will also use the abbreviation".

Eq 23, R is not an obvious choice for an operator, as it often represents relaxation.

We removed now the additional abbreviation 'R', and just write this with the corresponding sigma term from equation (4), see now Eq. 26.

L367, missing space. ".All"

This is corrected.

Could you move the figures where they are first mentioned?

Yes, this is done in the revised manuscript.

Reviewer 2 (Michael Bowman):

This is a excellent consideration of the the SIFTER signal under idealized conditions of perfect pulses and limited relaxation. The results are in good agreement with model experiments. They supply a theoretical basis for planning and for analyzing real SIFTER experiments. More realistic treatments will be made as needed to extend these results, for example, to finite width pulses, pulses with non-ideal turning angles, partial excitation of the spectrum, mixtures of monoradicals and biradicals. This work certainly deserves publication.

We are thankful to the reviewer for his positive evaluation of our work.

This paper is written in a verbally descriptive style where each formula and term is described verbally in preference to concisely describing it in equations. I find that style rather difficult to follow when the math is as extended as this. However, this style suits some people well and is prefered by them over styles that I would prefer. So I am not advocating a change of style and do not argue the merits of people's taste in styles. However, there are some things that the authors can do to make the paper more easily read and understood.

We thank the reviewer for the open yet understanding criticism of our manuscript style. We have improved readability with these comments in mind as described in detail below/above, such that the revised version should be easier to follow for readers of both styles.

One of these is concerned with the notation and terms in the equations. EPR has many official and unofficial standards of terminology, but with many conflicts between the different sets. For example, omega<0> is often the microwave frequency of the spectrometer instead of the dipolar splitting of radical pair 0. Also, omega<dd> is often the dipolar frequency at a particular distance r and angle theta, as defined in eq. 1 and used multiple times several pages later. Or it is the maximum frequency for a particular distance but the angular dependence is expressed separately. In addition, the many terms in the paper make it difficult to retain (or even find) their definition and use. So, I would find it a great help in reading this paper if there were a table listing all of the terms and meanings in sufficient detail to understand if omega<dd> is the dipolar splitting with angular dependence included, or if it is the dipolar interaction at a distance r with a seperate angular term. Such a table would also make it easier to see the relevance and role of terms, which is difficults because there are so many used in the paper.

In order to make the notation of the various dipolar coupling frequencies clear, we have included both a new Figure 1 and a new Table 1 in the revised version. Figure 1 explains the different spin-spin coupling frequencies in a graphical fashion. Table 1 provides an overview of key terms in the spin density matrices and detected signals together with references to the corresponding equations, and with some concise explanations.

The long verb al descriptions makes it difficult for me to understand the form and significance of terms until I reached the figures at the end of the paper. But then I had to go back and reread the first 3/4 of the paper. In know that when I read it again, I will be constantly refering to the figures as I read section 2 and 4. I would recommend putting some schemes or diagrams in section 2 to illustrate the terms and their relationships and reveal their significance. I think this is a case where each picture would be worth much more than a thousand words.

We thank the reviewer for the suggestion. Accordingly, in order to make it easier for the reader to get an overview of the different terms contributing to the SIFTER signal, we have included a new Figure 3 into the revised version to provide an overview of the different terms and their relation.

Finally, I notice some inconsistency in refering to equations and terms in the paper. Many of the equations are refered to later by their eq. number. But others, such as sigma<1> through sigma<3> are refered to by their name. I do not see that those terms ever appear on the right hand side of an equation, although I might have missed it. So, I am not clear why they would be given names in the first place.

We revised the manuscript to always refer to the corresponding equation number in such cases.

Editor comments/review (Stefan Stoll):

In addition to the reviewers' comments, I am adding a few remarks as well:

This is an interesting paper that adds theoretical insight about how dividing a SIFTER signal by a SIFTER signal without the second pi/2 pulse--called SIDRE here--is valid for removing unmodulated signal contributions.

[1] The main practical implication of this work is that the signal division approach is valid. This should be emphasized more.

This is likely a misunderstanding. The procedure that follows from our theoretical account is different from a one-step background division: First, the unmodulated part of the SIFTER signal has to be fitted and subtracted. Second, the remaining SIFTER signal with dipolar modulations has to be divided by a different background function. The unmodulated part of the SIFTER signal is indeed close to the SIDRE signal (the three-pulse refocused echo sequence with constant sum of the delay times). Therefore, and only for the purpose of analyzing the correspondence between the theory and experimental data, we discuss here the SIFTER traces divided by the SIDRE traces.

To emphasize this clearly, we added in the abstract of the revised manuscript (L9-11): "Our theoretical account suggests that the appropriate procedure of extracting the intra-molecular dipolar contribution requires fitting and subtracting the unmodulated part, followed by division by an intermolecular background function that is different in shape. This scheme differs from the previously used heuristic background division approach."

Furthermore, we added text in the Conclusions section stating (L563-566): "Our theoretical results predict that the modulated SIFTER signal is multiplied with one type of the intermolecular background function, while the unmodulated part of the SIFTER signal, which is also an intermolecular signal on its own, has a different shape. Accordingly, the appropriate background correction requires fitting and subtraction of the unmodulated part, followed by a division by a different background function, distinct from the unmodulated SIFTER signal."

[2] This division approach is not new. It was first proposed and succesfully utilized in Spindler et al, J.Magn.Reson. 280, 30 (2017), see Fig. 6 and discussion at the end of section 3.5 around eq.7. The authors do not cite nor disucss this originial prior work. This references should be cited, alongside the similarly early and relevant 2017 Denysenkov reference and the 2018 Bowen reference, in a prominent place such as the introduction.

We are happy to add such a discussion and the reference, with the above remark that the division approach actually turns out to be incorrect (of course the division approach was a very reasonable guess earlier, in the situation where no theoretical description was available). It is unfortunate that we missed this reference because our aim was actually to cite all works so far published on SIFTER (there are not too many of them). Now, we added a comment in the introduction section and extended the discussion of the previous background correction procedure (compare lines 415-419 in the first version of the manuscript). We have also re-ordered the introduction for clarity.

The comment in the introduction section reads (L55-58): "The main approach so far for the SIFTER intermolecular background correction has been based on two heuristic assumptions (Spindler et al., 2017). First that the background function can be factorised analogously as in DEER, and second, it has been recognised that the background must contain an additional term, for which similarity in shape has been found to the SIFTER-delay refocused echo sequence trace (SIDRE, see Figure 1(a)) (Spindler et al., 2017)."

Added text to discussion (L481-485): "We would like to reiterate here that the currently used heuristic background correction procedure, based on the analogy with DEER data analysis, unfortunately, does not match our theoretical predictions. The intermolecular background signal for the modulated part of SIFTER signal would decay slower than the SIDRE signal B_s(tau1,tau2). Therefore, background division with using SIDRE as a background function or with fitting the background function to the unmodulated part of the SIFTER signal would disturb the desired intramolecular dipolar modulations."

[3] The entire Section 2 is very difficult to follow, due to notational inconsistencies and omissions, combined with a verbose and at times meandering narrative. It's all probably rigorous and correct, but it is hard to tell. Since this section is the valuable novel contribution of this paper, I strongly encourage to rework it substantially for terseness and clarity.

Here are just a few comments on the initial parts of section 2, (comments similar in spirit likely apply to the latter part):

- The section starting at line 127 ("Second, ...") seems to more appropriately be placed with the discussion of interacting biradicals later in the manuscript.

We respectfully disagree with this: The purpose of that discussion is to explain, why we can start computing spin density propagation from a single arbitrarily selected spin, and then sum/average the obtained result over all spins in the sample. In our opinion, the place for this discussion is correct: at the beginning of the section where all multi-spin computations are described.

- There is a notational inconsistency between omega_0 (dipolar frequency in biradical with B-spin number 0) and \tilde\omega_l (dipolar frequency in biradical with B-spin number l) - why tilde in one case and not the other?

The single spin from which we start spin density propagation is marked as A spin, and all (intra- and inter-molecular) couplings related to this spin don't have tilde mark. All couplings with both spins being B-spins are marked with tilde. We don't see any inconsistency here, however, based on the request for improved clarity and comprehensibility, we added a new Figure 2 that illustrates the definitions of the different dipolar couplings considered here. The figure illustrates how the complexity of the notation ultimately arises from the complexity of the problem.

- The approximation leading to eq.(10) is handy, but its range of validity is not stated. \omega_n\tau_1 must be smaller than about 0.5 for it to be reasonable. This should be mentioned. Does this have any consequence in the context of the experimental data?

As this is strictly related only to intermolecular couplings, this approximation would be accurate under a wide range of experimental conditions. We added the requested remark, and an estimate that at an intermolecular distance of 10 nm this would still apply up to the SIFTER trace length (from the edge to the center) of 10 μs. The corresponding characteristic spin concentration for such intermolecular distances is on the order of 1 mM, which is higher than typical concentrations used in experiments with narrow-line radicals (see L184-186).

- The terms "filtering" and "pre-filtering" are used in several places, including the title. I have to admit that I do not completely understand what this term is meant to indicate, so I am not able to judge whether the associated statements are valid. What is the definition of filtering? Can this be clarified?

We thank the reviewer for pointing this out. The term ‘filtering’ here actually combines two views on the electron-proton processes, and as such it is a bit ambiguous by nature. The purpose is to indicate that electron-proton (electron-nuclei) interactions play a role in the formation of the SIFTER background, and that these contributions are different for the pathways with and without coherence transfer to another spin. To state this clearly, we added the following paragraph in the beginning of the section 2.2.2 (L210ff):

“Electron spins interact with surrounding nuclear spins and change their precession frequency according to the continuously ongoing configuration dynamics of the surrounding nuclei. It is common to call such a process spectral diffusion. By nature, this is a deterministic process, but due to the very large number of coupled nuclear spins and a random distribution of couplings, it demonstrates quasi-stochastic features. Accordingly, each pulse sequence can be seen as a path for a (quasi-)decay of electron coherence due to the nuclei-related dephasing, and at the same time it can be seen as a 'filter function' selecting certain electron-nuclear frequencies and suppressing others. The latter process is often called dynamical decoupling. The first interpretation can also be seen as a type of filtering, albeit a different one: local nuclear configurations around electron spins might differ in the characteristic decay times, due to the strengths of the couplings or due to the different statistics of nuclear spin-up and spin-down states. Thus, pathways within one pulse sequence would have different suppression factors for electron spins in different nuclear surroundings. To make the formulations easier, we will simply refer to 'filtering' of SIFTER signal contributions due to the electron-nuclear interactions without specifying the particular interpretation.”

- The discussion between eqs. (12) to (15) is confusing. It seems to start out with an ensemble average, but then some new notation is introduced, such \tilde{B}_S and B_t(\tau), without defining equations. What does the tilde indicate? What does the S subscript indicate (and the lower-case subscript s that appears elsewhere several times)? And what does t subscript in B_t represent? It's all a bit unpenetrable. Also, to what degree is eq.(15) going beyond just an ensemble average of the detected signal using the density from eq.(12)?

This is now clarified by adding spin Hamiltonians to each section, and a new Figure 3 as well as a new Table 1.

The tilde does not have a specific meaning and is now removed - it was used only to better distinguish B_S from B_t. Since this rather introduced confusion, in particular because tilde is used above to indicate the B-B dipolar frequencies, we removed the tilde mark for the SIDRE signal. B_t includes electron-proton interactions, B_2p only describes the intermolecular electron-electron contribution, B_S describes the electron-nuclear evolution in case of no coherence transfer during the SIFTER experiment. The different terms are now also listed in a new Table 1 as an overview. In a new Figure 3 the different terms are illustrated. These points were actually already explained in the manuscript, however, we hope that with the added spin Hamiltonians and figure and table, these explanations now suffice.

- Eqs.(9) etc. using the symbol V with incrementing subscripts to indicate density operators at various points in the sequence. Since the earliest Hahn papers, or even Bloch, V is universally used to indicate the signal intensity, i.e. something proportional to the trace of density times a detection operator). Why not use the standard notation \sigma for the density operator, as was done in section 2.1? Also, why not use the actual time points instead of the subscripts, like was done in Eqs.(2)-(6).

We changed for the operators to σ_V to still distinguish it from σ_S in the first section and kept for the detected signals the symbol ‘V’ as suggested. Time points were added.

- Eq.(17) is said to have "parabolic shape curved down" - as a function of what? It seems like it must be seen as a function of time t, where tau1 = tau1_0 + t and tau2 = tau2_0 - t. The scanned time t should be defined, probably together with the SIFTER sequence in Fig.1. Then, one can write tau1(t)*tau2(t) in Eq.(17), and this is then obviously parabolic in t.

Several changes we made in the revised version should make these points more clear. First, pulse sequence timings were added to Figure 1. Second, the new Figure 3 summarizes some key steps of SIFTER signal buildup and in particular includes a graph of D tau1 tau2 as function of t (new Fig. 3 f).

[3] Equation (40) seems to be the central result. Is it possible to add a figure that plots the various components of the SIFTER signal (and the SIDRE signal) and thereby assists in visualizing the anatomy of the signal?

The suggested figure has been added as the new Figure 3, please see the previous reply.

[4] The experimental figures 2 and 4 show the divisions of the SIFTER and SIDRE signals. While Eq.(40) decsribes the SIFTER signal, what is the expression for the SIDRE signal? Is it \tilde{B}(tau1,tau2)? It would probably be helpful to introduce a notation such as V_SIDRE(tau1,tau2) = ... for clarity.

SIDRE signal at these conditions is due to electron-nuclear interactions. As far as we know, there is no analytical solution for this case. We added the requested comment that V_SIDRE is equal to B_S(tau1,tau2) and included this signal in the new Table 1.

[5] The "SIDRE" signal is basically the 1D antidiagonal of the 2D signal of the refocused two-pulse echo, V(tau1,tau2). 2020 Bahrenberg et al show several plots of V(tau1,tau2), both experimentally and theoretically. It might be good to refer to this work a bit more explicitly in the context of the discussion of the SIDRE sequence and the SIDRE experimental data.

This is a good suggestion. We added the following short discussion (L246-249): ”The investigation of the refocused echo signal dependence on the two delay times indicates that the SIDRE signal has maximum intensity at tau_1 = tau_2, i.e. at t = tau_1 - tau_2 = 0, with t being the intrinsic SIFTER and SIDRE time variable (Bahrenberg et al., 2021). Note that, as indicated in the cited work, if either tau_1 or tau_2 is scanned without keeping the full evolution time tau_0 = tau_1 + tau_2 constant, then the maximum spin echo intensity comes at a time point different from tau_1 = tau_2.”