Kramer spec - determination of deconvolution formula

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Determination of deconvolution formula

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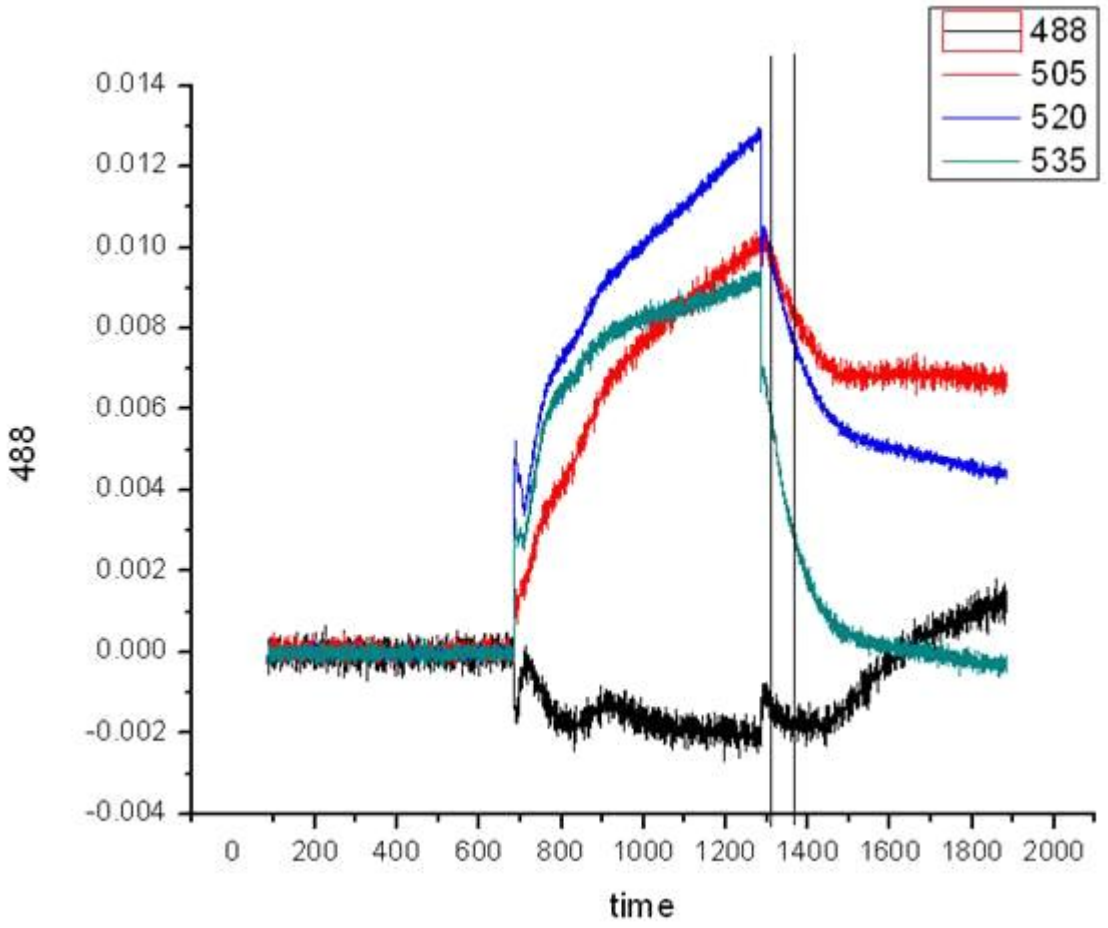
To obtain a deconvolution formula with 4 components (488, 505, 520, 535)

1. Dark adapt a leaf for >3 hours to deplete all the zeaxanthin in the leaves.
2. Run a script measuring A_{488}, A_{505}, A_{520} and A_{535} over a 30min-long LIRK.
3. Light adapt a leaf, then run a script measuring A_{488}, A_{505}, A_{520} and A_{535} over a short period time (e.g. 20 seconds) just to get a baseline (#1). Then take out the leaf from leaf chamber, (optional: put in a piece of black cloth), and run the same script again (#2).
4. LIRK data analysis:

Example graph



First normalize baseline using the first 10 minutes of darkness.

Zeaxanthin: Take down the value (absolute Y value) of each trace at the end of 2nd dark period (i.e. the end of the experiment), and put it down in the second column of the following matrix. We think this is mostly zeaxanthin.

ECS: Measure that abrupt increase in absorbances at the very beginning of the 10-min light period, and put it down in the third column of matrix. When baseline is normalized such that the end of the 1st 10-min dark period is set to 0, simply take down the absolute values of the increase in absorbance. The change in absorbance in this short time period is only attributable to ECS changes.

qE: Measure changes in aborbance of the four traces in the straight portion of the absorbance decay in the 2nd 10-min darkness (between the two black lines in the above graph), put down on the fourth column of the matrix. We think this change is caused by changes in qE, this is highly debatable because zeaxanthin also drops in darkness, and the study that showed relationship between qE and A535 did not carry out experiment under physiological conditions (Horton paper).

5. 488nm data analysis: substract the A_{488}, A_{505}, A_{520} and A_{535} of #2 from those of #1 ("#1 - #2") and then put down the four values in the first column of the matrix.
   Matrix

Ext'n Coeff.s	X	Z	E	Q
A488				
A505				
A520				
A535				

   The values in the matrix can be negative.

6. Normalize the values: normalize the first column holding A488 to 1; normalize the 2nd column holding A505 to 1; normalize the 3rd column holding A520 to 1; normalize the 4th column holding A535 to 1, these are our true "extinction coefficients" (they are not actual extinction coefficients, instead they are coefficients that describes the ratio of a species' absorbance at a certain wavelength to the species' peak absorbance. For example for zeaxanthin the coefficient at 520nm is $A_{520, zea}$ divided by $A_{505, zea}$ since peak absorbance of zeaxanthin is at 505nm).

Ext'n Coeff.s	X	Z	E	Q
A488	1			
A505		1		
A520			1	
A535				1

7. Plug the coefficients from the matrix into the following equations (i.e. "ε"s)

$$A488 = \varepsilon_{488, scatter} \cdot X + \varepsilon_{505, scatter} \cdot Z + \varepsilon_{520, scatter} \cdot E + \varepsilon_{535, scatter} \cdot Q$$
$$A505 = \varepsilon_{488, zea} \cdot X + \varepsilon_{505, zea} \cdot Z + \varepsilon_{520, zea} \cdot E + \varepsilon_{535, zea} \cdot Q$$
$$A520 = \varepsilon_{488, ecs} \cdot X + \varepsilon_{505, ecs} \cdot Z + \varepsilon_{520, ecs} \cdot E + \varepsilon_{535, ecs} \cdot Q$$
$$A535 = \varepsilon_{488, qE} \cdot X + \varepsilon_{505, qE} \cdot Z + \varepsilon_{520, qE} \cdot E + \varepsilon_{535, qE} \cdot Q$$

   Solve the simultaneous equation with four unknowns (i.e. XZEQ), treating A488, A505, A202 and A535 as known constants.

To obtain a deconvolution formula using only 3 components (505, 520, 535)

Follow the previous protocol, skip step 3 and 4, build a 3-way matrix, and solve it.

Notes:

1. We are really not sure what 488nm absorption means – it is proposed that A488 reflects leaf scattering – so using a 3-component formula is likely as good as a 4-component formula.