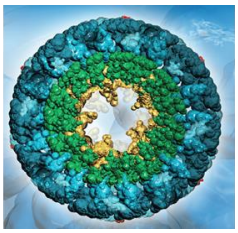
HORIBA Scientific

Fluorescence

Holistic Analysis of Mammalian Cell Proliferation using Fluorescence Spectroscopy



Application Note
Life Sciences

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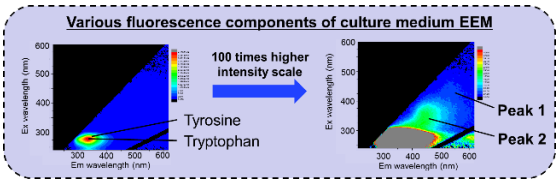
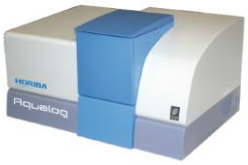
Abstract

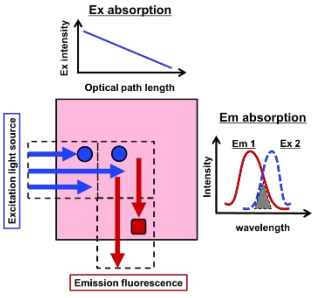
Monitoring culture media conditions is highly important for industries in order to improve cell proliferation for applications such as regenerative medicines and protein synthesis. Various chemical components of the medium have fluorescence characteristics, and thus Excitation Emission Matrix (EEM) measurement is well suited to measure several components simultaneously. Here, we applied EEM measurement to monitor cell culture medium conditions during cell proliferation.





Background

$$F_{ideal} = F_{obs}^{\frac{(Abs_{EX} + Abs_{EM})}{2}}$$



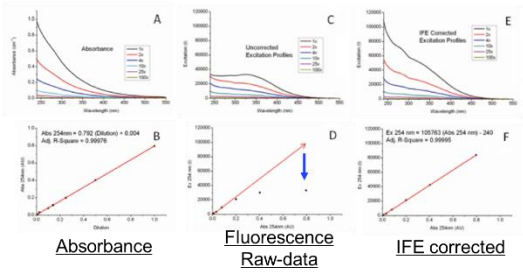


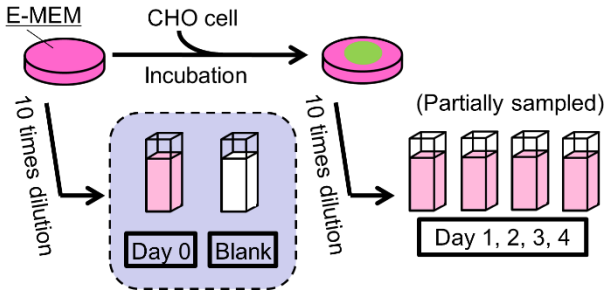
Figure 1: Samples often exhibit Inner Filter Effects (IFEs) which cause excitation and emission light absorption and increase fluorescence spectral distortion as a function of concentration. The Aqualog (HORIBA, Ltd.) measures excitation, emission, and absorption spectra simultaneously using a patented design to corrects data for IFEs.[1]

Experimental Setup

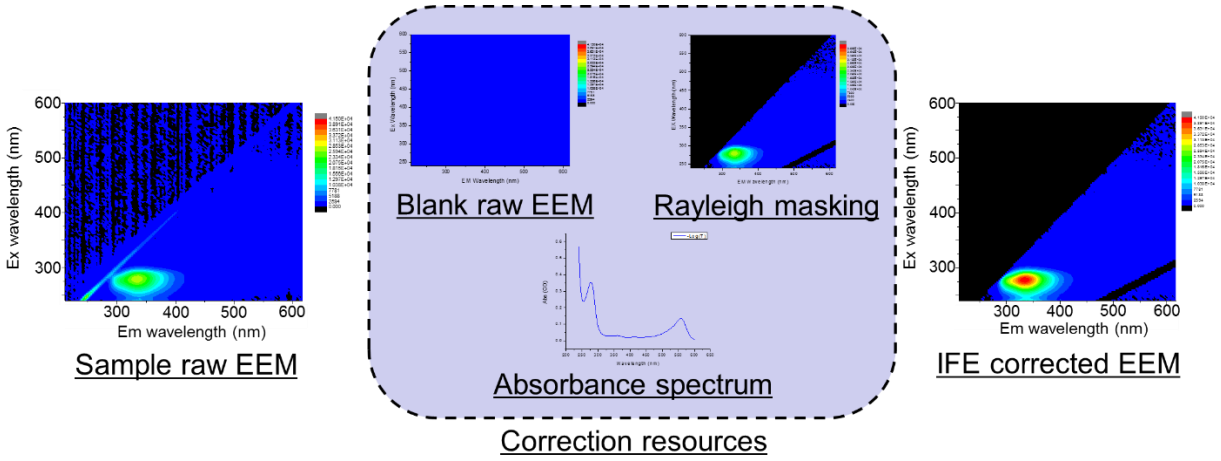
Culture medium: Eagle's minimal essential medium (E-MEM), Fetal Bovine Serum, Penicillin and streptomycin and Non-essential amino acids

Mammalian cell: Chinese Hamster Ovary (CHO-K1)

Two sample dishes were incubated for four days, and remaining E-MEM except the cell were partially sampled everyday.



Measurement Conditions



EEM acquisition was executed with following condition.

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

Measured EEM was applied following calculation to obtain corrected EEM.

- Wavelength dependence correction of monochromator and detectors
- Blank subtract and Rayleigh masking
- IFE correction by absorbance spectrum

Results

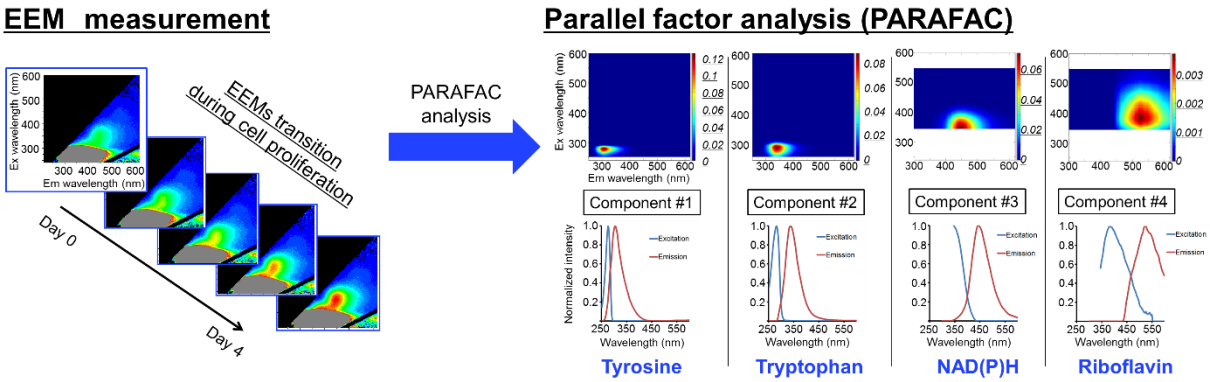


Figure 2: Fluorescence intensity (Ex: around 350 nm, and Em: around 450 nm) were increased continuously. All EEM data in the cell proliferation were analyzed using PARAFAC as a chemometric analysis. As a result, four spectral components were successfully extracted. Each component spectra looked similar to the known fluorescence materials indicated above.[2]

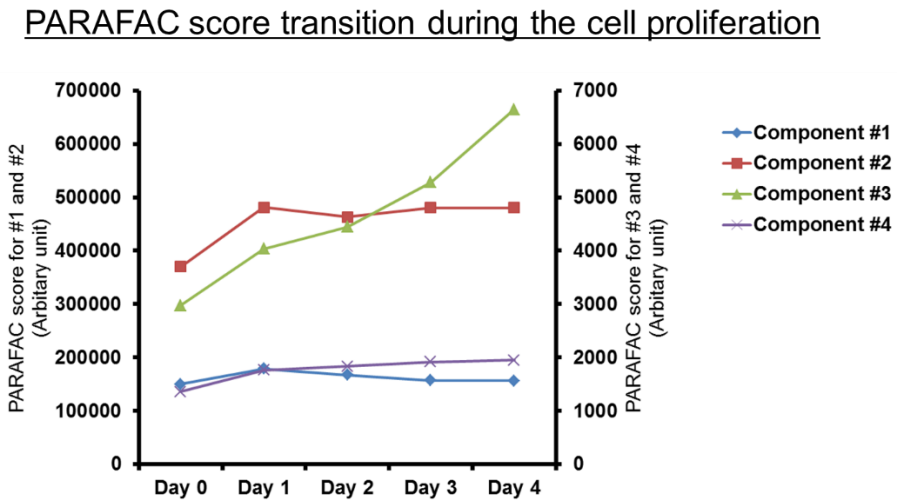


Figure 3: PARAFAC score of component #3, like NAD(P)H-like, increased continuously during the cell proliferation. The result shows that the component #3 is a key fluorescence indicator of the culture medium condition and cell proliferation. NAD(P)H is known to correspond to changes in cell environment.[3]

Conclusion

EEM measurement well characterized the culture medium condition in cell proliferation. PARAFAC analysis of the EEM data detected four fluorescence components simultaneously from overlapped contour graph. Furthermore, each fluorescence components was detected quantitatively by PARAFAC scores, and one of the components corresponded to NADPH which has important roles in metabolism. Therefore, EEM measurement clearly can offer useful information for monitoring the culture medium condition in the cell proliferation.

References

1. Adam M. Gilmore and Stephen M. 2013. Readout **No.41**. HORIBA Ltd.
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)