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ARL Spectral Fitting as an Application to Augment Spectral Data via Franck-Condon Lineshape Analysis and Color Analysis --Manuscript Draft--

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TITLE:

ARL Spectral Fitting as an Application to Augment Spectral Data via Franck-Condon Lineshape Analysis and Color Analysis

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SUMMARY:

This protocol introduces Franck-Condon Lineshape Analyses (FCLSA) of emission spectra and serves as a tutorial for the use of ARL Spectral Fitting software. The open-source software provides an easy and intuitive way to perform advanced analysis of emission spectra including excited state energy calculations, CIE color coordinate determination, and FCLSA.

ABSTRACT:

The ARL Spectral Fitting application provides a free, publicly accessible, and fully transparent method for performing Franck-Condon Lineshape Analysis (FCLSA) on spectral data, in addition to CIE color coordinate determination and basic spectral processing. While some of the features may be found in commercial software or academic research groups, we believe that ARL Spectral Fitting is the only application that possesses all three of the aforementioned attributes.

This program is intended as a standalone, GUI-based application for the use by an average laboratory researcher without requiring any coding knowledge or proprietary software. In addition to the standalone executable hosted on ARL GitHub, the associated MATLAB files are available for use and further development.

FCLSA augments the information found in luminescence spectra, providing meaningful insight into the relationship between the ground and excited states of a photoluminescent species. This insight is achieved by modeling spectra with two versions (modes) of an equation that are characterized by either four or six parameters, depending on which mode is used. Once

optimized, the value of each of these parameters can be used to gain insight into the molecule, as well as to perform further analysis (for example, the free energy content of the excited-state molecule). This application provides tools for easy by-hand fitting of imported data, as well as two methods for optimizing this fit—damped least-squares fitting, powered by the Levenberg-Marquardt algorithm, and derivative-free fitting utilizing the Nelder-Mead simplex algorithm. Furthermore, estimations of sample color can be performed and reported in CIE and RGB coordinates.

INTRODUCTION:

Photoluminescence measurements, comprising both fluorescence and phosphorescence spectra, are widely used throughout various academic fields and industrial applications¹. Photocatalysts are increasingly used in organic synthesis for the production of complex and valuable target molecules²⁻⁴. In order to determine the energetics of photocatalysts, the excited state energy is routinely estimated using emission spectra. The development of novel lighting materials, such as organic light emitting diode (OLED) luminophores, necessitates that the observed color output be characterized and reported^{5,6}. *Commission internationale de l'éclairage* (CIE) color coordinates are routinely used for this purpose⁷.

The purpose of the ARL Spectral Fitting application is to provide a quick and easy method to augment spectral data through meaningful analysis that is widely accessible both in terms of ease-of-use and availability (https://github.com/USArmyResearchLab/ARL_Spectral_Fitting). This software performs several routine spectral processing functions automatically for the user, including data normalization and conversion between wavelength, λ , and wavenumber, $\tilde{\nu}$, units with appropriate intensity scaling as shown in the equation below¹. The software is capable of handling a variety of input and output file formats. Several advanced analyses are easily performed using the software such as the calculation of CIE and chromaticity coordinates, color prediction, determination of the excited state free energy (ΔG_{ES}) in various units, and FCLSA for the determination of the FCLSA parameters⁸.

$$I(\tilde{\nu}) = \lambda^2 I(\lambda)$$

A graphical user interface (GUI)-based application was pursued because it allows any researcher to perform this analysis and requires no background knowledge of computer science. This application was written in MATLAB, using its App Designer tool. Outside of ARL Spectral Fitting, finding a publicly accessible implementation of an application, designed to perform Franck-Condon Lineshape Analysis is practically impossible. This is because research groups do not publicly-release their implementations, preferring instead to keep them proprietary.

Franck-Condon Lineshape Analysis (FCLSA) is often used in the photophysical characterization of novel compounds because of the rich information it conveys about the molecule⁹⁻¹⁴. Each of the four parameters (six if in double mode) gives information about the excited state of the molecule. The energy quantity, or 0-0 energy gap, (E_0) is the difference in zeroth energy levels of the ground and excited states of the molecule. The full width at half maximum ($\Delta\nu_{1/2}$) informs about the widths of individual vibronic lines. The electron-vibrational coupling constant, or Huang-Rhys

factor, (S) is a dimensionless calculation based on the equilibrium displacement between ground and excited states of the molecule¹⁵. Finally, the quantum spacing parameter ($\hbar\omega$) is the distance between vibrational modes that govern the nonradiative decay of a molecule.

The equations for single and double mode FCLSA are as follows:

$$I(\bar{\nu}) = \sum_{v=0}^N \left[\left(\frac{E_0 - v\hbar\omega}{E_0} \right)^3 \frac{S^v}{v!} \times \exp \left(-4 \ln 2 \left(\frac{\bar{\nu} - E_0 + v\hbar\omega}{\Delta\nu_{1/2}} \right)^2 \right) \right]$$

$$I(\bar{\nu}) = \sum_{v_M=0}^N \sum_{v_L=0}^N \left[\left(\frac{E_0 - v_M\hbar\omega_M - v_L\hbar\omega_L}{E_0} \right)^4 \left(\frac{S^{v_M}}{v_M!} \right) \left(\frac{S^{v_L}}{v_L!} \right) \times \exp \left(-4 \ln 2 \left(\frac{\bar{\nu} - E_0 + v_M\hbar\omega_M + v_L\hbar\omega_L}{\Delta\nu_{1/2}} \right)^2 \right) \right]$$

where the parameters are as previously defined. In the double mode equation, S and $\hbar\omega$ are separated into medium ($_M$) and low ($_L$) energy terms. $I(\bar{\nu})$ is the intensity at the wavenumber $\bar{\nu}$ ^{10,16-18}. In both equations, the summation is performed over N quantum levels with a default value of $N = 5$, as commonly used in the literature¹¹, but any integer can be specified in the ARL Spectral Fitting Software under **Settings | Fit**.

PROTOCOL:

1. Data import

1.1. To import data, press the **Import Data** button. Select the type of spectrum being imported—either excitation or emission.

1.1.1. Once a spectrum type is chosen, ensure that the MATLAB file explorer will appear. From this window, select the desired file and press **Open**. Supported file types include .TXT, .CSV, .XLS, and .XLSX.

NOTE: Some data processing is performed automatically before plotting the imported data. This includes: detection of x-axis units (wavenumber or wavelength) and conversion to wavenumber, if appropriate; intensity normalization of the highest peak to 1; calculation of the energy quantity, if appropriate; and estimation of the quantum spacing. These values are calculated based on detected data peaks with energy quantity assigned to the wavenumber value of the highest energy peak and quantum spacing based on the averaged distance between vicinal peaks, which necessitates the detection of at least two peaks.

1.2. To load any of the spectra, press the button corresponding to the desired spectrum, found under **Info | Sample Spectra**. Nine sample spectra come pre-packaged with the application.

1.3. To load and plot more than one spectrum at a time, activate the check box **Allow multiple data spectra on axes** under **Settings | General | Figure Settings**.

1.4. To select a different loaded spectrum than is currently active, press the **Select Spectrum to Fit** button and then choose the desired spectrum from the list shown on the newly visible **Select Spectrum** panel.

2. Data processing

NOTE: The user may wish to perform data processing prior to the fitting process. Available processes include:

2.1. Selection of a peak to act as a basis for normalization: To select a peak to act as the basis for intensity normalization, press the **Select peak for normalization** button found under **Settings | General**. Follow the instructions shown on screen. The default peak for intensity normalization is the highest intensity peak found during import.

2.2. Conversion between x-axis units: To convert the x-axis's units between wavenumber (cm^{-1}) and wavelength (nm), toggle the slider found under **Settings | X-Axis** to the desired mode (either **Wavenumber** or **Wavelength**). Intensity as well as x-axis units will be adjusted for all loaded spectra, using the equation given above.

2.3. Constraining of x-axis range: To manually constrain the x-axis range, select **Manually adjust X-axis and fit limits** under **Settings | X-Axis**. Then, use the revealed controls to specify the x-axis range. By default, the application will automatically expand and contract the x-axis range to fit all loaded data points.

2.4. Alternative E_0 calculation methods: To select an alternative calculation method for the energy quantity, select the desired method found under **Settings | Fit**. The default method is **Full FCLSA fit**. To change to another method, select the corresponding radial button and follow onscreen instructions¹⁹.

3. Manual fitting

NOTE: Based on the amount of structure visible in the spectrum, it may be highly advantageous to initialize the fitting parameters with appropriate estimates prior to optimization. This initialization can decrease the time required for optimization and helps to ensure that the values returned by optimization are realistic for the spectrum.

3.1. Plot the fit function with its current parameter values by pressing the **Plot Fit Function** button.

3.2. Using a combination of the coarse and fine adjustment buttons, sliders, and edit fields, adjust

parameter values to increase the goodness of fit for loaded data. By default, the coefficient of determination (R^2) is displayed in the upper-left-hand corner of the graph. Use this as a quantitative measure of the goodness of fit to guide the choice of parameter values.

NOTE: Because the energy quantity (E_0) and quantum spacing ($\hbar\omega$) are calculated by the application upon data import, it is recommended that these values be held constant or minimally varied when fitting by-hand.

3.3. Use default setting to utilizes the single mode Franck-Condon Lineshape Analysis formula as it is most relevant for room temperature spectra. If desired, such as when fitting 77 K spectra, toggle between the single and double mode in **Settings | Fit**.

NOTE: Overparameterization becomes a larger issue when fitting in double mode as opposed to single mode due to the increased number of free-floating parameter values. Broad, structureless emission spectra pose the largest issue to the fitting algorithms and may result in cross-correlation between FCLSA parameters, particularly between $\Delta\nu_{1/2}$ and S . When fitting spectra, it is imperative that the obtained FCLSA parameters are verified to be physically realistic using literature precedence as a guide.

4. Optimization

4.1. After satisfactory initial parameters are found, further optimization can be performed. To do this, press the blue **Optimize Fit** button. Optimization will run and replot the fit function with newly optimized parameter values.

4.2. Two optimization options are provided: the least-squares and simplex methods. To switch between these two methods, toggle to the desired method in **Settings | Optimization**.

4.3. If desired, customize the optimization method using the settings found under **Setting | Optimization**.

NOTE: In order to provide the user with control over the optimization routines, the following customization options are possible for both optimization methods:

4.3.1. Fix a parameter's values: To fix a parameter's value during optimization, press the check box in the edit field corresponding to the desired parameter.

4.3.2. Custom bounding of a parameter during optimization: To reveal the custom bounding options, activate the **Allow custom parameter bounding during optimization** check box in **Settings | Optimization**. To specify custom bounds for a parameter's value during optimization, use the controls revealed by pressing the **Custom bounds** button under the edit field corresponding to the desired parameter.

4.3.3. Custom end triggers for optimization: To adjust the maximum value of iterations,

termination tolerance on model value, or termination tolerance on coefficient values, activate the corresponding check box in **Settings | Optimization** and enter the desired value into the corresponding edit field.

NOTE: The following customizations are available only for the **Least-Squares** optimization:

4.3.4. Goodness-of-fit statistics: To display goodness-of-fit statistics (degree-of-freedom adjusted coefficient of determination, sum of squares due to error, degrees of freedom in the error, and root mean square error) after optimization is complete, activate the check box found under **Settings | Optimization**.

4.3.5. Robust fit options: To activate robust fit options, select the desired menu from the dropdown list under **Settings | Optimization**. By default, this option is off. If desired, activate Least Absolute Residual or Bisquare Weights fitting, which give lower weight to outlier data points.

4.3.6. Threshold data weighting: To preferentially weigh data points above a threshold intensity, choose an intensity to act as a threshold and a weight multiplier to apply to all points above that threshold. By default, this option is set to 'on', and threshold and weight multiplier are set to 0.1 and 1.2, respectively. These options are available in **Settings | Data Weighting**.

4.3.7. Extrema data weighting: To preferentially weight data points surrounding local extrema (peaks and valleys), select the number of data points surrounding each extremum to apply the preferential weights to, as well as what the weight multiplier should be for those points. These points are more feature rich as they are directly associated with the fitted parameters. Look for extrema data weighting settings under **Settings | Data Weighting** which is set to **on** as a default setting, and the number of points and weight multiplier are set to 5 and 5, respectively. To visually identify which data points are used in the preferential weighting, select **Fill salient data points**.

5. Chromaticity and free energy calculations

5.1. Ensure that the optimized fit of the data and the associated parameter values are satisfactory before proceeding to additional calculations. To perform these calculations, press the **Calculate** button, located at the bottom of the **Calculations** pane.

NOTE: The first value returned, labeled ΔG_{ES} (cm^{-1}), is the free energy of the excited state calculated using the equation shown below. The default unit for this value is inverse centimeters (cm^{-1}), but units of electronvolt (eV) and joule (J) are also available.

5.1.1. To change the unit, select the desired option from the dropdown list box found under **Settings | Calculations**.

NOTE: The calculation for free energy of the excited state, given by

$$\Delta G_{es} = E_0 + \frac{(\bar{v}_{1/2})^2}{16k_B T \ln 2}$$

is based on the energy quantity (E_0), full width at half-max ($\Delta\nu_{1/2}$), Boltzmann constant (k_B), and environmental temperature of the experiment (T). The value for experimental temperature is assumed to be 298K but can be specified as 77K or any other temperature²⁰.

5.1.1.1. To change the experimental temperature, choose the desired option under **Settings | Calculations | Experimental Temperature**.

NOTE: The second value returned is the CIE chromaticity coordinate, calculated from the currently selected spectrum. When plotted on a chromaticity diagram, this value shows the predicted color of the active data spectrum.

5.1.2. To display the chromaticity diagram with coordinate plotted, press the pop-out button (represented by a square with an arrow pointing towards the upper-right-hand corner) next to the **Chromaticity Coordinate** text box.

5.1.3. To check for the predicted color of the sample, use the third calculation, shown as a colored rectangle. This estimation is based off the same calculation that yielded the chromaticity coordinate. By default, use CIE Standard Illuminant D65 to make this prediction. To change the illuminant, select the desired option from the dropdown menu labeled **White Point** in **Settings | Calculations**.

5.2. To calculate CIE chromaticity coordinates and color values for multiple loaded spectra simultaneously, activate the corresponding checkbox found under **Settings | Calculations**.

NOTE: This setting is on by default. Once a second spectrum is plotted, the icon on the pop-out button next to the **Chromaticity Coordinate** label will change from the square with an arrow pointing to the upper-right-hand corner to three dots (•••).

5.2.1. Press the ••• button to reveal a panel labeled **Select Spectra**. Select the desired spectra from this panel and choose to **Export Values** as a table and/or **Display Diagram** to reveal the chromaticity diagram with all coordinates plotted and labeled.

6. Data export

6.1. Once again, ensure that the fit of loaded data is satisfactory, and that all desired calculations have been performed. To export both loaded and calculated data, press the **Export Data** button. There are six data export options: **Figure**, **Parameter Values**, **Spectrum Data Points**, **Fit Data Points**, **Color Values**, and **Chromaticity Diagram**.

6.1.1. To export the displayed plot as a figure pre-formatted for publication or presentation,

select Figure. This formatting can be disabled under **Settings | General | Figure Settings**. Supported file types include EPS (vector-graphics file), JPG, PNG, and PDF.

6.1.2. **To export all parameter values—with or without calculated values—as a table, select Parameter values.** Inclusion of calculated values is toggleable under **Settings | Calculations** and supported file types are .CSV, .TXT, .DAT, .XLS, and .XLSX.

6.1.3. **To export data of the currently selected spectrum as a series of x-y data points, select Spectrum data points.** x-values will use either wavenumber (cm^{-1}) or wavelength (nm) units, depending on how the graph is defined via the settings. Supported file types are the same as above in 6.1.2.

6.1.4. **To export the fit as a series of x-y data points, again dependent on the current mode of the axes, select Fit data points.** Supported file types are the same as above in 6.1.2.

6.1.5. **To export chromaticity and CIE coordinates as well as the predicted color as an RGB value, if enabled, select Color Values.** Supported file types are the same as above in 6.1.2.

6.1.6. **To export the chromaticity diagram with the chromaticity coordinates plotted on it associated with the loaded spectra, select Chromaticity Diagram.** Supported file types are .EPS, .JPG, .PNG, and .PDF.

REPRESENTATIVE RESULTS:

Using the fitting routine described above, Franck-Condon Lineshape Analysis was performed on two spectra that come pre-packaged with the application: the room temperature (292 K) and low temperature (77 K) emission spectra for 9,10-diphenylanthracene dissolved in toluene. Measurements were obtained using a spectrofluorometer with fluid solutions in 1 cm cuvettes and a standard cuvette holder for room temperature measurements. The low temperature measurements were obtained by immersing NMR tubes into liquid nitrogen in a dewar to generate frozen glass samples. All spectra were corrected for the detector response. A single mode fit was sufficient for the room temperature spectrum, while double mode was used to model the low temperature spectrum. Color analysis was performed on both spectra and found to yield similar estimates.

To fit the room temperature spectrum, by-hand adjustment was used after least-squares optimization with default customizations. The final parameter values obtained were as follows: $E_0 = 24380 \text{ cm}^{-1}$, $\Delta\nu_{1/2} = 1200 \text{ cm}^{-1}$, $S = 1.25$, $\hbar\omega = 1280 \text{ cm}^{-1}$. The resulting coefficient of determination calculated was 0.99947 as shown in **Figure 1**. Calculation of free energy of the excited state using these parameter values yielded a value of $25,000 \text{ cm}^{-1}$.

Simplex optimization was used to fit the low temperature spectrum. By-hand adjustment was not necessary after optimization. The final parameter values obtained were as follows: $E_0 = 24764 \text{ cm}^{-1}$, $\Delta\nu_{1/2} = 746 \text{ cm}^{-1}$, $S_1 = 1.13$, $\hbar\omega_1 = 1382 \text{ cm}^{-1}$, $S_2 = 0.31$, $\hbar\omega_2 = 651 \text{ cm}^{-1}$. The resulting coefficient of determination calculated was 0.9991 as shown in **Figure 2**. Calculation of free energy of the

excited state using these parameter values yielded a value of 25,700 cm^{-1} .

Color analysis of the low temperature spectrum yielded the following results: chromaticity coordinate = [0.15819, 0.03349], CIE coordinate = [0.19571, 0.041432, 1], and predicted RGB value = [67, 0, 233]. The values obtained for the room temperature spectrum were very similar to that of the low temperature spectrum with unperceivable color differences.

FIGURE AND TABLE LEGENDS:

Figure 1: Single mode fit of 9,10-diphenylanthracene (292 K): This figure shows the room temperature emission spectrum of 9,10-diphenylanthracene and its FCLSA fit function, achieved through least-squares optimization followed by by-hand adjustment of parameter values. This is an example of a loosely structured spectrum.

Figure 2: Double mode fit of 9,10-diphenylanthracene (77 K): This figure shows the low temperature emission spectrum of 9,10-diphenylanthracene and its FCLSA fit function, achieved through a simplex optimization. This is an example of a highly structured spectrum.

DISCUSSION:

This application provides an easy and rapid analysis of emission spectra through two main methods commonly used in the photophysical community. The first is Franck-Condon Lineshape Analysis (FCLSA), which gives insight into the energetics and vibronic coupling associated with decay of excited state molecules back to their ground states. This is achieved by optimizing parameter values to maximize the goodness of fit of a spectrum using one of two possible FCLSA modelling equations. The second method of analysis provides insight into the observed color of the light emitted from the molecule. By combining tristimulus color curves with provided intensity data, the CIE coordinate can be calculated. This determination allows for the highly accurate color prediction of both absorption and emission spectra.

Experimental photoluminescence spectra are commonly measured using a photomultiplier tube (PMT) or charge coupled device (CCD) as a detector and plotted as emission intensity versus wavelength (nm). Many photophysical characterizations, including FCLSA and calculation of the free energy of the excited state, are performed in wavenumber space, as demonstrated by the use of $\bar{\nu}$ (cm^{-1}) in the corresponding equations above. In addition to the x-axis conversion, the emission intensity as measured versus wavelength, denoted as $I(\lambda)$ must be converted to $I(\bar{\nu})$. This application automatically identifies the original x-axis units of imported spectral data as either wavelength (nm) or wavenumber (cm^{-1}). By default, the application then converts the spectral data, normalizes the spectrum to unity at the maximum intensity peak, and plots the spectrum as “Normalized $I(\bar{\nu})$ vs. wavenumber (cm^{-1})” to indicate that the correct intensity conversion was applied. Although it is recommended that all fitting be performed using wavenumber units, the application can also plot the spectrum as “Normalized $I(\lambda)$ vs. wavelength (nm)” by following the instructions in section 2 above.

There are two optimization algorithms available for use in the application. The default option is damped least-squares, which utilizes the Levenberg-Marquardt algorithm²¹. Combining a version

of gradient descent and the Gauss-Newton algorithm, this algorithm finds local, not necessarily global, minima. While this is a significant limitation, the algorithm offers advantages in its customizability—this method can take into account preferential weighting of data points, perform robust fitting, and display goodness-of-fit statistics²². The alternative method of optimization is derivative-free, powered by the Nelder-Mead simplex algorithm²³. This algorithm uses a heuristic method to return a global minimum of the given cost function (in this case, a sum of squared differences between predicted and observed intensities). The simplex method has been used for FCLSA before, though the code implementing it was never published²⁴.

Both the least-squares and simplex optimization methods work best for structured spectra that exhibit narrow, well defined, and symmetric peaks. As spectra become less structured, meaning that they lose symmetry and the peaks broaden, these methods lead to less robust fits where parameters can become highly correlated. Typically, spectra recorded at low temperatures or in rigid media are more structured compared to those obtained near room temperature or in fluid solution^{12,25,26}. The robust fit options included with the least-squares method can help to alleviate this issue. This problem can be significantly diminished if one or more of the parameters are fixed to a constant value during optimization. For instance, IR spectroscopy experiments can be used to determine relevant quantum spacing ($\hbar\omega$) values. Alternatively, relevant literature values can be used to set custom bounds for the parameters.

In some instances, the FLCSA fit, and parameters obtained from the optimization routines do not adequately represent the data even when robust fit options or fixed parameters are employed. This is a failure of the fitting algorithms and may be associated with the multiple FCLSA fitting parameters (potential overparameterization) or spectral shape of the data (featureless spectra). In these cases, further improvement of the fits may be obtained using a “by-hand fit” of the data with manipulation of the FCLSA parameters. The adequacy of such fits can be assessed visually and quantified by comparing goodness-of-fit statistics that are automatically included in the plot.

A general routine to follow for an accurate by-hand fit consists of the following five steps: First, determine an initial estimate for E_0 manually or automatically using one of the three methods provided. By default, the parameter’s value is assigned to the wavenumber associated with the highest intensity peak detected upon data import. Alternatively, the user can define E_0 as the wavenumber at which the emission spectrum intersects its corresponding excitation spectrum. The final method to determine E_0 uses the so-called X% Rule, where X = 1 or 10. In this method, E_0 is assigned to a wavenumber X% of the full width at half-maximum (FWHM) intensity of the most prominent data peak assuming a Gaussian band shape. The second step in the by-hand fitting protocol is to calculate $\hbar\omega$ based on quantum spacing observed in the structure of the emission spectrum. If possible, refer to the IR spectrum of the molecule and try to correlate the photoluminescence-based value to a strong band in the IR spectrum. Third, determine S based on the relative intensities of spectral peaks. Fourth, determine a rough $\Delta\nu_{1/2}$ based on bandwidth. Fifth, iteratively readjust S and $\Delta\nu_{1/2}$ as necessary.

The difficulty with performing FCLSA using broad, relatively featureless spectra was demonstrated through the fitting procedure for 9,10-diphenylanthracene in fluid solution at 292

K compared to that performed for the more structured spectrum obtained in frozen glass at 77 K. When fitting the room temperature spectrum, optimization returned an initial coefficient of determination of 0.9971 that was improved to 0.9994 through by-hand tuning of the parameters and visual inspection of the results. In contrast, by-hand fitting of the low temperature variant was unnecessary due to the fine structure of the spectrum that resulted in a coefficient of determination equal to 0.9991 after simplex optimization.

In many instances, both optimization routines (least-squares and simplex) return very similar results. This is indicative of them finding a global minimum for the FCLSA parameters. In general, the least-squares method tends to be better suited to data that is noisy, is not well structured, or contains many near-zero data points at the spectrum's tails. Conversely, the simplex method tends to return better fits than the least-squares method for data that is well structured and possesses few outlier points. In these cases, the simplex method typically requires little by-hand pre-optimization of parameter values and no adjustment after optimization. For those cases in which the data's noise or overall lack of structure prevents a high-quality fit using either of the provided optimization methods, it is recommended that the by-hand fitting method (see above) be employed with no subsequent optimization.

This application offers several advantages over previous implementations of Franck-Condon Lineshape Analysis. The first and most important advantage is that it is free, publicly accessible, and fully transparent. This is accomplished by posting the code to GitHub, providing access to anyone with a computer and internet connection (https://github.com/USArmyResearchLab/ARL_Spectral_Fitting). Not only can anyone access this application, but they can also view the underlying code. This provides an opportunity for community-sourced feedback and development. An additional advantage lies in the ease-of-use of this application. No background knowledge of computer science or command line interaction is required. Rather, this software employs a simple graphical user interface (GUI) that enables researchers of all backgrounds to perform the spectral analyses described above. Furthermore, this application provides the user multiple options for control over the optimization methods and can be used to determine the free energy of the excited state. Finally, the software calculates and reports several useful color values including chromaticity coordinates, CIE coordinates, RGB, and hexadecimal color codes. All of these analyses can be accomplished in seconds, requiring only that the user press a button.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure 1: Single Mode Fit of 9,10-diphenylanthracene (292 K)

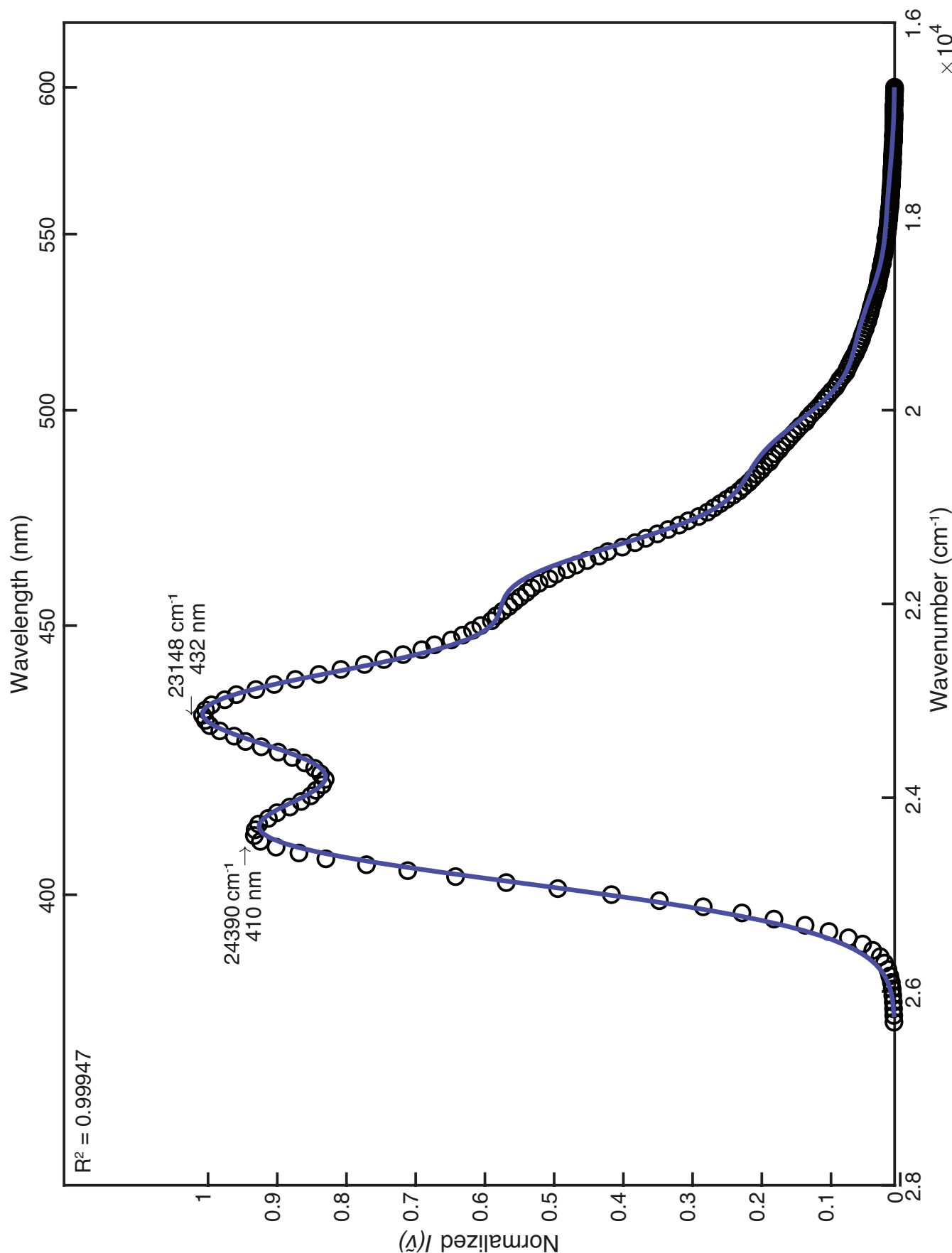
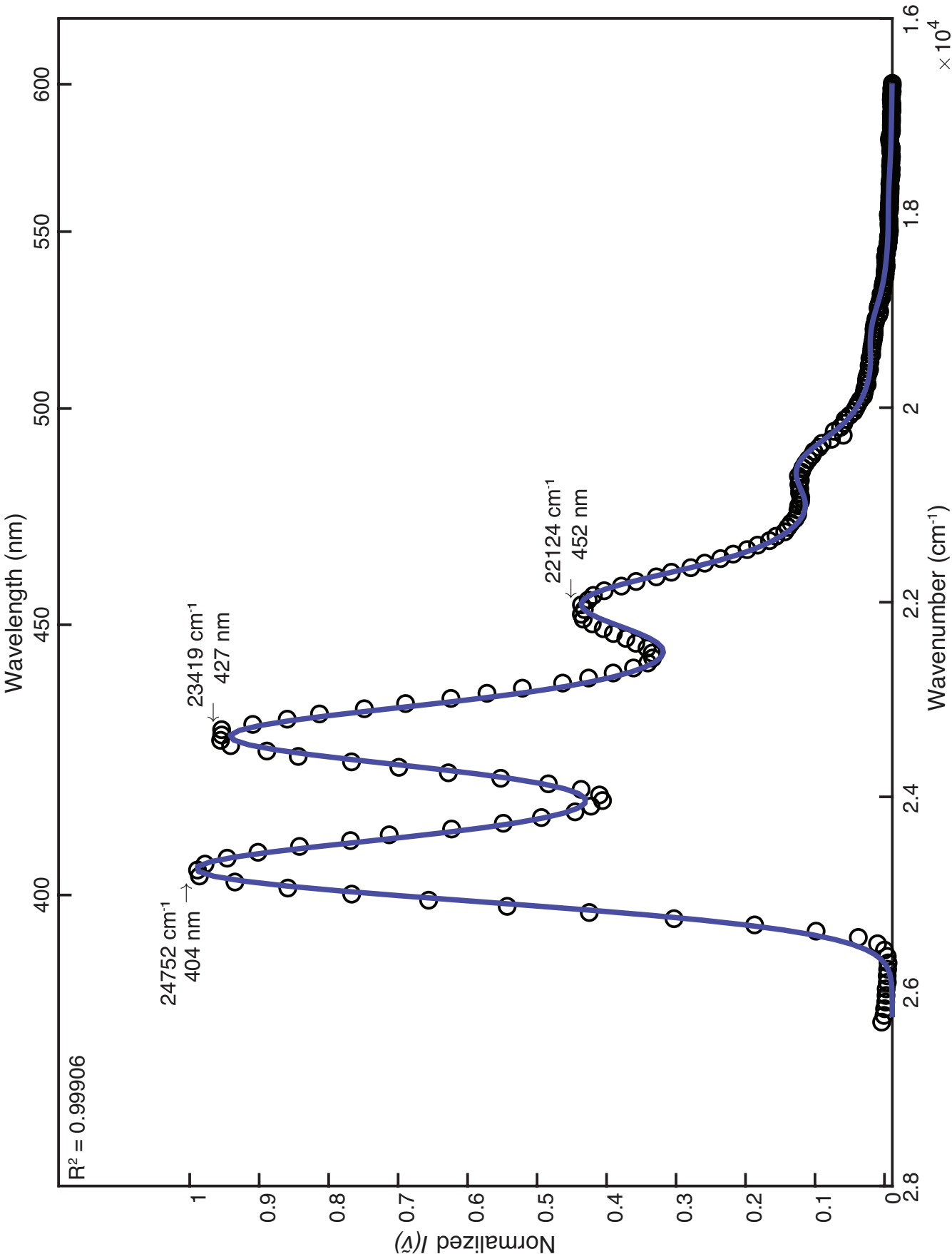


Figure 2: Double Mode Fit of 9,10-diphenylanthracene (77 K)



Name of Material/Equipment	Company	Catalog Number	Comments/Description
ARL Spectral Fitting	Army Research Laboratory	v1.0	https://github.com/USArmyResearchLab/ARL_Spectral_Fitting
MATLAB	MathWorks	R2020b	https://www.mathworks.com/products/matlab

spectral_Fitting/releases/tag/v1.0
html



DEPARTMENT OF THE ARMY
U.S. ARMY COMBAT CAPABILITIES DEVELOPMENT COMMAND
ARMY RESEARCH LABORATORY
SENSORS AND ELECTRON DEVICES DIRECTORATE
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ADELPHI, MARYLAND 20783-1138

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,

A handwritten signature in black ink that reads "Ryan M. O'Donnell".

Ryan M. O'Donnell, Ph.D.
Chemist
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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 $E = h\nu$ 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 ($\bar{\nu}$, 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:

$$I(\bar{\nu}) = \lambda^2 I(\lambda)$$

Therefore, we updated the software and manuscript figures to label the y-axis as either "Normalized $I(\bar{\nu})$ " 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 $\bar{\nu}$;

[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 $[\text{Ru}(\text{bpy})_3]^{2+}$ (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.

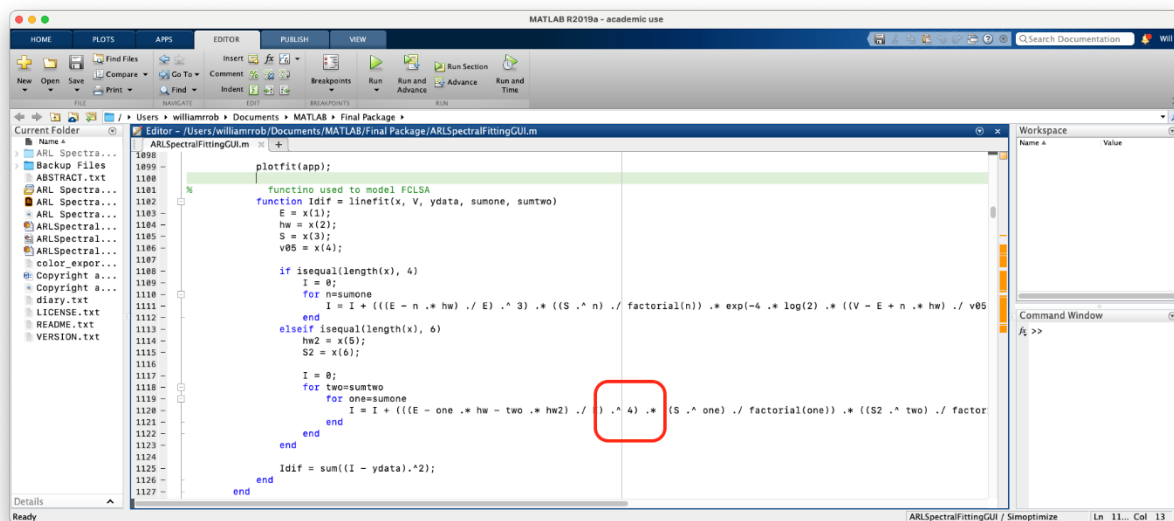
[RMO] These are indeed major concerns and are addressed at the end of this document to provide ample space for our response.

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.

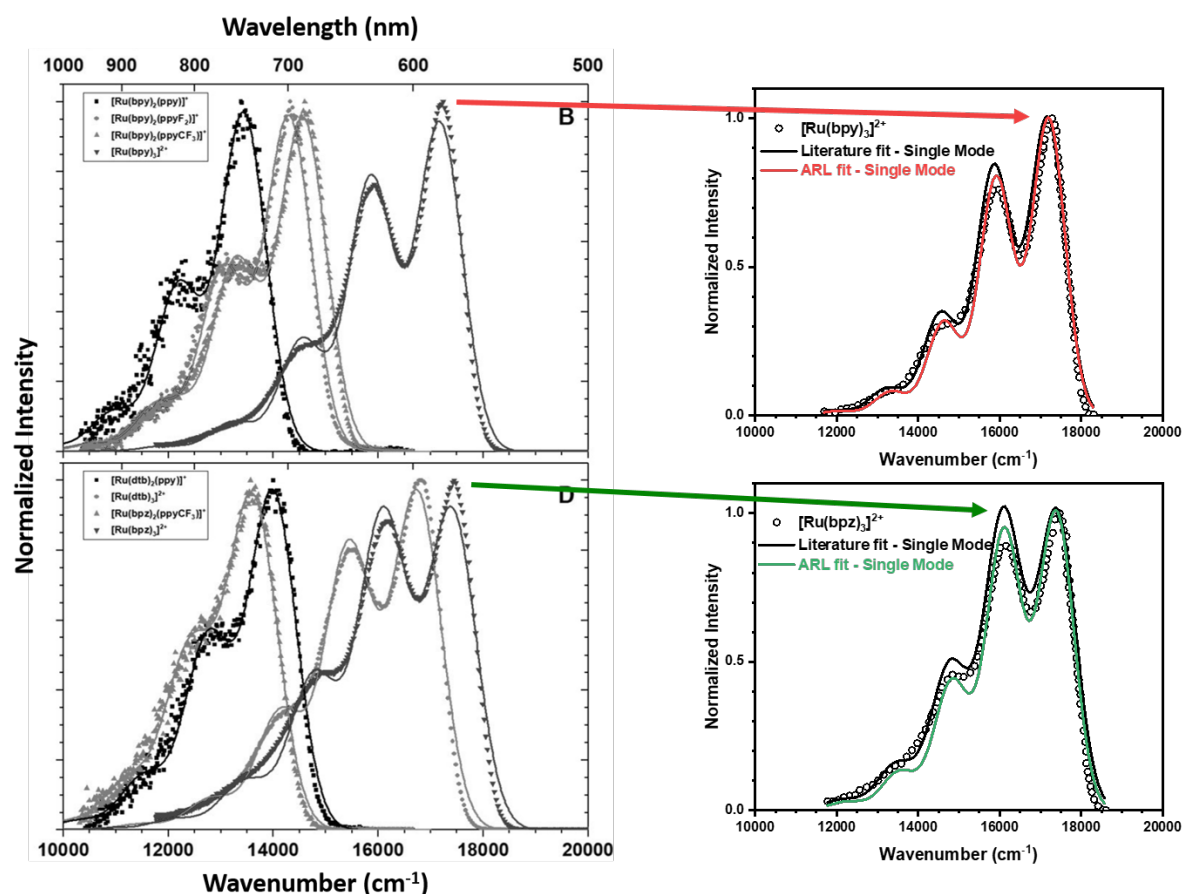
Major Concerns:

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.

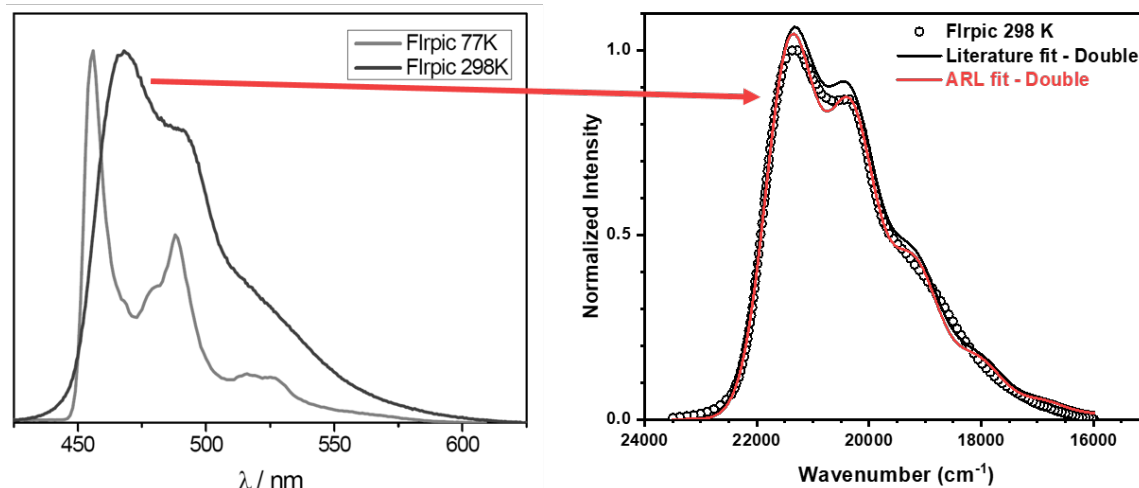
[RMO] This is disappointing to hear and we are surprised by this comment. Without knowledge as to what spectra the reviewer used for fitting, we can only speculate as to the sources of error. We believe that the fitting algorithms implemented in the software (both Least-Squares and Simplex routines) 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 $[\text{Ru}(\text{bpy})_3]^{2+}$ and $[\text{Ru}(\text{bpz})_3]^{2+}$ 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.

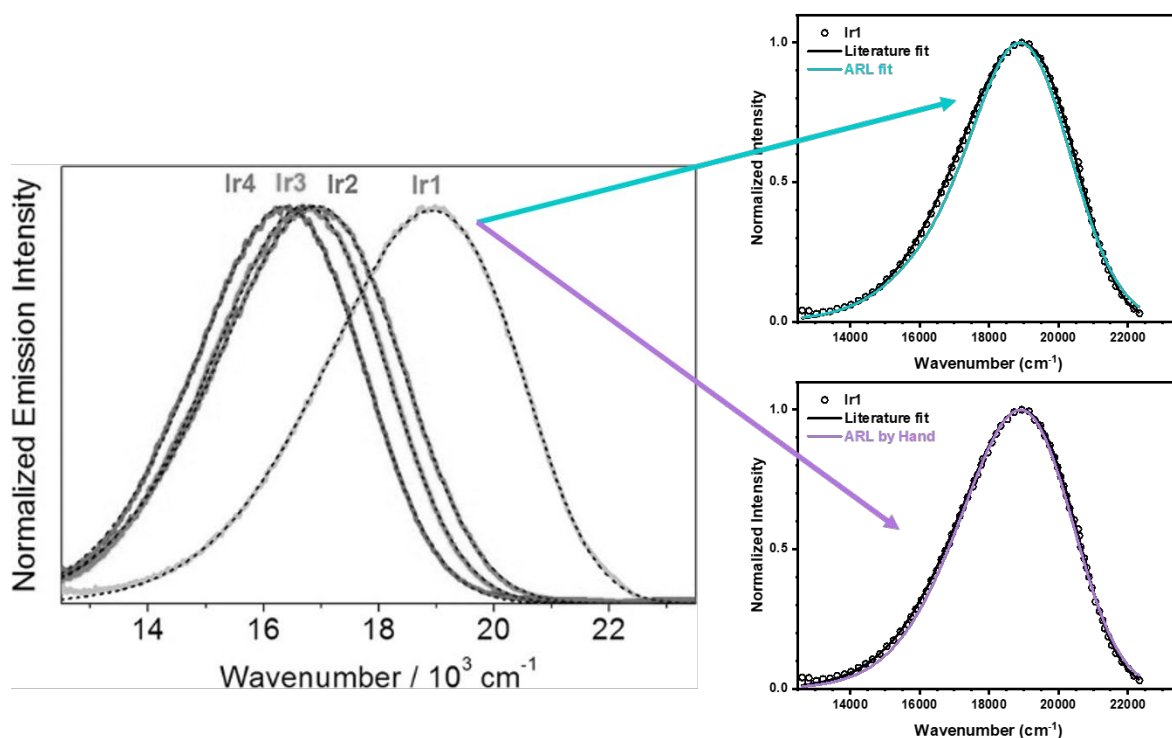


Next we reproduced the literature double mode fit for $[\text{Ir}(\text{F}_2\text{ppy})_2(\text{pic})]$ reported by Zanoni and co-workers (DOI: 10.1039/c5nj01352f). The Supplemental Figure S8 is copied at left and shows the 298K spectral data that was digitized on the right (open circles) along with 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