Measurement of autofluorescence associated with fermentation stress of wine yeasts by spectral cytometry

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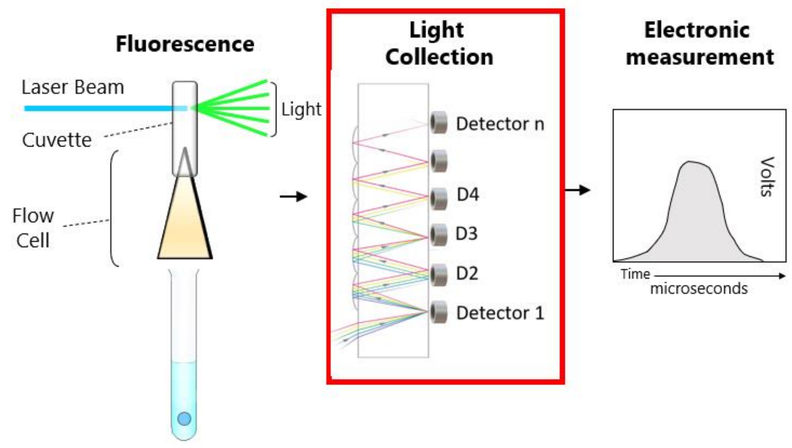
Introduction and objectives

Spectral cytometry is an advanced flow cytometry technique that uses detectors to capture the entire fluorescence emission spectrum, allowing for more accurate identification and quantification of fluorescent signals in cells. It offers superior spectral resolution and increased sensitivity compared to traditional flow cytometry methods.

Our experience with the use of flow cytometry to monitor alcoholic fermentations has taught us that with the aging of the strains within the wine matrix, a significant autofluorescence appears, especially on dead cells, inducing errors and false results if not distinguished from the background.

We tried to determine whether this fluorescence was associated with a labelling artifact or if it was associated with an evolution with the maturation of the process.

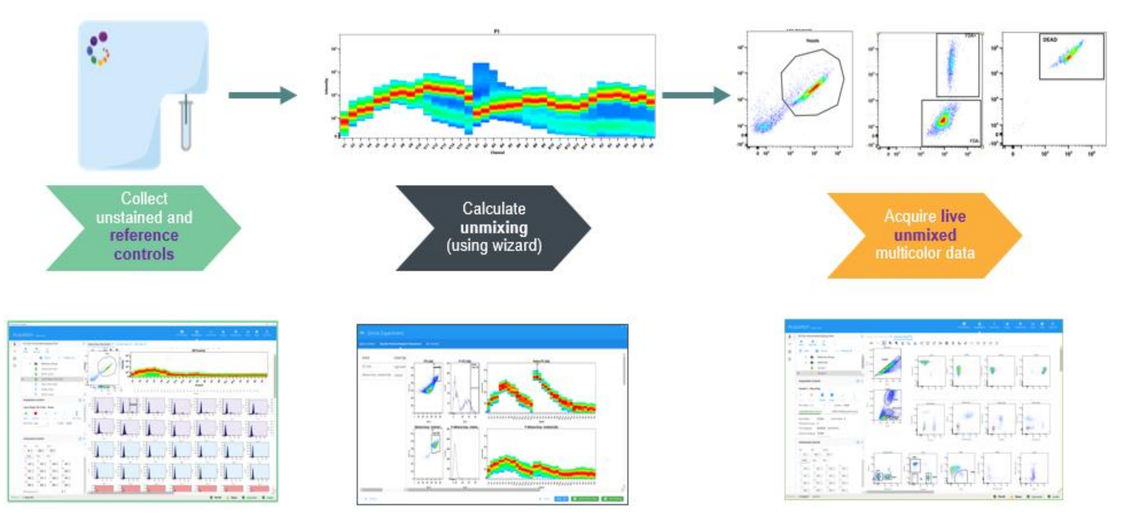


Materials and methods

A series of wines from various geographical origins at different stages of alcoholic fermentation or ageing were analysed using the Levia Test method on Cytek® Guava® Easycyte™ flow cytometer, to assess the number and physiological state of the yeasts.

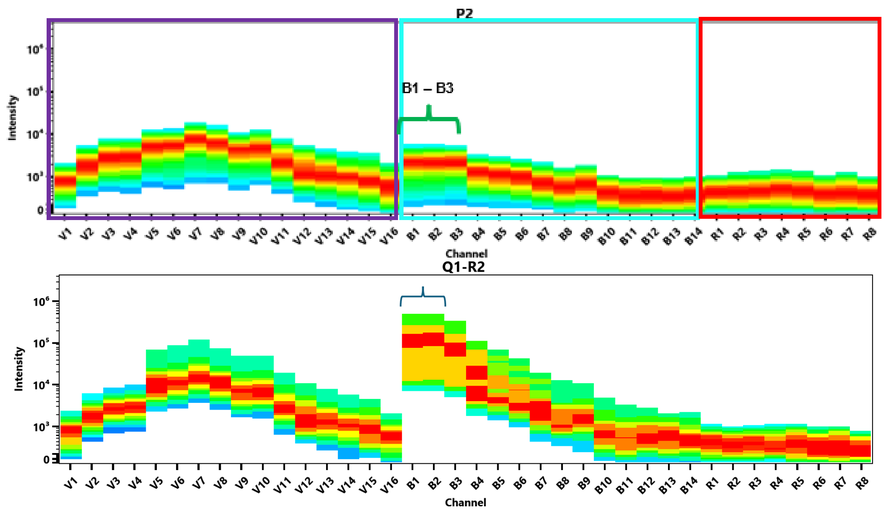
The same wines were analysed on a Cytek® Northern Lights ™ cytometer using the same method and without any fluorescent dye to evaluate autofluorescence generated by violet/ blue or red laser excitation.

At the same time, yeasts in culture were subjected to thermal, chemical or nutrient stresses and analysed according to the same protocols to mimic the ageing of the wine.

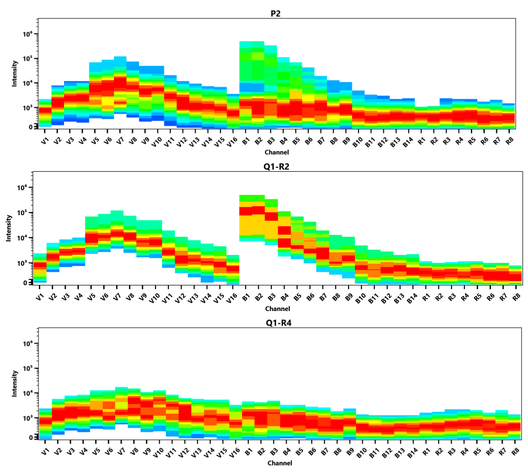


Results

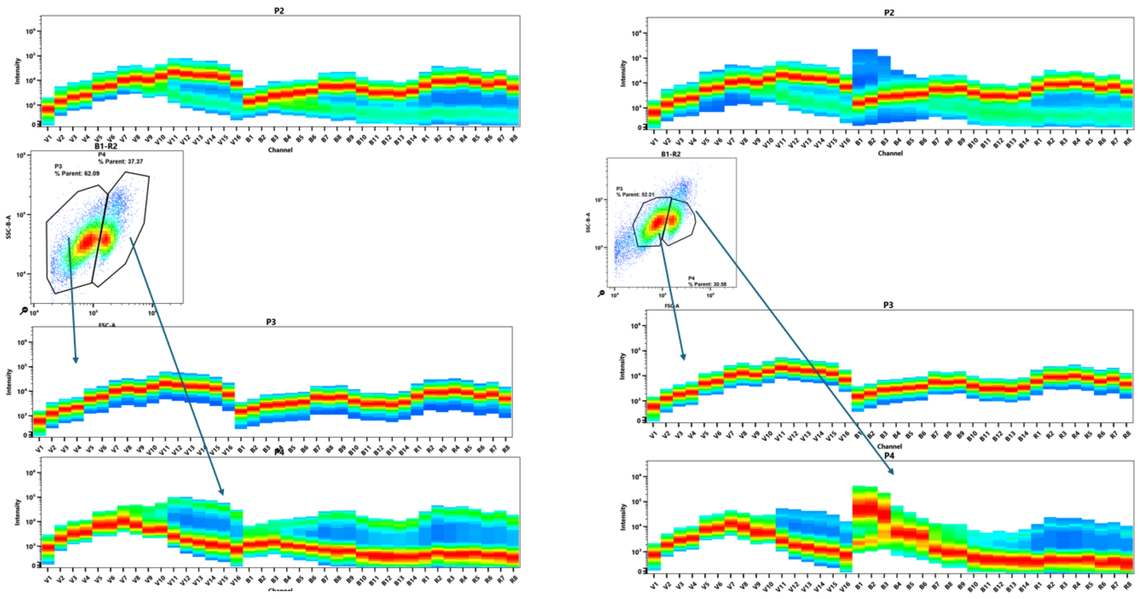
1. The FDA-specific fluorescence spectrum used in the Levia Test is easily used to visualize active cells
2. Autofluorescence is observed mainly in the red channels regardless of the excitation laser and only if the yeasts have evolved with the wine matrix.
3. Cells cultured in culture medium under different stresses, including after transfer into wine, do not exhibit the same autofluorescence.
4. The fluorescence signature is constant on all yeasts observed in wines
5. The fluorescence signature is present in red wines and to a lesser extent in white wines
6. It is possible to monitor the state of viability, stress and cell death by associating this signature with the fluorescence intensity of the enzymatic activity



Fluorescein is analysed on channel B1 to B3.
The colored cadran show the channels associated to each laser.
Up : no FDA
Down : FDA associated to active yeasts



Population of live and dead cells obtained by stress in culture. Analysed with Levia Test
Up : Whole population
Middle : Living cells
Down : Dead cells

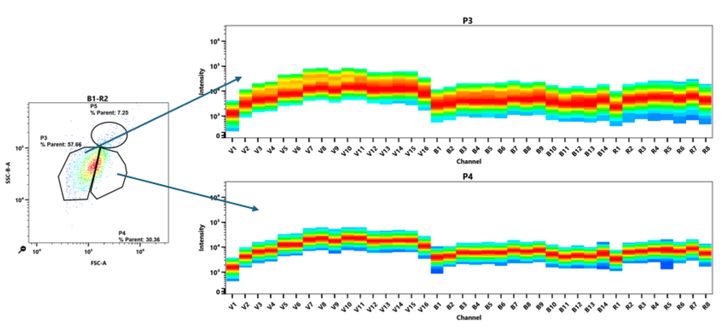


Wine analysed without dye (Left) : the red autoflourescence is clearly associated to the shape shifting induced by the matrix corresponding to dying cells.
Wine analysed with FDA (Right) : the autofluorescence is correlate with poor or no fluorescence, indicating no metabolism and/or impaired membrane.

Conclusions

Over the past ten years, Amarok Biotechnologies has developed a strategy for controlling alcoholic fermentations based on flow cytometry. Our experience has taught us that each matrix has its own unique characteristics. In the context of winemaking, we quickly abandoned the use of red fluorochromes in classical cytometry because we were using a mortality signature which, associated with the green fluorescence generated by the degradation of fluorescein diacetate, made it possible to distinguish between living, damaged or dead cells. This work carried out with spectral cytometry confirms our technical choices and supports the accuracy of our analysis.

Additional studies could be interesting to determine the nature of these fluorescent molecules as well as their precursors. Moreover, it seems obvious that this phenomenon is linked to the concomitance of several different stresses associated with this particular matrix, which makes the study more complex.



Example of an intermediate situation where the yeasts have almost no detectable viability with the appearance of the red autofluorescence signature marking their decline.