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30 March 2021

Dear Dr. Bajaj,

We greatly appreciate your consideration and the insightful reviewer comments and suggestions for our manuscript, JoVE62425 "ARL Spectral Fitting as an Application to Augment Spectral Data via Franck-Condon Lineshape Analysis and Color Analysis."

We hope that we satisfactorily address the remaining editorial and technical comments through our manuscript revision and the responses embedded below. We look forward to your response and are happy to provide any further clarification as necessary.

Sincerely,

Ryan M. O'Donnell

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EMBEDDED RESPONSES TO REVIEWER COMMENTS FOLLOW:

Editorial comments:

1. The editor has formatted the manuscript to match the journal's style. Please use the attached file for revision.

[RMO] The authors concur with all proposed formatting changes and new title.

2. Please address all the specific comments marked in the manuscript.

[RMO] All comments have been addressed in the revised manuscript.

3. Please address all reviewer comments as well - (reviewer2's screenshots are attached with this email. Please review and address comments accordingly).

[RMO] See below for specific responses.

Reviewers' comments:

Reviewer #1:

The work can be accepted as it is.

Reviewer #2:

The manuscript was revised well. However, revisions are still required since the authors did not respond the reviewer's comments satisfactorily as below.

1. Original comment: The reviewer installed the application and performed the spectral fitting for the reviewer's data and test data including in the application. The resulting fits, however, did not reproduce the spectral band shape at all even when the reviewer used the values obtained by his original fitting program as the initial parameters. The fitting algorithm may not work well.

The reviewer tried fittings again. The spectra were reproduced by employing adequate parameters, indicating that the spectral "simulation" does work well. Upon a fitting, however, the simulation deviated from the targeted spectrum.

[RMO] We confirmed the Reviewer's outputs by following along with their screenshots as a guide. We concur that the fits returned through optimization are indeed worse than the fits obtained through "by-hand" fitting and admit that the fitting algorithm may not work well for all data sets, although we believe this to be a generic problem for FCLSA. In our attempts to alleviate these issues, we implemented two separate optimization routines with various control parameters including the ability to fix parameters and set boundary conditions (See Lines 374 – 383). The potential issues with obtaining satisfactory FCLSA fits are discussed at length in the manuscript (Lines 385 – 424) with suggestions for obtaining accurate by-hand fits using the calculated R^2 values as a guide. Furthermore, we note that this is an issue for the community at large as the software routines used to fit FCLSA spectra are not publicly available nor discussed in detail in the literature. Our intent with this software and manuscript is to provide open-source code and software that can be utilized by spectroscopists and improved through community interaction. We welcome any suggestions the Reviewer may offer for further development of our fitting routines.

2. Original comments: The band shape of an emission spectrum in the vertical irradiance (energy) scale differs from that in the photon number scale. The application does work for the latter type of spectra. The confusing vertical axes should be noted in both manuscript and application.

The authors' answer is based on the x-axis scale of a spectrum (wavelength or wavenumber). The reviewer's comments, however, is about the y-axis scale. The band shape of an emission

spectrum depends on the vertical-axis scale owing to the E = hν relationship, and the Franck-Condon analysis is applicable to spectra only in the photon-number scale.

[RMO] We believe that there must be some lingering miscommunication or understanding on our part and hope the following adequately addresses the Reviewer's comment. Our software takes experimentally measured photoluminescence spectra as an input, typically measured as counts per second (y-axis) as measured via photomultiplier tube or CCD camera versus wavelength in nanometers (x-axis). FCLSA are typically performed in "energy space" by converting from wavelength (λ, nm) to wavenumber ($\overline{\upsilon}$, cm^{-1}) which our software does and allows the user to toggle back and forth between both units (nm and cm^{-1}). In doing so, we account for the dispersion differences associated with conversion of the emission spectra by using the equation given on Line 74:

$$I(\overline{\upsilon}) = \lambda^2 I(\lambda)$$

Therefore, we updated the software and manuscript figures to label the y-axis as either "Normalized $I(\overline{\upsilon})$" or "Normalized $I(\lambda)$" for enhanced clarity when plotted against either wavenumber or wavelength, respectively.

Original Author Responses to Reviewer Comments from 18 February 2021 included below for reference:

Editorial comments:

Editorial Changes

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

[RMO] No known spelling or grammatical issues found in revised manuscript.

2. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note.". E.g. line 116-117: "..select the desired…" instead of "..user will select…" etc.

[RMO] Manuscript updated throughout with the imperative tense.

3. If button clicks/menu selections are identified (e.g., quotes or cursive text has been used), change them to bold text. Use either | or > between the clicks/selections, and do not use ◊ or other symbols. Example: "File ◊ Options ◊ Advanced" becomes File > Options > Advanced or File | Options | Advanced

[RMO] Manuscript updated with bold text and ">" notation.

4. Line 253: Insert the equation in the note.

[RMO] Inserted the equation into the note, see Line 260.

5. Cite the figures in-text with only their number. E.g.: line 329: "….Figure 1." instead of "….Figure 1. Single mode fit…".

[RMO] Updated so that only the figure numbers are referenced, Lines 338 and 344.

6. Cite the in-text references before ending the sentence. E.g. line 61: "…industrial applications 1." Instead of "…industrial applications.1" ; line 368: "…Levenberg-Marquardt algorithm21." instead of "…Levenberg-Marquardt algorithm.21 " etc.

[RMO] In-text references moved into the sentence structure.

7. Remove "&" from the references. Follow the JoVE style for citation: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Full Source. Volume (Issue), FirstPage – LastPage(YEAR).]

[RMO] References fully updated to follow JoVE style and verified for accuracy. *There is an error in the JoVE EndNote Style file (https://www.jove.com/files/JoVE.ens) that uses ampersands instead of commas in Bibliography > Author Lists > Author Separators > before last.*

Reviewers' comments:

Reviewer #1:

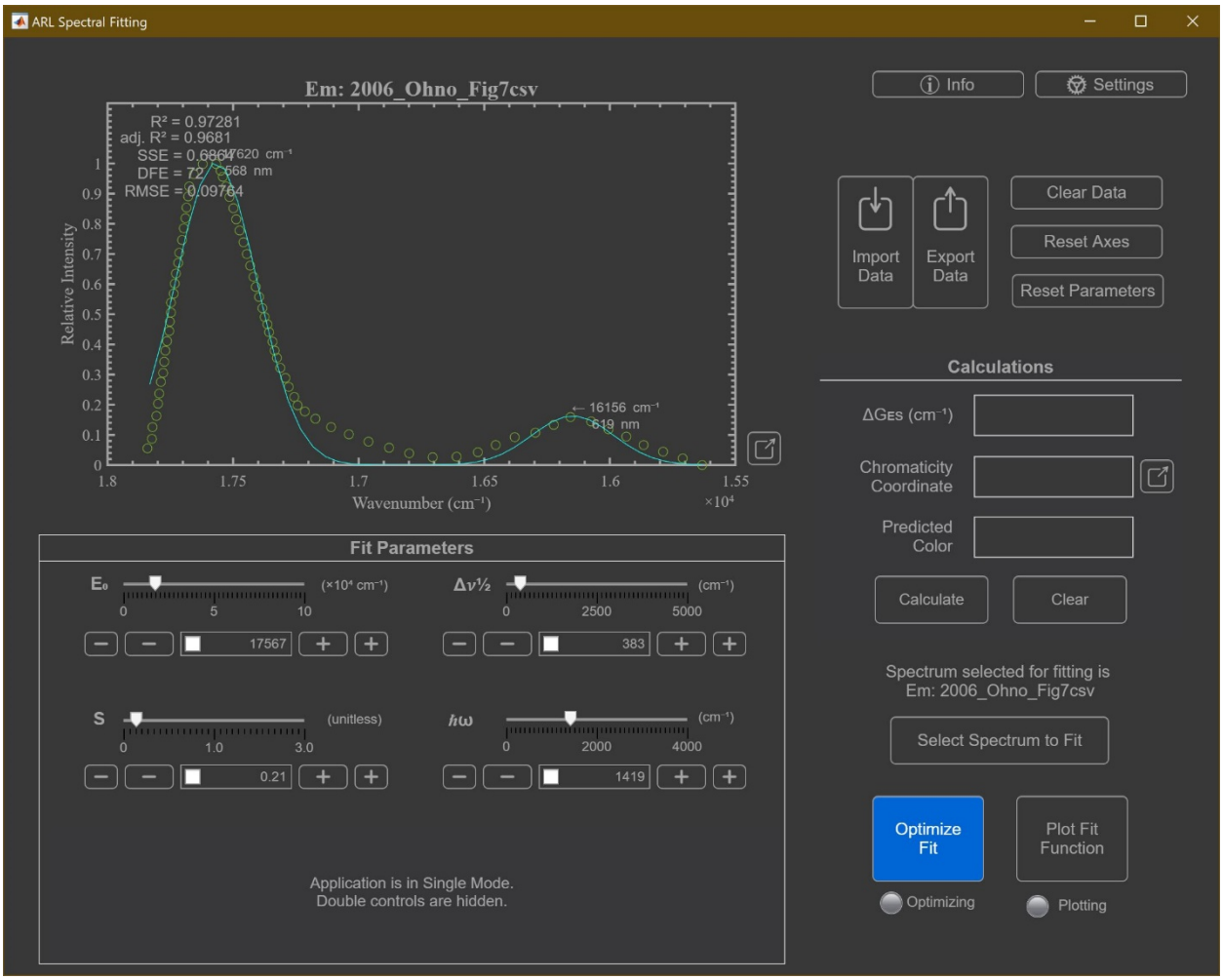
In this paper, William et al developed a software for the photoluminescence spectra fitting. The authors give a detailed manual and provide two examples. I found the software is useful. This manuscript can be consideration of publication on JOVE, if the following comments can be

considered.

The key information for the ARL development is based on FCLSA, the two equations on Page 2. There are three points of these equation for clarify:

1. there is a mistake in the single mode equation. The E in this equation should be replace by /nu^{/bar};
[RMO] The typo was corrected and now $\bar{\nu}$ is used in the equation on Line 101.

2. the authors suggested that the single mode equation can be used for the fitting of PL spectra measured at room temperature while the two modes equation should be used for 77K. Does this mean that the APL software can only be useful for the two temperatures? What about other temperature, for example 4K?
[RMO] Technically, any temperature can be used with the software although the majority of spectra obtained and fitted via FCLSA in the literature are at either room temperature (298K) or liquid nitrogen temperatures (77K). Calculation of the excited state free energy is temperature-dependent and the value can be set in the software to the experimental temperature as discussed in Lines 258 – 264. The single mode equation is best used for data with minimal band "structure." This is typically observed for room temperature measurements but is also sample dependent. For spectra with greater structural resolution, typically observed in frozen matrices at 77K or in other rigid media, the two-mode equation can be more useful. The issue here is with potential overparameterization of the data set, as mentioned in the Note on Lines 186-189 and throughout the Discussion section. The ability for the current version of our software to fit low temperature (<77K) spectra is dependent on the molecule and structure observed in the spectra. We demonstrate that our software is capable of adequately fitting the 2K spectral data for $[Ru(bpy)_3]^{2+}$ (DOI: 10.1021/ic052068r**) using literature data as shown below.**



3. The two equations sum the vibrational state up to 5. This makes sense for the PL fitting of organic molecular. However, this treatment is not reasonable for the fitting of PL of inorganic solid, which the vibration phonon energy is much smaller. Therefore, I think that the ARL software can only be used in the region of organic emitter. Such information must be reflected in the manuscript. Also, is it possible that the capability of the ARL software can cover the PL fitting of inorganic emitter?

[RMO] The software defaults to the most commonly used literature value of N=5 for the summation of the vibrational states. However, Line 108-109 was updated to reflect the capacity for user input. Although the software and manuscript focus on organic and transition metal complexes, particularly with regards to the implementation of FCLSA, we believe these to be the majority use case in the literature. Furthermore, we are not currently aware of any restrictions as to why an inorganic emitter could not be fit using the current version of our software. As our intent is to promote community interaction and feedback for future versions, we welcome any further comments or suggestions from the reviewer.

Reviewer #2:

Manuscript Summary:

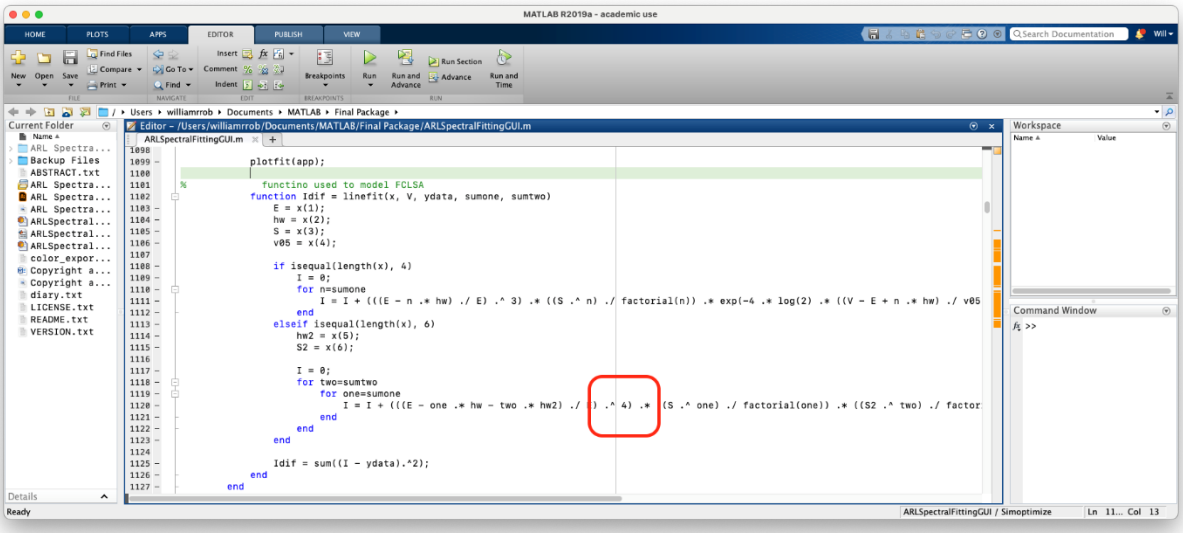
This manuscript reports an application and its protocol of the Franck-Condon band shape

analysis for emission spectra. Although the analysis gives us various information about the ground and excited states of a sample, it is applicable only in the research group with a specialist(s). This application and manuscript are hugely helpful for researchers in photochemistry and related fields since the user is easily accessible to the Franck-Condon analysis and spectral parameters. Thus, the reviewer recommends that this manuscript is potentially publishable in the Journal after the revisions below.

Major Concerns:
The reviewer installed the application and performed the spectral fitting for the reviewer's data and test data including in the application. The resulting fits, however, did not reproduce the spectral band shape at all even when the reviewer used the values obtained by his original fitting program as the initial parameters. The fitting algorithm may not work well.
[RMO] These are indeed major concerns and are addressed at the end of this document to provide ample space for our response.

The band shape of an emission spectrum in the vertical irradiance (energy) scale differs from that in the photon number scale. The application does work for the latter type of spectra. The confusing vertical axes should be noted in both manuscript and application.
[RMO] We understand that the band shape of an emission spectrum does differ when viewed in photon number or energy scale and our application works for both types of spectra. This is captured in our manuscript and software as the spectra are converted to the appropriate intensity via the equation given on Line 80. The original x-axis is determined upon data import (wavelength or wavenumber) based on the x-axis values and then plotted in wavenumber scale with the conversion applied, if appropriate. Thus, wavenumber spectra are imported with no changes while the intensity conversion is applied to wavelength spectra. We do concur that the vertical axes labeling may be slightly confusing and have updated the manuscript and software to display them more accurately as "Normalized Intensity" or "Intensity" dependent on whether normalization was applied or not.

The cubic part in the equation for two-mode fitting would be the forth power, not cubic.
[RMO] This typo has been corrected in the manuscript. We verified that the typo was only present in the manuscript as the software uses the correct fourth power as highlighted.

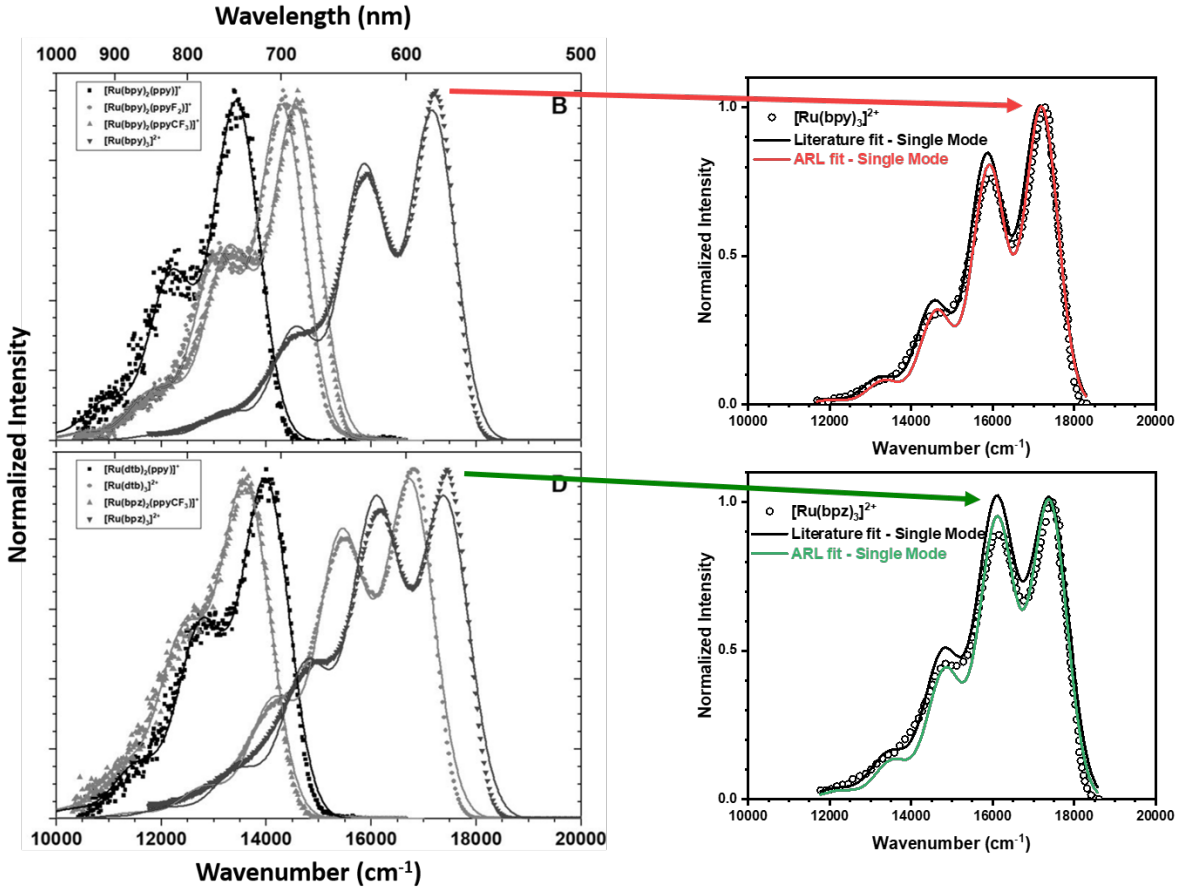


Minor Concerns:
Physical quantities in the equations should be given in italic.
[RMO] Physical quantities in the equations have been italicized.

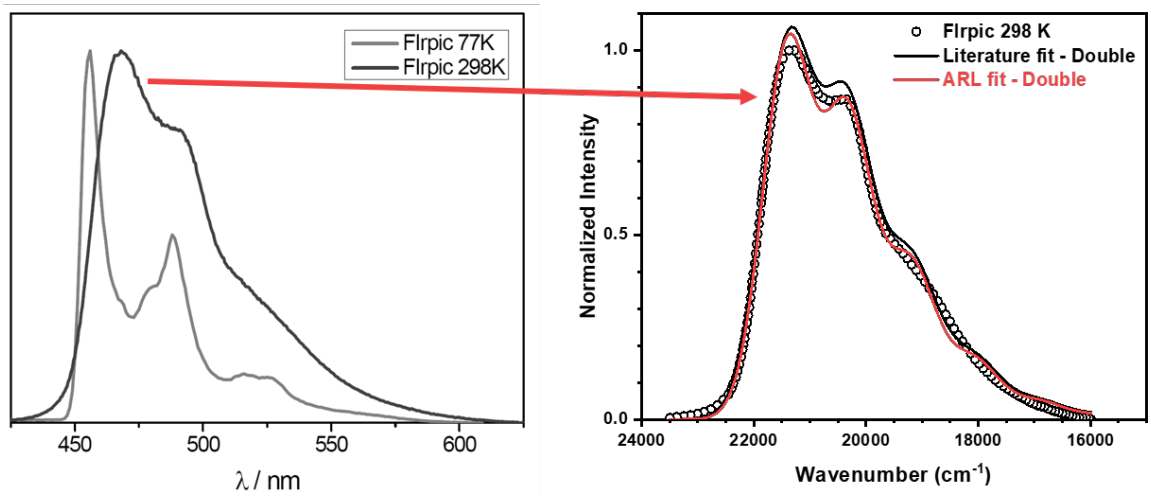
Major Concerns:
The reviewer installed the application and performed the spectral fitting for the reviewer's data and test data including in the application. The resulting fits, however, did not reproduce the spectral band shape at all even when the reviewer used the values obtained by his original fitting program as the initial parameters. The fitting algorithm may not work well.
[RMO] This is disappointing to hear and we are surprised by this comment. Without knowledge as to what spectra the reviewer used for fitting, we can only speculate as to the sources of error. We believe that the fitting algorithms implemented in the software (both Least-Squares and Simplex routines) perform an excellent job of fitting emission spectra with visible structure. We admit that broad, structureless spectra pose issues to most fitting algorithms due to the potential for overparameterization resulting from the 4 FCLSA fitting parameters. Fitting functions can become stuck in local minima or even "shallow" global minima due to a high degree of correlation between parameters. This is why we introduced parameter initialization along with the ability for users to: customize optimization settings; introduce custom parameter boundaries; and weight data. Below we demonstrate the robustness of our program via comparison to literature data that we digitized using WebPlotDigitizer (https://apps.automeris.io/wpd/**) and show how parameter correlation can drastically affect the results for broad, featureless spectra. We were also able to replicate our fits using code written in Mathematica and can provide that information upon request. It is incumbent upon the user to ascertain "reasonable" FCLSA parameters based on literature precedence and other experimental data when interpreting broad, featureless spectra.**

Spectral data for several polypyridyl ruthenium complexes containing cyclometalated ligands from Motley and co-authors are provided below with experimental data given as scatter points and FCLSA fits overlaid as solid lines (DOI: 10.1021/acs.inorgchem.7b02321**).**

We chose to first plot the single mode FCLSA fits for $[Ru(bpy)_3]^{2+}$ and $[Ru(bpz)_3]^{2+}$ using the literature parameters and our software to demonstrate that we can reproduce the previously published data. We then fit the experimental data using our Least-Squares routine and obtained the solid red and green fits to the data. All of the parameters we obtained were within 10% error or less from the published results. Our fits were characterized by R^2 values that were similar or improved compared to the literature fits. All FCLSA parameters and R^2 values are tabulated below for reference.

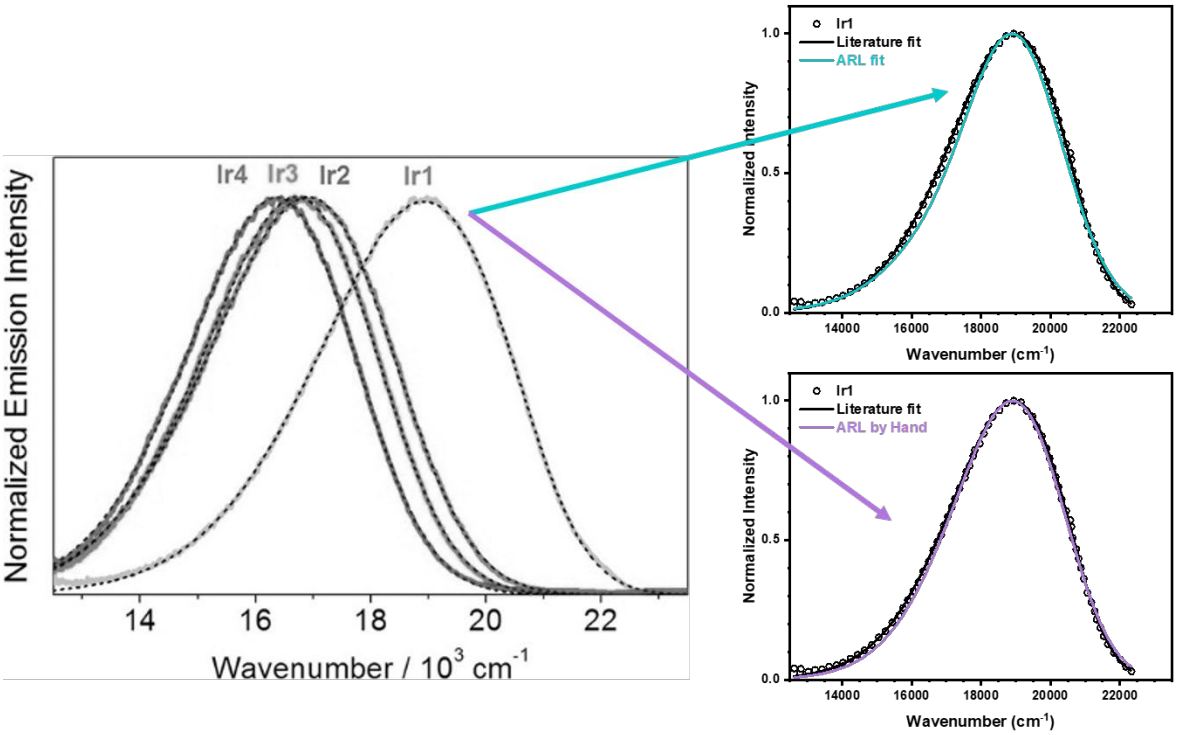


Next we reproduced the literature double mode fit for $[Ir(F_2ppy)_2(pic)]$ reported by Zanoni and co-workers (DOI: 10.1039/c5nj01352f**). The Supplemental Figure S8 is copied at left and shows the 298K spectral data that was digitized on the right (open circles) along with the FCLSA fit using the published parameters (solid black line). After performing our Least-Squares fitting routine, we obtained the fit overlaid in red. As demonstrated by the comparison of the FCLSA fitting parameters in the table, our software was well within experimental error of the published literature values.**



The previous two examples demonstrated the robustness of our software for fitting spectra with visible structure in the spectra. We now demonstrate how broad, structureless data can be fit using our software but is subject to overparameterization. Note that this is a mathematical issue that will be encountered by any fitting routine. The experimental emission spectra (solid lines) and overlaid FCLSA fits (dashed lines) from Zanoni and co-workers (DOI: 10.1021/ic500070s**) are shown at left. First we reproduced the spectral data (open circles) and published fit (solid black line), top right, to demonstrate that our software can accurately plot the double mode FCLSA fits. Using our software's Least-Squares fitting routine, we obtain the teal solid line which "fits" the spectral data. However, the actual parameters, as tabulated below, drastically differ from the published results as well as from literature precedent thus demonstrating that just a good fit does not necessarily bear physical relevance without interpretation. Using a "by-hand" fit and paying attention to the R^2 value, we were able to obtain the fit shown in solid purple at the bottom right with physically realistic parameters and an excellent R^2 value. What is interesting is that we purposefully used FCLSA values similar to those reported in the literature for Ir2 (see table). In the published manuscript by Zanoni and co-workers, Ir1 is a relative outlier when comparing its $\Delta\nu_{1/2}$ and S parameters to those for Ir2, Ir3, and Ir4. We believe that this is actually an artifact of their fit and the high degree of parameter correlation present in their spectral data. We then performed the same analysis for Ir2, not shown, and found that using a "by-hand" fit we could fit the Ir2 data using FCLSA parameters very similar to those published for Ir1. Thus, we demonstrated that in this set of data, $\Delta\nu_{1/2}$ and S are highly correlated. Care should be taken by experimentalists to be cognizant of this issue. While we noted the problematic possibility of overparameterization in the manuscript, experimentalists and reviewers should be responsible for determining**

the most physically realistic FCLSA parameters when fitting broad, structureless spectra.



Complex	Fit Source	E_0 (cm^{-1})	$\Delta\nu_{1/2}$ (cm^{-1})	$\hbar\omega_1$ (cm^{-1})	S_1	$\hbar\omega_2$ (cm^{-1})	S_2	R^2
$[Ru(bpy)_3]^{2+}$	10.1021/acs.inorgchem.7b02321	17,170	1,010	1,310	1.06			0.98917
	ARL - Least Squares	17,200	954	1,287	1.01			0.99272
	Difference	0.2%	-5.5%	-1.8%	-4.7%			*0.00355*
$[Ru(bpz)_3]^{2+}$	10.1021/acs.inorgchem.7b02321	17,400	1,090	1,310	1.26			0.99127
	ARL - Least Squares	17,389	1,013	1,282	1.18			0.99124
	Difference	-0.1%	-7.1%	-2.1%	-6.3%			*-0.00003*
Firpic	10.1039/c5nj01352f	21,400	1,080	2,380	0.15	1,070	1.00	0.99895
	ARL - Least Squares	21,406	1,038	2,282	0.14	1,068	0.98	0.99881
	Difference	0.0%	-3.9%	-4.1%	-6.7%	-0.2%	-2.0%	*-0.00014*
Ir1	10.1021/ic500070s	19,920	2,350	1,380	1.34			0.99968
	ARL - Least Squares	18,984	3,259	2,531	0.29			0.99756
	Difference	-4.7%	38.7%	83.4%	-78.4%			*-0.00125*
	ARL - By Hand	19,570	2,810	1,390	0.93			0.99889
	Difference	-1.8%	19.6%	0.7%	-30.6%			*-0.00079*
Ir2	10.1021/ic500070s	17,570	2,810	1,390	0.93			0.99988
	ARL - By Hand	17,850	2,350	1,380	1.25			0.99912
	Difference	1.6%	-16.4%	-0.7%	34.4%			*-0.00076*