General Overview

The program phasor_calculations.m is a supplement to the publication "Chemical complexity of the retina addressed by novel phasor analysis of unstained multimodal microscopy." [CHEMPH_111091] In that publication, we introduced the super-phasor unmixing (SPU) method to unmix seven spectroscopic signals in the spectral range 300-690 nm. The performance of SPU was found to be significantly superior to fully constrained linear spectral unmixing especially in regions of high spectral overlap. This program can serve as a tool for implementing the super-phasor analysis. The reading of [CHEMPH_111091] is highly encouraged before implementing phasor_calculations.m as that publication provides much greater detail and context than these instructions. All equation numbers in these instructions reference [CHEMPH_111091].

phasor_calculations.m has the ability to do two things. The first is to convert spectral coordinates into phasor coordinates (eqs. 1 and 2) and perform a simple phasor analysis to find percent compositions (eq. 3). This function works with two and three pure components. The second function of phasor_calculations.m is to perform super phasor analysis (eq 4). This function works with two and three super-phasors. Further details on the program's variables are addressed in Variable Specifications. A pseudo-run of the program for a seven component analysis is provided in Implementation.

Variable Specifications

[phasor_coords, percentages] = phasor_calculations(pure_spectra, exp_spectrum, lambda_min, lambda_max)

Input Variables

pure_spectra: An n x 4, n x 6, 2 x 2 or 2 x 3 matrix used to hold the flourescence spectra expected to combine to form the mixed experimental spectrum.

An n x 4 matrix should be used when two pure spectra are involved and an n x 6 matrix should be used for three pure spectra. These matrices should take the form of [λ , I , λ , I , λ , I] across the 4-6 columns with n rows.

The 2 x 2 and 3 x 2 matrices are used for super phasor analysis where each index represents super-phasor G and S coordinates. These matrices should be of the form [G, S] and have two columns with each row containg a set of super-phasor coordinates.

exp_spectrum: A m x 2 matrix used to hold the measured mixed flourescence spectrum. This matrix should take the form [λ , I] with m rows.

lambda_min: the minimum wavelength for calculations

lambda_max: the maximum wavelength for calculations

Output Variables

phasor_coords: a 4 x 2 or 3 x 2 matrix that returns the various calculated phasor coordinates. The columns in this matrix are of the format [G, S]. The first 2-3 rows are the phasor coordinates for the pure spectra and are returned according to their input order in the variable pure_spectra. The last row is always reserved for the set of phasor coordinates belonging to exp_spectrum.

percentages: a 3 x 1 or 2 x 1 matrix that returns the percent composition associated with each pure component. The order is once again determined by the order in pure_spectra.

Implementation

$spec1 = [\lambda_a \quad I_a \quad \lambda_b \quad I_b \quad \lambda_c \quad I_c]$ (nx6)

$spec2 = [\lambda_d \quad I_d \quad \lambda_e \quad I_e]$ (nx4)

$spec3 = [\lambda_f \quad I_f \quad \lambda_h \quad I_h]$ (nx4)

$expSpec = [\lambda_z \quad I_z]$ (nx2)

$(coords1, percent1) = phasorcalculations(spec1, expSpec, 300, 500)$

$$coords1 = \begin{bmatrix} G_a & S_a \\ G_b & S_b \\ G_c & S_c \\ G_{z1} & S_{z1} \end{bmatrix}$$

$$percent1 = \begin{bmatrix} a \\ b \\ c \end{bmatrix}$$

$(coords2, percent2) = phasorcalculations(spec2, expSpec, 500, 650)$

$$coords2 = \begin{bmatrix} G_d & S_d \\ G_e & S_e \\ G_{z2} & S_{z2} \end{bmatrix}$$

$$percent2 = \begin{bmatrix} d \\ e \end{bmatrix}$$

$(coords3, percent3) = phasorcalculations(spec3, expSpec, 650, 800)$

$$coords3 = \begin{bmatrix} G_f & S_f \\ G_h & S_h \\ G_{z3} & S_{z3} \end{bmatrix}$$

$$percent3 = \begin{bmatrix} f \\ h \end{bmatrix}$$

$$super = \begin{bmatrix} G_{abc} & S_{abc} \\ G_{de} & S_{de} \\ G_{fh} & S_{fh} \end{bmatrix}$$

$G_{abc} = a \cdot G_a + b \cdot G_b + c \cdot G_c$ *the other G and S values follow the same pattern

*the formation of *super* is not included in the program and must be done by the user

$(coords4, percent4) = phasorcalculations(super, expSpec, 300, 800)$

$$coords4 = \begin{bmatrix} G_{abc} & S_{abc} \\ G_{de} & S_{de} \\ G_{fh} & S_{fh} \\ G_{z4} & S_{z4} \end{bmatrix}$$

$$percent4 = \begin{bmatrix} abc \\ de \\ fh \end{bmatrix}$$

$totalcontributions_a = abc \cdot a$ * the other total contribtion values follow the same pattern.