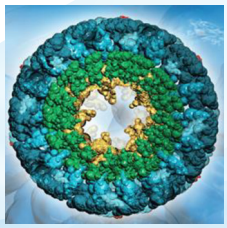
HORIBA

Fluorescence

Monitoring Culture Medium Conditions During Cell Proliferation Using the Veloci BioPharma Analyzer

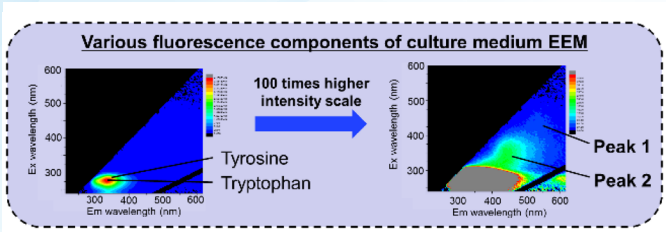


Application Note

FL-2025-1-28

Abstract

Monitoring culture media conditions is critical for optimizing cell growth in the Biopharma industry. The health of cells has drastic impacts on the yield and quality of APIs in regenerative medicine, monoclonal antibody production (mAB), protein synthesis, and many more applications. Various chemical components of culture media exhibit fluorescence, making Excitation Emission Matrix (EEM) measurement a powerful tool for simultaneous analysis of multiple components. This study demonstrates the application of EEM measurement to monitor cell culture medium conditions during the proliferation of mammalian cells.



Background

Culture media components often cause fluorescence spectral distortions due to the Inner Filter Effect (IFE), which results from self-excitation and emission light absorption along the cuvette path length. The Veloci BioPharma Analyzer measures excitation, emission, and absorbance spectra simultaneously using a patented design that corrects for IFEs. This capability enables accurate characterization of fluorescence signals in complex biological samples without chromatographic separation, and allows for qualitative and quantitative analysis of these components.

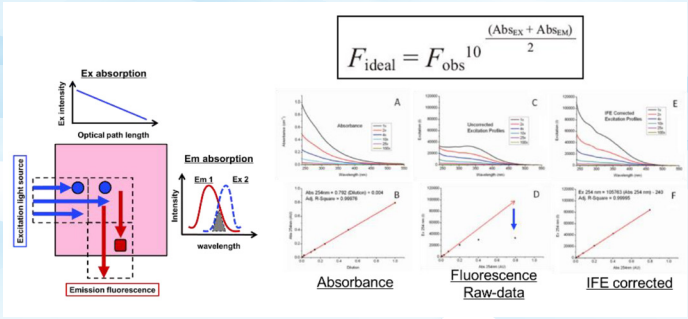


Figure 1: Samples often exhibit Inner Filter Effects (IFEs) which cause excitation and emission light absorption and increase fluorescence spectral distortion as a function of concentration.

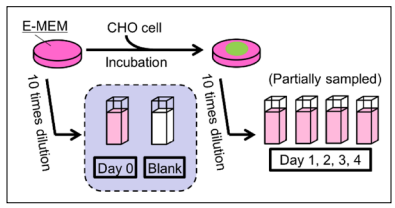
Experimental Setup

Culture Medium:

- Eagle's Minimal Essential Medium (E-MEM)
- Fetal Bovine Serum (FBS)
- Penicillin and streptomycin
- Non-essential amino acids

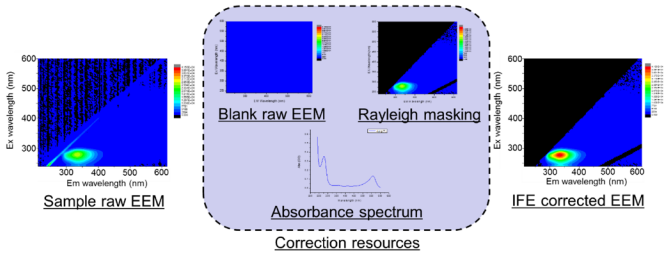
Mammalian Cell:

Chinese Hamster Ovary (CHO-K1)



Two sample dishes were incubated for four days. Partial samples of the remaining E-MEM (excluding cells) were collected daily for analysis.

Measurement Conditions:



Excitation range: 240–600 nm, 5 nm slit (bandpass)
Emission range: 211–617 nm

Corrected EEMs were obtained through:

- Wavelength-dependent corrections of monochromator and detectors
- Blank subtraction and Rayleigh masking
- IFE correction using absorbance spectra

Results

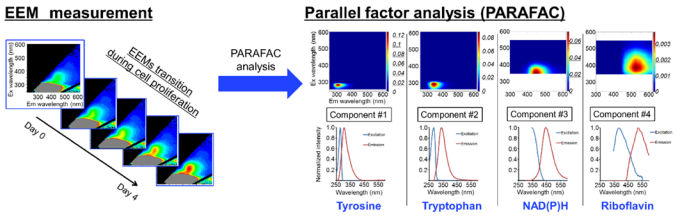


Figure 2: Fluorescence intensity (Ex: ~350 nm; Em: ~450 nm) increased steadily during the experiment. All EEM data were analyzed using Parallel Factor Analysis (PARAFAC), which successfully extracted four spectral components. These components corresponded to known fluorescent materials in the culture media.

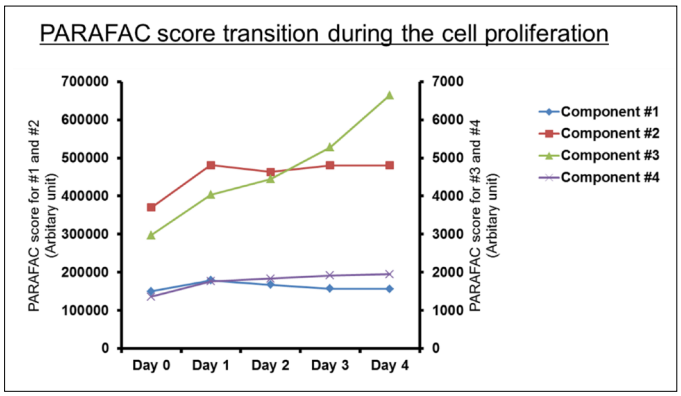


Figure 3: PARAFAC analysis revealed that the score of Component #3, resembling NAD(P)H-like fluorescence, increased continuously during cell proliferation. NAD(P)H is known to reflect metabolic activity and changes in the cellular environment, highlighting its significance as an indicator of cell proliferation and culture medium conditions.

Conclusion

EEM measurement using the Veloci BioPharma Analyzer effectively characterized culture medium conditions during cell proliferation. PARAFAC analysis enabled the detection of four distinct fluorescence components and provided quantitative insights through component scores. These plots indicate that Tyrosine, Tryptophan, NADPH, and Riboflavin can be monitored across the cell growth stages. Notably, one component, NAD(P)H, plays a crucial role in cellular metabolism. It should be noted that these compounds are very difficult to measure with Raman spectroscopy and that the A-TEEM results are providing complementary information about the sample.

References

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2. S.M.Faassen and B.Hitzmann, Sensors, 15, 10271-10291 (2015)
3. D.W.Zabriskie and A.E.Humphrey, Appl. Environ. Microbiol., 35, 337-343 (1978)

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