

Interactive comment on “Technical Note: Dissolved organic matter fluorescence – a finite mixture approach to deconvolve excitation-emission matrices” by A. Butturini and E. Ejarque

Anonymous Referee #2

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The fluorescence properties of dissolved organic matter (DOM) are increasingly being used to provide additional qualitative information on the dynamics of DOM in aquatic environments. These UV-Visible fluorescence properties can be mapped by scanning across a range of excitation and emission wavelengths to generate an excitation-emission matrix (EEM), which represents the combined fluorescence signal from DOM. Traditionally these fluorescence “landscapes” have been characterised by visual inspection, so called “pick picking” (reviewed in Coble 2007), identifying clear shoulders and peaks which are common across a range of environments. More recently a range

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of alternative methods with different qualitative and quantitative approaches have been applied (e.g. multivariate and multi-way analysis and neural network techniques). This contribution puts forward an alternative approach, which is essentially a 2D Gaussian curve-fitting exercise (a skewed Gaussian is applied). As far as I can understand this represents a computational-parallel to manual peak picking. The location of and number of peaks are determined by derivative analysis of the fluorescence landscape of a sample, rather than by eye as in the traditional approach.

The major concerns I have with the submission are: 1. The major constraint with the approach is that it assumes that the asymmetric Gaussian equation is appropriate to characterise excitation and emission properties of organic fluorophores. Has this been tested? Can you provide a simple example of how the approach fits to a single fluorophore, for example quinine sulphate? QS has two major excitation maxima and a shoulder, and a single emission peak. Would this be modelled as three peaks? 2. It would be more convincing to see this approach demonstrated on a simple mixture of fluorophores rather than the natural DOM samples only. I recommend that this is included. It would make it easier to follow how one determines the number of peaks necessary. 3. What does the output tell us? The primary goal of applying such an approach is data reduction. To separate the valuable trends in the data from the noise. The output appears to be still quite complex. Many of the peaks identified are inter-correlated. This can be due to the fact they co-vary in the dataset or due to the fact that they represent different maxima of the same fluorophore. An extension of this point is it is unclear to me how the Voroni diagram approach works? How sure are you of the assignments it has made? What determines whether a peak from a sample belongs to one or the other groups? 4. How does the approach guard itself against redundant results? What do the residuals of the fit look like? I do not see the r^2 values as being the most appropriate or only way to assess fit. 5. Is the sudden shift downstream from peak 4 to peak 5 in figure 5 also apparent from plots of the EEMs or emission spectra (from ex 240 nm). Likewise for the sudden appearance of peak 7. Some comparison between the measured and modelled data would be useful. 6. Finally, the discussion

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of the results is very superficial and similar to what can be gleaned from traditional peak picking. Do the authors really consider that the peaks are shifting and do they have any explanations for why the fluorescence peaks would shift? Are there changes in pH or metals that are causing this? Or is it the loss of one type of material and the gain of another downstream?

Although the approach is intriguing, I find that the presentation, explanation and validation is too limited to recommend this for publication. I encourage the authors to further develop the presentation addressing how robust the peak fit results (numbers and positions) and classifications (grouping) are. This could be done by including fits on simple mixtures of known fluorophores and addressing the variability in the fits on

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