Mathematical correction methods of inner filter effects affecting 3D fluorescence spectra

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Abstract

- **Fluorescent Excitation Emission Matrix (FEEM)** gathers successive measurements of the fluorescence intensity emitted by a solution. Each entry corresponds to a distinct couple of excitation and emission wavelength (i, j).
- **Our contributions:** We propose two linearization methods of FEEM affected by Inner Filter Effects (IFE): The Controlled Dilution Approach (CDA) introduced in [1] and a new Mirrored Cell approached (MCA). We then present some experimental MCA results obtained from laboratory mixtures of three fluorophores.
- **Key points of the proposed approaches:**
  - **Measure a second FEEM** from the same sample under different experimental conditions.
  - Does not require absorbance measurement, only fluorescence.

Fluorescence Spectroscopy

- **FEEM analysis** A set of FEEM $\mathbf{F}^{(1)} \cdots \mathbf{F}^{(K)}$ from K mixtures of N fluorescent components (fluorophores) is traditionally [1-2-3] modelized as:

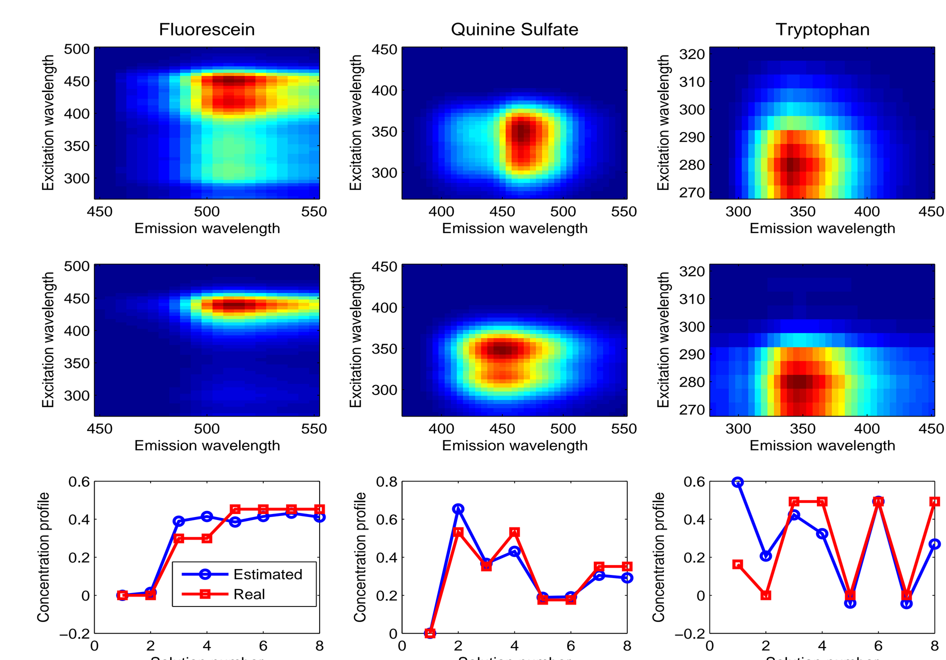
$$F_{i,j}^{(k)} = H_{i,j}^{(k)} \underbrace{\sum_{n=1}^{N} c_{k,n} \varepsilon_{i,n} \gamma_{j,n}}_{L_{i,j}^{(k)}} \qquad (1)$$

- $c_{k,n}$: contribution of fluorophore n in solution k.
- $\varepsilon_{.,n}$: excitation spectrum of fluorophore n
- $\gamma_{.,n}$: emission spectrum of fluorophore n

- **Trilinear model.** Providing that concentrations are small enough one can assume $\forall i, j, k \ H_{i,j}^{(k)} = 1$. Then (1) defines a Cannonical Poliadic (CP or PARAFAC) decomposition [4] and allows to easily estimate $c_{.,n}$, $\varepsilon_{.,n}$ and $\gamma_{.,n}$ for each fluorophore [5].

Inner Filter Effects (IFE)

- **In practice,** Inner Filter Effect (IFE) due to light absorption into the sample cell cannot be neglected [6]: $H_{i,j}^{(k)} \neq 1$ and CP decomposition becomes unappropriated:



CP decompositon of uncorrected FEEM measured from 8 mixtures of three fluorophores: estimated components (top), real components (middle) and concentration profiles (bottom).

Thereby FEEM have to be linearized first. In other words: estimate $\mathbf{L}$ from $\mathbf{F}$ and a model of IFE.

Controlled Dilution Approach [1]

Classical IFE linearization methods imply strong dilution series [7] and/or absorbance measurements [8]. We want to avoid this.

- **Outline:** the second FEEM, $\mathbf{F}_d$, is obtained from the controlled dilution of the considered sample. The dilution factor p can be chosen arbitrarily small(usually we take $p = 2$). IFE model yields:

$$\begin{cases} F_{i,j} = L_{i,j} e^{-\sum_{n=1}^{N} (\varepsilon_{i,n} + \varepsilon_{j,n}) c_n} \\ (F_d)_{i,j} = \frac{1}{p} L_{i,j} e^{-\sum_{n=1}^{N} (\varepsilon_{i,n} + \varepsilon_{j,n}) \frac{c_n}{p}} \end{cases}$$

- **CDA estimate of L**

$$\hat{L}_{i,j}^{CDA} = \left(\frac{(p(F_d)_{i,j})^p}{F_{i,j}} \right)^{\frac{1}{p-1}}$$

- **Main features**
  - Require only fluorescence measurement
  - Very simple numerical correction

Mirrored Cell Approach 1/2

- **Outline:** the second FEEM, $\mathbf{F}_m$, is obtained from the same sample but put into a mirrored cell. We suppose that i and j span the same wavelength domain of size I. R_m is the reflection coefficient of the mirrored facets. IFE model [9] yields:

$$\begin{cases} F_{i,j} = g_i g_j L_{i,j} & \text{with} \quad g_i = e^{-\sum_{n=1}^{N} \varepsilon_{i,n} c_n} \\ (F_m)_{i,j} = h_i g_i h_j g_j L_{i,j} & \text{with} \quad h_i = \left(1 + R_m g_i^2\right). \end{cases}$$

Now defining $Y_{i,j} = \log \frac{(F_m)_{i,j}}{F_{i,j}}$ and $x_i = \log h_i$ we obtain: $Y_{i,j} = x_i + x_j$.

In a matrix form we have $\mathbf{y} = \mathbf{S}\mathbf{x}$ with

$$\mathbf{y} = \begin{bmatrix} (Y_{1,1}) \\ (Y_{1,2}) \\ (Y_{1,2}) \\ \vdots \\ (Y_{1,I}) \\ (Y_{2,2}) \\ (Y_{2,3}) \\ (Y_{2,4}) \\ \vdots \\ (Y_{I,I}) \end{bmatrix}; \quad \mathbf{S} = \begin{bmatrix} 2 & 0 & \cdots & \cdots & \cdots & \cdots & 0 \\ 1 & 1 & 0 & \cdots & \cdots & \cdots & 0 \\ 1 & 0 & 1 & 0 & \cdots & \cdots & 0 \\ & & & \vdots & & & \\ 1 & 0 & \cdots & \cdots & \cdots & 0 & 1 \\ 0 & 2 & 0 & \cdots & \cdots & \cdots & 0 \\ 0 & 1 & 1 & 0 & \cdots & \cdots & 0 \\ 0 & 1 & 0 & 1 & 0 & \cdots & 0 \\ & & & \vdots & & & \\ 0 & \cdots & \cdots & \cdots & \cdots & 0 & 2 \end{bmatrix}; \quad \mathbf{x} = \begin{bmatrix} (x_1) \\ (x_2) \\ \vdots \\ \vdots \\ \vdots \\ (x_I) \end{bmatrix}$$

Mirrored Cell Approach 2/2

A least squared estimator of vector $\mathbf{x}$ is then given by

$$\hat{\mathbf{x}} = (\mathbf{S}^\intercal \mathbf{W} \mathbf{S})^{-1} \mathbf{S}^\intercal \mathbf{W} \mathbf{y},$$

where $\mathbf{W}$ is a suitable weighting matrix and we deduce $\hat{\mathbf{h}} = e^{\hat{\mathbf{x}}}$. Value of R_m is estimated by optimization of a suitable criterion. Its wavelength dependence is neglected.

- **MCA estimate of L**

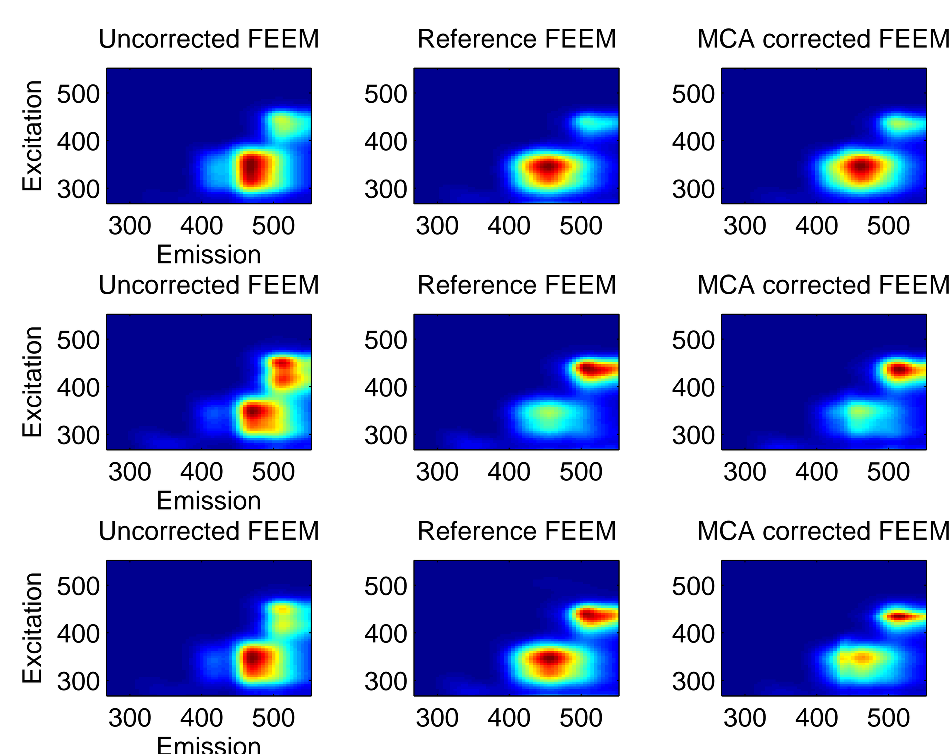
$$\hat{g}_i = \sqrt{\frac{\hat{h}_i - 1}{R_m}};$$

$$\hat{L}_{i,j}^{MCA} = \frac{F_{i,j}}{\hat{g}_i \hat{g}_j} = \frac{R_m F_{i,j}}{\sqrt{(h_i - 1)(h_j - 1)}}.$$

- **Main features**
  - Require only fluorescence measurement
  - Does not require any sample manipulation

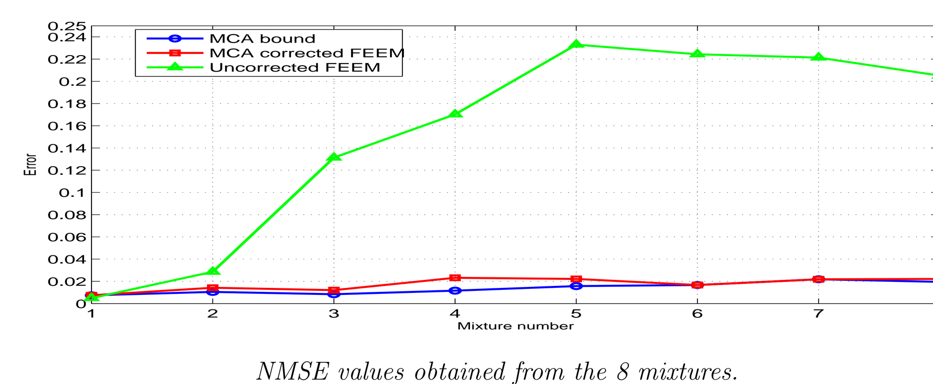
Examples of MCA results

- MCA was applied to 8 FEEM measured from concentrated mixtures of fluorescein, quinine sulfate and tryptophan. 3 examples are given below.
- The Normalized Mean Squared Error (NMSE) were computed between MCA linearized FEEM and reference FEEM (obtained from strong dilution) for each mixture. NMSE values are plotted along with those obtained from the uncorrected FEEM (first figure on the next box).
- Eventually the CP decomposition is applied to the MCA linearized FEEM (second figure on the next box).

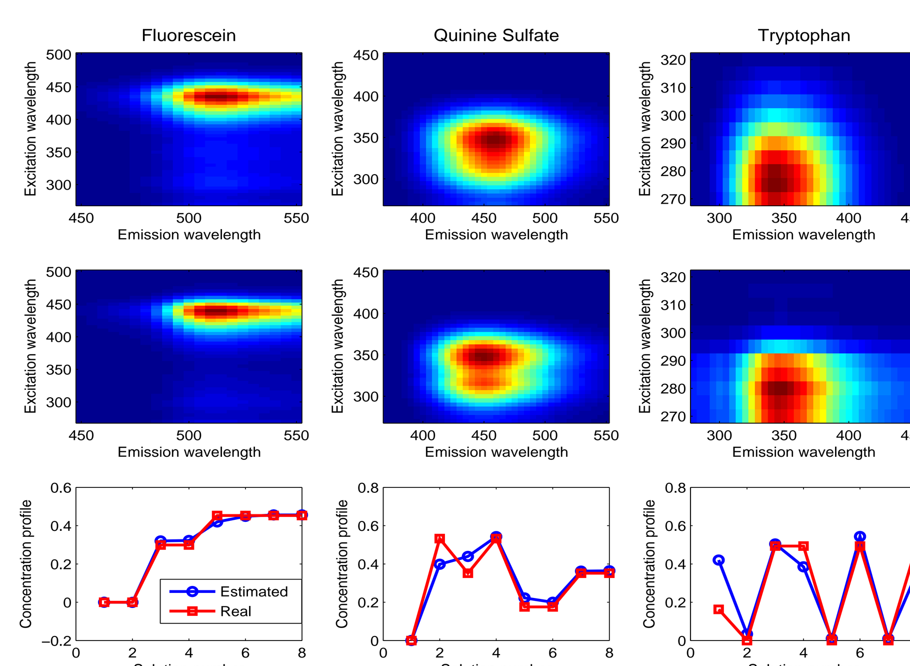


Comparison between uncorrected, reference and MCA linearized FEEM.

Examples of MCA results



NMSE values obtained from the 8 mixtures.



CP decompositon of MCA corrected FEEM from 8 mixtures of three fluorophores: estimated components (top), real components (middle) and concentration profiles (bottom).

Conclusion

Two simple IFE correction methods which only require an fluorescence measurements have been described, including a new approach using a mirrored cell. MCA is completely original and has been validated on known mixtures of three fluorophores. Both allow to linearize the measured FEEM even in the case of strong IFE and appear as a suitable pretreament before advanced FEEM analysis.

References

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