# APPLICATION NOTE

# Study of Natural Organic Matter components by 3D- Solid Phase Front Face Fluorescence (3D-SPFFF) spectroscopy



Tryptophan

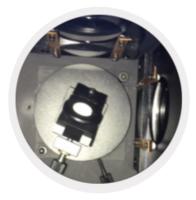
Cellulose

## **AUTHORS**

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#### EQUIPMENT

SmartFluo-QY spectrofluorometer (Zolix Instruments Co.) equipped with a solid sample holder



## **INTRODUCTION**

Natural Organic Matter (NOM) is an important component of aquatic environments. Many works have shown its central role in the mobility and the availability of aquatic pollutants. This major property is related to ability of NOM to form weak or strong associations/complexes with organic pollutants (pesticides, pharmaceutical compounds) or heavy metals.

Several decades of researches have shown that NOM is a complex set of variable compounds derived from plants/humus decaying and/or generated from primary production of aquatic microorganisms. Consequently, characterisation of NOM properties has always been a challenge for scientist. Nevertheless, some characterisation technics have already provided useful information. In particular, fluorescence spectroscopy has allowed the differentiation of some of the specific components composing NOM.

Recent advance in the sensitivity of fluorescence equipment has provide the possibility to measure either dissolved or solid samples, providing a new field for the study of solid NOM properties.

This work describes one application of 3D-Solid Phase Front Face Fluorescence (3D-SPFFF) spectroscopy to characterize some components representative of solid NOM. Thus, fluorescence emission excitation matrices were performed on simple or complex model compounds.



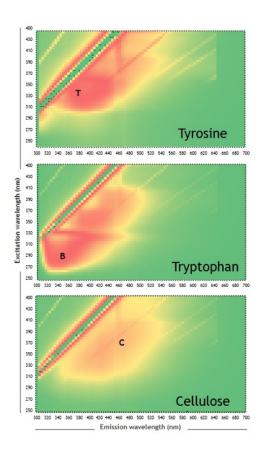


Figure 1. Fluorescence Excitation-Emission Matrix of simple model compounds

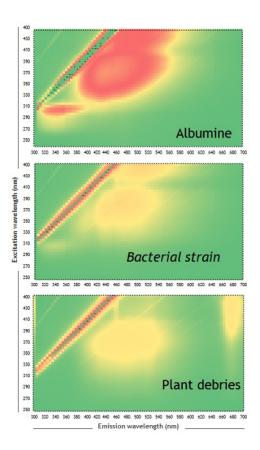


Figure 2. Fluorescence Excitation-Emission Matrix of complex model compounds

#### Reagents

Pure reference compounds (Tyrosine, Tryptophan, and Cellulose) at reagent grade were purchased from Acros Organics. Two model complex compounds were prepared by freeze drying: decaying plant litter (<200  $\mu$ m) was collected in a hardwood forest; colonies of Shewanella oneidensis were grown on PCA agar plate. Albumin (protein at analytical grade) was purchased from Sigma Aldrich.

#### Sample preparation

5 mg of sample was formatted in pellet (diameter of 1 cm; thickness 0.2 mm) with a manual hydraulic press working at 5 tons/cm2.

# **RESULTS AND DISCUSSION**

Fluorescence excitation-emission matrix of solid reference compounds highlight specific fluorophores for each sample (figure 1). The Excitation/Emission couples of these fluorophores are in accordance with the tables proposed by Cobles (1996) defining the origins of NOM (peak B, T, and C).

Figure 2 shows that the application of the SPFFF to complex samples also works. Thus, Albumin is characterized by an intense Tyrosine-like fluorophore in adequacy with the proportion of this amino acid (5g/100g -Spahr et Edsall, 1964) in this protein. Plant debris are characterized by low fluorophores of cellulose and chlorophyll pigments (at 430/680 nm), coherent with the rapid degradation of these elements in soils. Distinct fluorophores have also been highlighted in the bacteria sample.

# CONCLUSION

The SmartFluo-QY with solid holder is a powerful instrument to characterize different component of solid NOM. The specificity of the fluorophores identified by SPFFF offers many possibilities for studying their behaviour, evolution or reactivity.

Coble, P.G. (1996). Marine Chemistry, 51, 325–346 Spahr P.F. et Edsall J.T. (1964). J. biol. Chem., 239, 850-854



