



Uncertainty of dilution gauging using fluorescent tracer - The FUNY Framework

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FUNY, Rammeverk for beregning av usikkerhet i vannføringsmålinger
der vannføringen er målt vha. fluorescens-fortynningsmetoden

Alexandre Christophe Hauet

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Sammendrag:

Denne rapporten beskriver beregning av usikkerhet i vannføringsmålinger der vannføringen er målt vha. fluorescensfortynningsmetoden, dvs. målinger der man bruker Uranin eller Rhodamin. Dette blir gjort ved å se på usikkerhetsforplanting ("Propagation of uncertainty") for målingene. Først blir hvert enkelt bidrag til usikkerheten identifisert og modellert eller beregnet, og deretter blir den samlede usikkerheten beregnet. Rammeverket for beregning av usikkerhet kalles FUNY (Fluorescens UNcertainty), og operasjonelt bruker vi software med samme navn. FUNY viser både total usikkerhet og de enkelte bidragene, slik at vi ikke bare får vite kvaliteten (usikkerheten) til en måling, men også *hvorfor* kvaliteten er som den er. Dette er en stor hjelp for å øke kvaliteten.

Usikkerhetsberegningen er basert SUNY-rammeverket: Uncertainty of salt discharge measurement - The SUNY Framework (Rapport 29/2020, ISBN: 978-82-410-2050-6).

SUNY er i sin tur basert på ISO/TS 25377:2007 - Hydrometric Uncertainty Guidance (HUG) og JCGM Evaluation of measurement data - Guide to the expression of uncertainty in measurement (GUM). BIPM, 2008.

Software (pr aug 2020) https://alexandrehaudet.shinyapps.io/SUNY_Salt_Dilution/

This report describes the calculation of uncertainty in discharge measurements where the discharge is measured using the fluorescence dilution method, that is, Uranine or Rhodamine. This is done by looking at the propagation of uncertainty for these measurements. First, each individual contribution to the uncertainty is identified and modeled or calculated, and then the total uncertainty is calculated. The framework for calculating uncertainty is called FUNY (Fluorescence UNcertainty), and operationally we use software with the same name. FUNY shows both total uncertainty and the individual contributions, so that we not only get to know the quality (uncertainty) of a measurement, but also why the quality is as it is. This is a great help to increase the quality.

The uncertainty calculation is based on the SUNY framework: Uncertainty of salt discharge measurement - The SUNY Framework (Rapport 29/2020, ISBN: 978-82-410-2050-6)

SUNY, in turn, is based on ISO / TS 25377: 2007 - Hydrometric Uncertainty Guidance (HUG) and JCGM Evaluation of measurement data - Guide to the expression of uncertainty in measurement (GUM). BIPM, 2008.

Software (as of Aug 2020) https://alexandrehauey.shinyapps.io/SUNY_Salt_Dilution/

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Forord

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Report

Uncertainty of dilution gauging using fluorescent tracer: The FUNY Framework

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1 Definition of the measurement method

The main principle of the dilution method is based on the conservation of mass [6]. In the so-called slug injection method [7], a known amount of tracer M_{inj} (in kg) is injected as a near-instantaneous gulp at one location in the stream. The tracer can be a salt (most often NaCl) or a fluorescent tracer (Uranine or Rhodamine). The tracer is mixed in the stream water thanks to advection, eddy diffusion and turbulences mostly, rapidly throughout the depth of the stream and less rapidly across the stream width, resulting in a tracer cloud traveling downstream with the general flow. After the mixing distance is reached, i.e. where the tracer has been completely mixed across the stream width, the mass flux of tracer at a time t , $q(t)$ (in kg/s), is equal to:

$$q(t) = Q(t) \cdot Cc(t) \quad (1)$$

where $Q(t)$ is the river discharge at time t (in m^3/s) and Cc is the added tracer concentration (in kg/m^3). Under the assumption of a steady flow, the integration of the tracer flux over the duration of the cloud (between the beginning and ending time of the cloud, T_b and T_e respectively) gives the injected mass M_{inj} :

$$\int_{T_b}^{T_e} q(t) \cdot dt = M_{inj} = \int_{T_b}^{T_e} Q(t) \cdot Cc(t) = Q \cdot \int_{T_b}^{T_e} Cc(t) \quad (2)$$

Knowing M_{inj} and measuring $Cc(t)$ allows to estimate the discharge Q using Eq. 2.

As the direct measurement of the tracer concentration is impossible, a proxy is used, like the measurement of the conductivity for the salt or the measurement of fluorescence for the Uranine or Rhodamine. Using Fluorescent tracer, $Cc(t)$ is determined from the discrete time interval measurement of the additional fluorescence $Cd(t) - Cd_0$ (in mV) as :

$$Cc(t) = [Cd(t) - Cd_0] \cdot CF \quad (3)$$

where $Cd(t)$ is the fluorescence (in mV) measured at time t and Cd_0 is the natural fluorescence (in mV) of the stream flow, also called the base fluorescence. The calibration factor CF is established from a calibration procedure that consists in adding standard additions of a solution of known concentration (the calibration solution) to a known volume of stream water and to measure the corresponding fluorescence following this protocol:

1. Prepare a calibration solution of known concentration Cc_{calib} .
2. Fill a flask with a known volume V_{flask} of river water. Measure the fluorescence of the river water Cd_0 , corresponding to a concentration $Cc_0 = 0$.
3. Pipette a known volume of calibration solution V_{pip} , and pour it in the flask. Measure the fluorescence Cd_i .

- Repeat the step 3 several times (classically four or five times) and for each addition i compute the concentration Cc_i as:

$$Cc_i = \frac{i * V_{pip} * Cc_{calib}}{V_{flask} + i * V_{pip}} \quad (4)$$

- Fit a linear regression as $Cc_i = CF * Cd_i + b$.

The slope of the relation between Cc_i and Cd_i provides the required value for CF . Then, combining Eq.2 and Eq.3 leads to:

$$Q = \frac{M_{inj}}{\int_{T_b}^{T_e} CF \cdot [Cd(t) - Cd_0] \cdot dt} \quad (5)$$

The fluorescence decreases with temperature significantly, following an exponential law. The measured $Cd(t)$ must be compensated to a reference temperature, often 20 or 25°C. The record of tracer concentration with time at the measurement location is called a tracer wave. Figure 1 illustrates the measurement process.

Several m (typically two) fluorescence sensors are used in order to verify that the mixing length is reached, producing m different values of discharge Q_m using equation (5). The final discharge is the average of the Q_m .

2 Uncertainty

2.1 Framework and notations

The approach proposed for the computation of fluorescent tracer dilution uncertainty is called FUNY for Fluorescent tracer UNcertainty, and follows the main steps proposed by the Hydrometric Uncertainty Guidance [3] based on the Guide to the expression of Uncertainty in Measurement, GUM [4]:

- Inventory of error sources (see section 2.2)
- Evaluation of the standard uncertainty of each input quantity (see section 2.4)
- Determination of the combined uncertainty (see section 2.3)

In the following study, we express all the uncertainties in terms of relative uncertainties, in %. The following notations are used:

- u is the standard uncertainty (at a level of confidence of 68%)
- U is the expanded uncertainty (at a level of confidence of 95%)

The expanded uncertainty U is the standard uncertainty multiplied by a coverage factor $k = 2$, as recommended by the GUM ([4]), corresponding to a 95% probability level and assuming a normal distribution of errors.

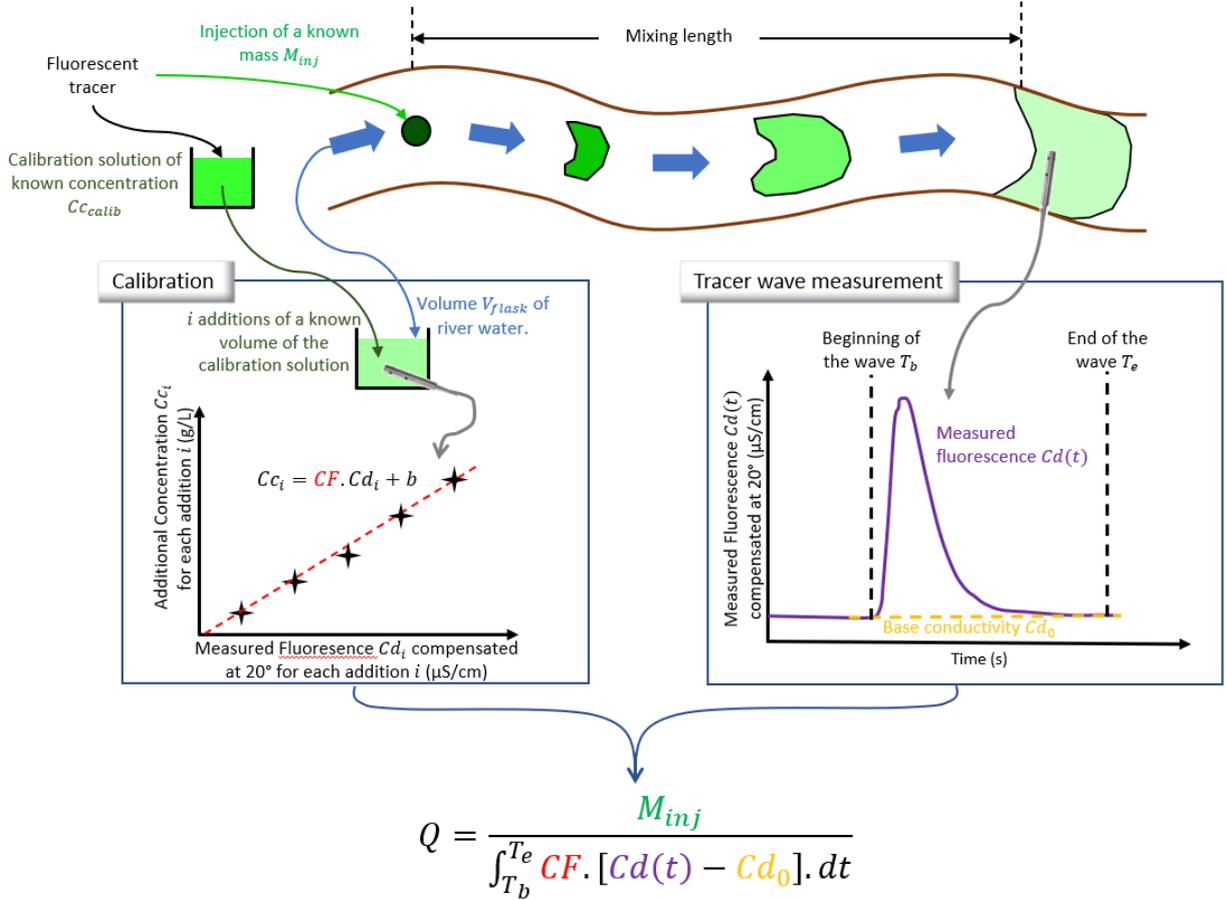


Figure 1: Principles of a dilution measurement

2.2 Inventory of error sources

From equation (5), we identify :

- Uncertainty in the mass of tracer injected $u(M)$
- Uncertainty in the measurement of time $u(dt)$
- Uncertainty in the correction of temperature $u(temp)$
- Uncertainty in the Fluorescence to Concentration law $u(CF)$
- Uncertainty if a measured fluorescence is out of the range of the Fluorescence to Concentration law $u(range)$

- Uncertainty in the computation of the area under the fluorescence curve, which includes four components:
 - Uncertainty in the computation of the base fluorescence $u(Cd_b)$, especially when the fluorescence base is not stable in time
 - Uncertainty in the signal to noise of the fluorescence $u(Cd_t - Cd_b)$, including the variability of the signal and the resolution of the probes.
 - Uncertainty in the sampling $u(samp)$ related to the number of points measured during the passage of the tracer wave.
 - Uncertainty in the setting of the end of the tracer's wave, $u(limits)$

Some other error sources can be considered, like:

- Uncertainty due to a not perfect mixing of the tracer $u(mix)$, if the mixing length between injection and the probes is not reached
- Uncertainty due to a loss or a gain of tracer between the injection and the probes $u(tracer)$, if tracer can be adsorbed for example
- Uncertainty due to unsteadiness of the flow $u(steady)$, i.e. variation of discharge during the measurement
- Uncertainty due to systematic residuals errors $u(s)$

2.3 Combination of the uncertainty sources

Following the GUM [4], for a measurement model written $y = f(x_i)$ the uncertainty sources $u(x_i)$ can be propagated using a first-order Taylor series approximation to compute the combined uncertainty $u(y)$, leading to :

$$u_c^2(y) = \sum_{i=1}^N u^2(x_i) + 2 \cdot \sum_{i=1}^{N-1} \sum_{j=i+1}^N u(x_i) \cdot u(x_j) \cdot r(x_i, x_j) \quad (6)$$

where $r(x_i, x_j)$ is the covariance between the inputs x_i and x_j . In the following, the correlation between the input values are neglected and $r(x_i, x_j) = 0$.

From equation (5), the propagation of relative uncertainty can be written as :

$$u^2(Q) = u^2(M) + u^2(dt) + u^2(temp) + u^2(CF) + u^2(range) + u^2(Cd_t - Cd_b) + u^2(Cd_b) + u^2(samp) + u^2(limits) + u^2(s) \quad (7)$$

With the additional error sources, equation (7) becomes :

$$u^2(Q) = u^2(M) + u^2(dt) + u^2(temp) + u^2(CF) + u^2(range) + u^2(Cd_t - Cd_b) + u^2(Cd_b) + u^2(samp) + u^2(limits) + u^2(mix) + u^2(tracer) + u^2(steady) + u^2(s) \quad (8)$$

If the measurement is realized using m probes each called k , it is important to distinguish:

- The errors not related to the number of probes, which are related to the injection, the hydraulic conditions and to what can happen between the injection and the probes : $u(M), u(mix), u(tracer), u(steady)$
- The random uncertainties related to the probes, that would reduce and trending to zero when increasing the number of sensors, i.e. the errors in the computation of the area under the tracer wave: $u(temp), u(Cd_t - Cd_b), u(dt)$ and $u(samp)$
- The systematic errors related to the probes, that would not reduce when increasing the number of sensors, i.e. errors on the calibration $u(CF), u(range)$, on the base fluorescence $u(base)$ and on the user input for the begin and end time of the wave $u(limits)$

For m probes, random uncertainties related to the probes are expressed as:

$$u_{random}^2 = \frac{1}{m^2} \sum_{k=1}^m u_{random}^2(k) \quad (9)$$

The systematic errors related to the probes are expressed as the variance mean :

$$u_{systematic}^2 = \frac{1}{m} \sum_{k=1}^m u_{systematic}^2(k) \quad (10)$$

Finally, the combined relative uncertainty using m probes k is expressed as:

$$u^2(Q) = u^2(s) + u^2(M) + u^2(mix) + u^2(tracer) + u^2(steady) + \frac{1}{m} \left(\sum_{k=1}^m u^2(CF)_k + \sum_{k=1}^m u^2(range)_k + \sum_{k=1}^m u^2(base)_k + \sum_{k=1}^m u^2(limits)_k \right) + \frac{1}{m^2} \left(\sum_{k=1}^m u^2(dt)_k + \sum_{k=1}^m u^2(temp)_k + \sum_{k=1}^m u^2(Cd_t - Cd_b)_k + \sum_{k=1}^m u^2(samp)_k \right) \quad (11)$$

The result of the measurement, Q , is associated to an expanded uncertainty $U(Q)$, using a coverage factor k to multiply the combined uncertainty, as:

$$U(Q) = k \cdot u(Q) \quad (12)$$

A coverage factor of $k = 2$, corresponding to a 95% probability level, is used, as recommended by the GUM [4].

2.4 Estimation of the uncertainty sources

2.4.1 Uncertainty in the mass of injected tracer $u(M)$

The uncertainty in the mass of injected tracer $u(M)$ is expressed as a percentage of standard deviation of the mass. A default value of 0.5% can be used, but the user can decide to input another value.

2.4.2 Uncertainty in the measurement of time $u(dt)$

The sampling frequency dt is generally about 1s for dilution measurement. The uncertainty of time measurement $u(dt)$ using modern quartz timer is negligible, so we assume $u(dt) = 0$, but the user can decide to input another value.

2.4.3 Uncertainty in correction of the temperature $u(temp)$

The fluorescence decreases with the temperature as an exponential function. When using Uranine as a tracer, the difference of fluorescence ΔCd for a change Δt of temperature is:

$$\Delta Cd = e^{(-0.004 \cdot \Delta t)} \quad (13)$$

As the TQ-TRacer does not encompass a temperature probe, the user must estimate Δt as the maximum possible difference of temperature between the calibration flask and the river. The uncertainty associated to the temperature $u(temp)$ is computed using a rectangular distribution as:

$$u(temp) = \frac{e^{(-0.004 \cdot \Delta t)}}{2 \cdot \sqrt{3}} \quad (14)$$

2.4.4 Uncertainty in the Calibration Factor $u(CF)$

The Calibration Factor (CF) is computed following the standard addition framework described in section 2.2. The following error sources affect the computation of CF:

- Uncertainty of the volume of the flask $u(V_{flask})$. It can be computed using the flask tolerance f_t usually indicated on it (depending on the flask quality class, for example $f_t = 0.25mL$ for a class A 500mL flask) associated to a probability law. A rectangular law is recommended, then the standard deviation is $u(V_{flask}) = f_t/\sqrt{3}$
- Uncertainty of the volume of the pipette $u(V_{pip})$. As the pipetting operation is realized in the field, we assume that the uncertainty must take into account the pipette uncertainty $u(pip)$ and a so-called operator and environmental effect $u(op)$.
 - $u(pip)$ is computed using the tolerance of the pipette p_t depending on the pipette quality class, usually $p_t = 1\%$ and a rectangular probability law as $u(pip) = p_t/\sqrt{3}$

- $u(op)$ is related to the operator skills and to the conditions that can impact the quality of the pipetting (mainly the temperature). From repetitive experiments conducted by [9], typical value of $u(op)$ are about 2%. The volume of the pipette are calibrated for a temperature of $20^{\circ}C$, and pipetting at low temperature results in smaller volume (this effect is reduced if the temperature of the pipette, the air and the water are the same). The default value for $u(op)$ is set to 2%, but the user can decide to input another value.

$u(V_{pip})$ is finally computed as $u(V_{pip}) = \sqrt{u^2(pip) + u^2(op)}$.

- Uncertainty of the concentration of the calibration solution $u(C_{calib})$, that depends on the quality of the solution. The default value for $u(C_{calib})$ is set to 1%, but the user can decide to input another value.

We propose to compute the uncertainty $u(CF_{protocol})$ using random sampling simulations

as described by the algorithm 1.

Algorithm 1: Compute $u(CF_{protocol})$

for $i \leftarrow 1$ **to** 100000 **do**

- Pick randomly 1 flask volume $V_{flask}(i)$ in a normal law distribution with mean equal to V_{flask} and a standard deviation $u(V_{flask})$;
- Pick randomly 1 concentration for the calibration solution $C_{calib}(i)$ in a normal law distribution with mean equal to C_{calib} and a standard deviation $u(C_{calib})$;

for $j \leftarrow 1$ **to** N *standard additions* **do**

- Get the fluorescence $Cd_{i,j}$ measured from the measurement file ;
- Pick randomly 1 pipette volume $V_{pip}(i, j)$ in a normal law distribution with mean equal to V_{pip} and a standard deviation $u(V_{pip})$;
- Compute the total added mass of tracer
 $M_{add}(i, j) = M_{add}(i, j - 1) + (V_{pip}(i, j) * C_{calib}(i))$;
- Compute the total volume in the flask $V_{flask}(i, j) = V_{flask}(i, j - 1) + V_{pip}(i, j)$;
- Compute the concentration $C_{c_{i,j}} = M_{add}(i, j) / V_{flask}(i, j)$;

end

- Fit a linear regression on $C_{c_{i,j}} = CF_i * Cd_{i,j} + b$;

end

- Compute $u(CF_{protocol})$ as the standard deviation of the relative value of CF_i :
 $u(CF_{protocol}) = sd(\frac{CF_i}{CF})$;
-

The uncertainty of the linear regression $u(reg.CF)$ is computed for each probe using :

$$u(reg.CF) = \frac{1}{CF} \cdot \sqrt{\frac{\frac{1}{N} \sum_{i=1}^N (\hat{C}d_i - Cd_i)^2}{\sum_{i=1}^N (Cc_i - \bar{C}c)^2}} \quad (15)$$

where $\hat{C}d_i$ is the estimation of the fluorescence from Cc_i using the linear model. $u(reg.CF)_k$

is computed for each probe k , and added to $u(CF_{protocol})$ to compute $u(CF)$ as:

$$u^2(CF) = u^2(CF_{protocol}) + \frac{1}{m} \cdot \sum_{k=1}^m u^2(reg.CF)_k \quad (16)$$

2.4.5 Uncertainty if out of the range of the Fluorescence to Concentration law $u(range)$

The relationship between the concentration and the fluorescence is supposed to be linear and is computed as $Cc = CF * Cd + b$ (see section 2.4.4). This hypothesis is not true: the value of CF slightly decreases when Cd increases. If the range of fluorescence covered by the calibration and the measurement are consistent, i.e. if the value of the wave's peak is close to the maximum value of the calibration, the non-linearity effect is negligible. Two cases must be considered:

- If the wave peak is low compared to the maximum of calibration, an adapted coefficient CF_{adapt} is computed using a linear fit on the calibration points up to the value of the wave's peak. $u(range)$ is computed as the relative difference between CF_{adapt} and CF (fitted on the whole range), as $u(range) = \frac{CF_{adapt} - CF}{CF}$
- If the wave peak is higher than the maximum of calibration, then $u(range)$ is arbitrary set as 15% of the relative area of the wave higher than the maximum of calibration. The default value of 15% can be modified by the user.
- If the wave peak is lower than the minimum of calibration, then $u(range)$ is arbitrary set as 15% of the relative area of the wave lower than the minimum of calibration. The default value of 15% can be modified by the user.

More work would be needed to have an objective value of the uncertainty when the wave is above or below the calibration range. The default value of 15%, quite high, allows to alert the user that the rules-of-thumb of dilution were not respected, with a wave out of the range of the calibration.

2.4.6 Uncertainty in the mixing $u(mix)$

Uncertainty of the mixing $u(mix)$ is estimated by the relative maximum difference between the discharge computed by the different probes Q_p in the river divided by the measured discharge, and by using a Gaussian probability distribution:

$$u(mix) = \frac{max(Q_p) - min(Q_p)}{Q_p \sqrt{2}} \quad (17)$$

If the measurement is realized with only one probe, $u(mix)$ is set to 15% arbitrarily. This default value of 15%, quite high, allows to alert the user that the rules-of-thumb of dilution were not respected by using only one probe. If the measurement is realized in a well-known site, for which the user is confident that the mixing length was reached, the value can be reduced to 5%.

2.4.7 Uncertainty in the tracer conservation $u(tracer)$

Uncertainty in the tracer conservation $u(tracer)$ must reflect the possibility for a loss or a gain of tracer between the injection and the probes. Loss of tracer may arise during the injection, if a part of the mass of tracer does not reach the river, or if there is sedimentation of the tracer. One could also consider tracer adsorption, or chemical reactions that could eat up tracer. A gain of tracer can happen if a source of tracer is present between the injection and the probes. The value of $u(tracer)$ is so extremely related to the site specificity, and can be hard to assess. The default value for $u(tracer)$ is set to 0%, but the user can increase it if loss or gain of tracer is considered.

2.4.8 Uncertainty in the flow steadiness $u(steady)$

Ideally, discharge measurements are made while the discharge is steady. If the flow is either increasing or decreasing during the course of making the measurement, then an additional uncertainty occurs. As proposed by [2], the uncertainty due to unsteady flow $u(steady)$ can be estimated from an existing rating curve for the measurement site using a stage measurement at the beginning (h_0) and at the end (h_f) of the measurement and a rectangular probability law as:

$$u(steady) = \frac{a_{steady}}{\sqrt{3}} \text{ with } a_{steady} = \frac{|Q(h_0) - Q(h_f)|}{Q_{measured}} \quad (18)$$

This approach is simplistic, and does not take into account the uncertainty of the discharges estimated from the rating curve. We consider that this additional uncertainty is not of prime importance in the framework developed. By default, $u(steady)$ is set to 0%, but the user can adjust it using equation (18).

2.4.9 Uncertainty in systematic errors $u(s)$

Uncertainty in systematic errors $u(s)$ expresses the residual systematic errors that remain after best calibration of the probes. The only way to assess the value of $u(s)$ would be to compare a perfect dilution measurement to a true value of discharge. Both being impossible, $u(s)$ is set arbitrary to 1.5%, a value consistent with the systematic errors affected to other discharge measurement like ADCP [8].

2.4.10 Uncertainty in the signal to noise of the fluorescence $u(Cd_t - Cd_b)$

The quality of the integration of the tracer wave $\int_{T_b}^{T_e} [Cd(t) - Cd_0] \cdot dt$ depends on the ratio between the additional fluorescence $Cd(t) - Cd_0$ and the noise of the signal. Following [10], this noise is computed as the maximum value between the variability of the fluorescence measurement before and after the tracer wave, i.e. the standard deviation of the fluorescence before and after the tracer wave $s(Cd_b)$, and the resolution of the sensor R associated to a rectangular probability distribution, as:

$$Noise = \max \left[s(Cd_b), \frac{R}{2\sqrt{3}} \right] \quad (19)$$

$s(Cd_b)$ is computed over a period of 20 sampling points before the beginning of the wave, and 20 sampling points after the end of the wave. If the end of the wave is the last recorded point of the measurement, then $s(Cd_b)$ is only computed on the 20 sampling points before the beginning of the wave.

We consider that the noise is not random. For example, noise due to air bubbles passing through the fluorescence probes will create systematic lower values of fluorescence, adding a noise biased lower than the signal. $u(Cd_t - Cd_b)$ is computed for each probe as the area between the tracer wave and the tracer wave plus the *Noise* value computed with equation (19) (as illustrated in figure 2), divided by the area of the tracer wave.

If several probes m are used, a value of $u(Cd_t - Cd_b)$ is computed for each probe. The probes are considered independent, and the final value of $u(Cd_t - Cd_b)$ is the root of the sum of $u^2(Cd_t - Cd_b)$ of each probe.

2.4.11 Uncertainty in the computation of the base fluorescence $u(Cd_b)$

If the base is not stable in time, and if some variations occur during the wave passage, the area under the curve $\int_{T_b}^{T_e} [Cd(t) - Cd_0] \cdot dt$ will be uncertain. The base fluorescence is usually not measured during the wave passage (using an upstream probe, for example), so $u(Cd_b)$ can not be assessed objectively. The default value for $u(Cd_b)$ is set to 0%, but the user can increase it.

2.4.12 Uncertainty in the sampling $u(samp)$

The quality of the computation of the integral of the tracer wave is related to the duration of the wave and the sampling frequency of the probes. For very short tracer wave, enough measurement points are needed to define correctly the shape of the wave and its area. To compute the uncertainty due to the sampling $u(samp)$, we propose to use the Interpolated Variance Estimator (IVE) developed by [1] and [5]. $u(samp)$ is computed for each probe using the relative difference ΔCd between each fluorescence measurement $Cd(t)$ at time t and

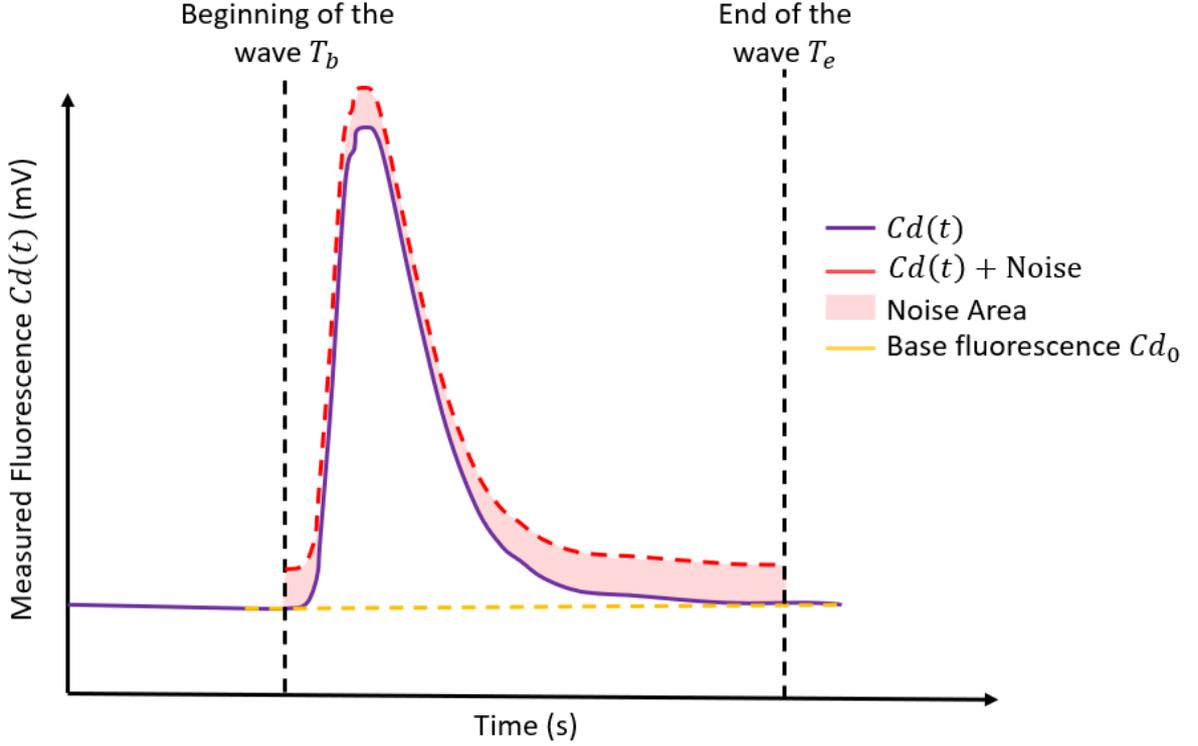


Figure 2: Computation of $u(Cd_t - Cd_b)$ using the relative area between the tracer wave and the tracer wave plus the *Noise* computed with equation (19).

its linear interpolation using the previous and next points ($Cd(t_{-1})$ and $Cd(t_{+1})$ respectively) divided by $Cd(t)$, as:

$$u(samp) = \sqrt{\left(\frac{1}{n-3}\right) \sum_{t=2}^{n-1} \frac{\Delta Cd^2}{2 \cdot (1 - \omega + \omega^2)}} \quad (20)$$

where n is the total number of time step of the measurement, ω is $\frac{t-t_{+1}}{t_{+1}-t_{-1}}$. A value of $u(samp)$ is computed for each probe. The probes are considered independent, and the final value of $u(samp)$ is the root of the sum of $u^2(samp)$ of each probe.

2.4.13 Uncertainty in the setting of the end of the tracer wave $u(limits)$

Considering the usual shape of tracer waves (sharp rising and slow falling), the error in the setting of the beginning of the wave T_{begin} is negligible (it is always obvious to detect the

beginning of the wave). Setting the ending limit T_{end} is more complex, as the fluorescence falls slowly down to the base. The uncertainty due to the setting of the ending of the wave $u(limits)$ is computed as follow:

- The user defines a standard deviation associated to the positioning of the ending of the wave, $sd(end)$. $sd(end)$ is expressed as a percentage of the wave duration. Operationally, we propose that the user choose between 3 ratings describing his ability to locate the end of the wave (good, fair or poor) with associated values of $sd(end)$ of 5%, 10% and 20% of the duration of the wave, respectively.
- From the beginning of the wave, we compute the cumulative area under the curve until the end of the measurement. This cumulative area is divided by the maximum cumulative value, to get the cumulative proportional area $CPA(i)$ for every i sampling points.
- We compute the absolute difference between values of CPA at T_{end} and at $T_1 = T_{end} + (T_{end} - T_{begin}) * sd(end)$ and $T_2 = T_{end} - (T_{end} - T_{begin}) * sd(end)$.
- $u(limits)$ is expressed as the maximum of the difference between the CPA values, associated to a Gaussiann probability law :

$$u(limits) = \frac{\max [|CPA(T_{end}) - CPA(T_1)|, |CPA(T_{end}) - CPA(T_2)|]}{\sqrt{2}} \quad (21)$$

- If T_{end} is located at the last recorded point, then $T_2 = T_{end} - (T_{end} - T_{begin}) * 2 * sd(end)$ and equation (22) is expressed as:

$$u(limits) = \frac{\max [|CPA(T_{end}) - CPA(T_2)|]}{\sqrt{2}} \quad (22)$$

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