Sensitive fluorescence instrumentation for water quality assessment

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Abstract: We are developing the instrumentation for *in situ* measurement of several characteristic parameters of water quality using direct and immunofluorescence techniques. The instruments and our first results are presented.

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1. Introduction

During the past decades, water quality assessment went through an immense development driven by the emerging water quality problems at a global scale on one hand, and by the appearance of both novel technologies yielding an elevated number of measureable parameters at an increasing sensitivity. We aim to assess and monitor the quality of artificial and natural freshwater in a complex, systematic and – occasionally – *in-situ* setup.

The standard processes measure the physical and chemical parameters of water (conductivity, pH, temperature, turbidity, total organic carbon – TOC, etc.) in well-defined protocols. We are to use fluorescence techniques to measure non-specific organic parameters, such as biological/chemical oxygen demand (BOD/COD) and TOC, as well as to determine the density of phytoplankton and phytobenthic algae based on Chlorophyll-*a* (Chl-*a*) content and the presence of polycyclic aromatic hydrocarbons (PAHs). The targeted sensitivity is greater than the wastewater standards for non-specific organic parameters up to the drinking water standards (e.g. TOC < 3 mg/l). Although the algae density can be determined via microscopic cell counting, in many cases it can be deduced from Chl-*a* fluorescence related to the overall chlorophyll content. In samples originating from natural water, the correlation between the algae count and Chl-*a* content can be strong (e.g. from River Danube) or weak (e.g. from Lake Balaton), whereas the correlation between biomass and Chl-*a* content is usually strong. [1]

2. Objective

2.1. Basic optical design rules

To achieve high sensitivity, it is necessary to accompany signal averaging with active noise reduction methods, e.g. by a synchronous detection of a low photon count, modulated probe light separated from the actinic light. Otherwise, it is important in every case to properly block the spectral range of the excitation with a high contrast ($T_{max}/T_{min} >> 10^6$) optical interference filter. The passband with high transmission ($T_{max} \sim 1$) must be optimized to the spectral properties of the emission, whereas the stopband should block the reflected and scattered light with the given contrast.

Since fluorescence emission is orders of magnitude smaller than the excitation in general, the relative sensitivity is in the range of 10^{-4}… 10^{-3} depending on the contrast of the filter. In case of a living sample, the lower boundary of this range can be achieved with measuring the maximum of the characteristic temporal fluorescence response. In this case, the algae density is proportional to the difference of the maximum and steady state intensities. To reach this sensitivity it is necessary either to use sufficient I_{exc} excitation intensity, or to increase the $\Delta\lambda$ passband width of the filter. It is important to mention that in dense samples, saturation in fluorescence emission occurs, which can be compensated with the change of the filter or the excitation intensity. Since the response of the sample depends on the excitation, the $I_{exc}\Delta t$ dose should also be considered.

2.2. Realised instruments

Using our earlier knowledge base on earlier instrument developments – especially of the FluoroMeter Modul (FMM) family – for the Chl-*a* fluorescence measurement of plant leaves [2, 3], a new instrumentation was realized with different sensing methods: (1) The first method is designed to fit the 96-well enzyme-linked immunosorbent assay (ELISA) microplate format, which is the gold standard in chemical, biochemical, medical and environmental diagnostics. The samples are illuminated with high power LEDs of different wavelengths and the emitted fluorescence is measured with silicon photodiodes having large active area. The necessary high spectral blocking and contrast is achieved by a combination of dichroic and bandpass filter (see Fig. 1 A, B).

(2) The second method uses a standard cuvette. The sample is excited with a Xenon flash lamp combined with an optical bandpass filter. The fluorescence is measured with (2a) a high-sensitivity, TE cooled CCD fiber-optic spectrometer or (2b) a photomultiplier tube combined with a set of optical interference filter. Method (2a) is appropriate to capture the fluorescence spectrum and thus more sufficient to distinguish between different fluorescence components. In contrast, method (2b) is more cost efficient, although its spectral resolution is limited by the optical bandpass filters available (Fig. 1 C).

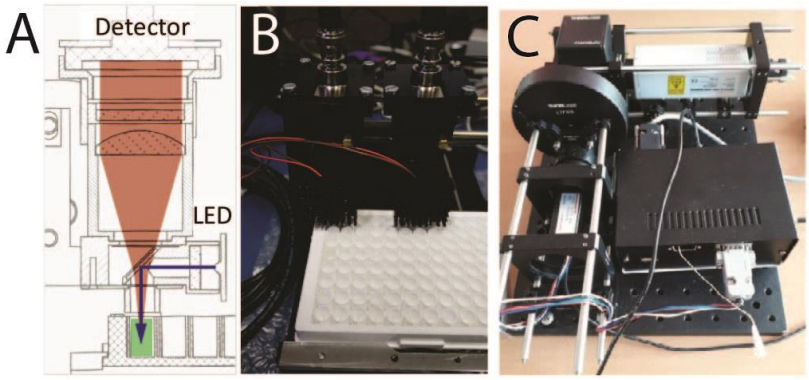

Fig. 1. (A) Schematic of the ELISA plate compatible sensor head. (B) Photo of the ELISA plate compatible instrument. (C) Photo of the cuvette based instrument.

3. Preliminary results

The first results show that we can cover 4 orders of magnitude with a modified FMM setup for algae (Fig. 2 A) and with the cuvette designed setup for tyrosine sample (Fig. 2 B). We estimate a higher dynamic range and lower limit of detection by an increase of the range of excitation light intensity and by a better spectral blocking using collimated beams from precise apertures, respectively. Using fluorescence spectroscopic measurements, we detected algal densities determined with traditional protocols in surface water [4] and found similar fluorescence excitation-emission maps (Fig.2 C) similar to available data [5].

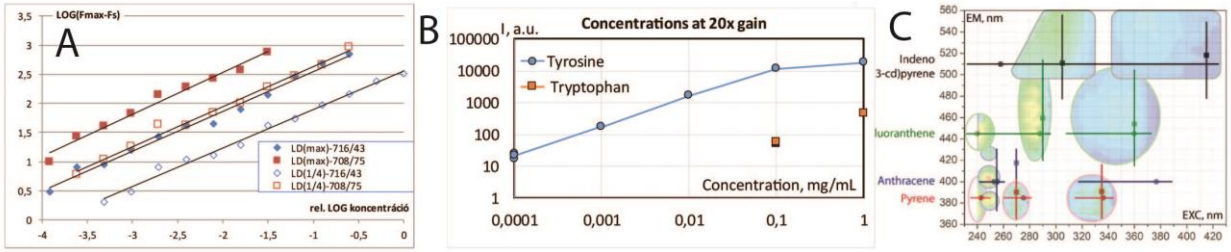

Fig. 2. (A) Fluorescence signal of an original and a diluted algal sample using different excitation light levels and detection wavebands, in log-log scale. (B) Fluorescence signal of tyrosine and tryptophan samples depending on the concentration, both on logarithmic scale. (C) Excitation-emission map of 4 selected PAHs.

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5. References

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