



Turbidity correction in bbe fluorometers

Impact of turbidity on the fluorescence signal

Influence at the excitation light

Turbidity particles in a water sample influence the excitation light in two ways. One part of the excitation light will be absorbed, the other part will be reflected by the particles. Therefore the fluorescence emission will suffer from disproportioning and weakening of the excitation light. Especially the excitation close to 650nm and 700nm will be reflected into the fluorescence detectors while the absorption effect will be strongest for the shorter wavelength around 470nm. Overestimating the excitation between 590nm and 610nm will cause an overestimation of cyanobacteria in the fitted result. Therefore high turbidities lead to false determinations or even cause "ghost algae" of cyanobacteria whilst green algae will appear to be underestimated. The presence of yellow substances (FDOM) in the sample causes the opposite effect: the signal at 470nm would be amplified so green algae could be expected to be over-represented.

Influence at the fluorescence signal

In contrary to the excitation the fluorescence detection is estimated exclusively at 700nm (Phyco-line instruments estimate the signal at 650nm and 700nm). The influence of turbidity shows mainly an absorption and the related weakening of the fluorescence signal. It is merely impossible to predict and therefore correct the signal for this effect so the error remains as a limitation of the method. Though the effect plays a major role at high turbidity readings exceeding 150 FTU it could be ignored for the correction interval below 150 FTU.



FluoroProbe

bbe is offering an option for determining the transmission through the water sample within the instruments chamber for the bbe FluoroProbe. A 700nm LED is built into the instrument light path which transmits light through the sample onto the photo-detector. This enables the instrument to detect very small concentrations of turbidity substances with good precision and resolution.

AlgaeTorch

The bbe AlgaeTorch is not equipped with a transmission sensor due to its construction, which differs from the bbe FluoroProbe's construction. Therefore the AlgaeTorch is equipped with a 700nm LED in parallel to the excitation LEDs within the torch's front compartment. Turbidity is measured as scattered light through the fluorescence sensor and calibrated for formazine turbidity units (FTU).

ALA, Chlorophyllsensor

The bbe ALA and the chlorophyll sensors (aka AOA-sensor) lack a 700nm LED. Therefore transmission is determined for all implemented wavelengths (370nm, 470nm, 525nm, 570nm, 590nm and 610nm). So the instrument detects the direct turbidity-impact for every single wavelength.

Implementation of the turbidity correction for the ALA, the chlorophyll sensors, the FluoroProbe and the AlgaeTorch

In these instruments the turbidity is recorded as transmission. For the correction of the excitation it was necessary to convert the transmission values to turbidity values in FTU at first. The relation between transmission and turbidity could be described with the polynom:

$$f(x) = a*x^2 - b*x + c \quad (1)$$

Though the transmission is calibrated into the instrument, the formula is universal for the conversion in all bbe fluorometers, so are the parameters a, b and c.

After converting the transmission into turbidity the correction follows the same pattern in all bbe fluorometers.

In the first step the LED specific offsets of the instrument will be corrected. This way the reflection of the excitation light caused by turbidity particles could be corrected.



$$\text{offset corr.} = \text{Turbidity} * \text{LED brightness} * \text{reflection constant} + \text{offset} \quad (2)$$

The result out of formula (2) is the corrected offset. The turbidity value will be multiplied with the LED brightness and the reflection constant. The instrument specific offset (calibrated in distilled water) will be added up. This way the disproportioning of the fluorescence signal caused by reflection could be corrected by simply subtracting the corrected offset. The calibrated fingerprints contain the true LED brightness. It is derived from all calibrated fingerprints and normalised for 1 $\mu\text{g/L}$. It will be stored as a calibration specific instrument constant within the parameter set for each distinct LED.

In a second step the LED specific raw signal will be corrected for the turbidity-caused weakening of the excitation light after subtracting the turbidity corrected offset.

$$\text{raw value corr.} = (\text{raw value} - \text{offset corr.}) + (1 + \text{weakening constant(LED)} * \text{Turbidity}) \quad (3)$$

After the correction of the LED specific raw values the results are fitted with the instrument specific fingerprints for the algae-classes.

Avoiding the error which appears due to the disproportioned fluoresce signal is the major advantage of correcting the raw signals for turbidity. This could not be achieved by correcting the readings after the fit, especially if “ghost algae” appear like turbidity caused cyanobacteria.



PhycoProbe

The bbe PhycoProbe's light path differs from the construction of the bbe FluoroProbe. Instead of the transmission LED the PhycoProbe contains a second sensor for phycocyanin (650nm). Therefore it is not equipped with a transmission sensor. Instead a 700nm LED was integrated together with the excitation LEDs to equip the PhycoProbe with a scattering sensor for turbidity detection. The PhycoProbe is calibrated for formazine turbidity units (FTU).

PhycoLA, PhycoSens

The bbe PhycoLA and PhycoSens implement both solutions, transmission detection for all excitation wavelengths and a 700nm scattering detection. This offers a very precise detector for turbidity and a two step turbidity correction.

Implementation of the turbidity correction into bbe PhycoLA, PhycoSens and PhycoProbe

Right from development of the new bbe Phyco-line, care was taken for the implementation of the correction for turbidity effects. First the raw values are corrected following the model of the standard fluorometers with the help of pre-defined parameters.

Within the bbe Phyco-line not only the fit algorithm was expanded, the sensor unit was expanded, as well. Due to the advanced possibilities the turbidity became an additional fingerprint comparable to yellow substances or phycocyanin and therefore part of the fit. This way also correcting for the colour properties of the turbidity particles became possible additionally to the bare brightness effects. As a result the fit became more stable and less susceptible to a disproportional raw signal.