Comments from reviewers and answers

Modifications that were applied to the manuscript:

As suggested by all reviewers:
- We agree with the reviewers that the use of signal deconvolution techniques have already proven their efficiency to “separate” overlapping signals, which is the case in this study. However, the fluorescent species that are present in plant cell cultures are still much higher than the ones that have been purified and identified. It becomes then highly tedious to model a noise level that showed to be highly dynamic and that could reach higher fluorescence intensities than that of the known species. A lot of work is thus still required in the identification of the molecules playing a major role in the noise. Intensive calibration studies have to be performed preparing different mixtures of these compounds once purified and identified and then using a PLS multivariate calibration method as proposed by del Olmo et al. (1996) and Capitan-Valley et al. (2000). This work is thus presented as the first step in the development of an on-line monitoring tool using 2-D fluorescence spectra. The scope of this work was clearly stated in the Introduction and the Conclusion.
- New figures were added showing 2-D spectra obtained.
- A table was added grouping the specific rates of increase for cell growth and for the fluorophores monitored by the probe. This was a very useful exercise and this table has been used to clearly show the probe potential to monitored on-line cell growth and other culture parameters.
- Text and references have been added on the importance of using mathematical tools to improve the probe capacity to estimate plant cell culture parameters.

Reviewer A:

- Fig 1 and fig 2: done
- 2D excitation spectra
- Fig 3 and 4: shake flasks added. Culture conditions are fully exposed in Material and Methods section.
- Fig 5-8: bioreactor added. Culture conditions are fully exposed in Material and Methods section.
- Was there any signal quenching observed? No quenching from medium components were observed as shown by the linear correlation between fluorescence intensity and alkaloid concentration (Fig 2) and p 14. There could have been some quenching during the culture and it has been mentioned in the text.
- Conclusion: see p 21.

Comment:
A mathematical treatment of the signal…. Such a treatment, as already observed in the literature, will benefit to this study only since the sensor would be calibrated with all
fluorescent compounds. This is not yet possible, as molecules have to be separated, identified with chromatographic methods before determining of their 2D fluorescence spectrum. Explanations have been added in the text and particularly in the conclusion section.

Reviewer B:

PLS methods are discussed in the conclusion section.
- "superflous details on culture manipulation": When it was possible, references to already published methods were used. However, changes made from published techniques and original methods were fully described to help the reader to be able to redo the experiments in the conditions of this study.

Reviewer C:

(1) Multiwavelengths: multiwavelength was used since it has been seen to be commonly used in literature.
(2) Units: Done
(3) Done
(4) Done
(5) Done
(6) Done
(7) Done
(8) Reference has been added on the protein parameter and more work has to be done, as indicated.
(9) Changes are made p 17
   Comments p18. No changes were made on this page but explanations were added in the Conclusion section.
(10) See p 20-21

Reviewer E:

Plant cells produce a lot of fluorescent alkaloids and most of them are unavailable as standard to allow off-line quantitative analysis of these compounds and thus, accurate calibration of the sensor. In a future work, we plan to separate by semi-preparative chromatography most of them in a sufficient amount to make by our self the standard solutions. More calibration work has to be done preparing different mixtures of these compounds and then using a PLS multivariate calibration method as proposed by del Olmo et al. (1996) and Capitán-Vallvey et al. (2000). This approach may eventually allow the monitoring of individual fluorescent metabolites in plant cell culture such as major alkaloids and physiological parameters.