Discharge measurement using the fluorescent dye dilution method with the FL30 field fluorometer

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1 Introduction

This manual has been assembled to facilitate discharge calibration measurements using the fluorescent dye dilution method using the FL30 field fluorometer. This is a first draft towards the development of a comprehensive instructional manual. It is our hope that the manual will continue to be improved. Please send suggestions to Helen Dahlke at `hdahlke@ucdavis.edu`.

Note that since all routine measurements such as meteorology and hydrology are carried out using standard time, it is important to make sure you are aware of the daylight savings time. This is particularly important when comparing the stage record with the discharge measurements since an erroneous one hour offset may introduce significant errors in your stage-discharge relationship.

2 Basic theory of the fluorescent dye dilution method

Note! This section only provides information on the theoretical basis of the tracer dilution method. None of the equations stated in this section have to be calculated when conducting the discharge measurements using the FL30 field fluorometer.

In the fluorescent dye tracer-dilution method of measuring discharge, a fluorescent tracer solution is injected into the stream to be diluted by the discharge of the stream. Based on measurements of the rate of injection, the concentration of the tracer in the injected solution, and the concentrations of the tracer at a sampling cross-section downstream of the injection site, the stream discharge can be computed.

2.1 Theory of the sudden-injection method

Discharge measurements in any stream are performed to establish a stage-discharge relationship, i.e. the water level measured at any location is related to the volume of water that is passing this cross-section at a certain time. These discharge measurements are performed using the sudden-injection method, where an instantaneous injection of a slug of tracer solution is added to the stream and an accounting of the total mass of tracer at the sampling cross-section is made.

If a slug of tracer solution is instantaneously injected into a stream, sampling of the stream at the downstream sampling cross-section will produce a concentration-time curve similar to that shown in Figure 2. The equation for computing stream discharge, which is based on the principle of the conservation of mass, is

$$Q = \frac{V_I C_I}{\int_0^\infty (C - C_b) dt} \tag{1}$$

where

Q is the discharge of the stream,

V_I is the volume of the tracer solution injected into the stream,

C_I is the concentration of the tracer solution injected into the stream,

C is the measured tracer concentration at a given time at the downstream sampling site,

C_b is the background concentration of the stream, and

t is time.

The term $\int_0^\infty (C - C_b) dt$ is the total area under the concentration-time curve. In practice the term $\int_0^\infty (C - C_b) dt$ can be approximated by the term

$$\sum_{i=1}^{n} (C_i - C_b)(t_{i+1} - t_{i-1})/2, \tag{2}$$

where

i is the sequence number of a sample,

N is the total number of samples, and

t_1 is the time when a sample, C_i, is obtained.

2.2 Factors affecting the accuracy of tracer-dilution methods

Even if it is assumed that measurements of concentrations and of the injection rate are error free, there still remain three factors that affect the accuracy of tracer-dilution methods of measuring discharge. Those factors are stream turbidity, loss of tracer between the injection site and the downstream sampling site, and incomplete mixing throughout the stream cross section before the downstream sampling section is reached.

2.2.1 Turbidity

Turbidity may either increase or decrease the recorded fluorescence depending upon the relative concentrations of tracer and turbidity (Feuerstein and Selleck, 1963). The FL30 is automatically correcting for the turbidity effect if calibrated in the field using water from the stream immediately before conducting the discharge measurement.

2.2.2 Loss of tracer

The estimation of stream discharge using the fluorescent dye tracer dilution method is based on equations of the conservation of mass. Consequently, the accuracy of the computed discharge is adversely influenced if some of the tracer is lost due to sorption or chemical reaction between the tracer and/or the streambed material, suspended sediments, dissolved material in the streamwater, plants, and other organisms. Photochemical decay is also a source of tracer loss which varies with the tracer material used and its residence time in direct sunlight. Loss from that source is usually negligible for Rhodamine WT dye but it is a factor that needs to be considered when using Uranine dye. Its residence time in direct sunlight is limited to only a few hours.

2.2.3 Criteria for satisfactory mixing

Tracer-dilution methods require complete vertical and lateral mixing at the sampling site. Vertical mixing is usually accomplished quite quickly compared to lateral mixing of the tracer. Therefore the distance required for lateral mixing is the primary concern and consideration when conducting experiments. The mixing distance varies depending on the hydraulic characteristics of the stream reach. Ice cover, for example, significantly reduces the mixing capacity of a stream reach. One formula commonly used as a guide for determining the required length, L, between the injection site and sampling section is:

$$L = 0.13C\frac{(0.7C + 6)}{g}\frac{b^2}{h}, \tag{3}$$

where

b is the average width of the wetted cross section,

h is the average depth of flow,

C is the Chezy coefficient for the reach ($15 < C < 50$, and

g is acceleration of gravity.

Equation 3 is intended for use where injection is made at a single point in midstream positions.

Before making a discharge measurement using the tracer-dilution method, the proposed measurement reach should be calibrated to determine the length required for adequate mixing and the possibility of significant tracer loss. This can be done by injecting the dye at various distances upstream from the sampling site. Take adequate samples at the sampling-site to define the entire concentration-time curve and determine the percentage of mixing, P_m, using the following equation:

$$P_m = 100 - \{|X_A - X_m|Q_A + |X_B - X_m|Q_B + |X_C - X_m|Q_C + \ldots\}\frac{50}{X_m Q}, \tag{4}$$

where

X_A,X_B,X_C,... are the areas under the concentration-time curves;

X_m is the mean area under the concentration-time curves;

Q_A,Q_B, Q_C,... are the subsection discharges applicable to the points A, B, C,...;

Q is the total discharge.

2.3 Fluorescent dyes

Fluorescent dye is the most popular type of tracer used in the U.S.A. for measurement of streamflow. The cost of the dye is relatively small because the quantity of dye needed for a discharge measurement is relatively small. In addition, modern fluorometers are capable of detecting dye concentrations of 1 ppb (1 μg/l) or less.

Fluorescence occurs when a substance absorbs light at one wavelength and emits it at another, usually longer, wavelength. Fluorescent dyes used as tracer are strongly fluorescent and typically organic dyes of the rhodamine family. They are commercially available. Fluorescent dyes used for discharge measurements should (1) have a high detectability range, (2) have little effect on flora and fauna, (3) have a low sorption tendency, (4) have a low photochemical decay rate, (5) be soluble and disperse readily in water, (6) be chemically stable, (7) be inexpensive, (8) be easily separated from common background fluorescence, and (9) be easy to handle.

Temperature has a significant effect upon the fluorescence intensity of dyes. Fluorescence decreases with increasing temperature. Thus, it is important to measure the temperature of the sample when measuring the fluorescence. If the same temperature is used for all samples, no temperature corrections will be needed. If temperatures cannot be held constant, temperature corrections, as given in Table 1 for Rhodamine WT dye, should be applied to dial readings or to correct concentrations.

2.3.1 Rhodamine WT

Rhodamine WT stands for rhodamine water tracing dye. Rhodamine WT is a synthetic red to pink colored, water-soluble dye having brilliant fluorescent qualities with molecular formula $C_{29}H_{29}N_2O_5ClNa_2$ and CAS Number: 37299-86-8. It is also known as Acid Red #388. Rhodamine dyes are soluble in water, methanol, and ethanol. The excitation and emission wavelength of Rhodamine WT are 530 and 555 nm. The dye is commercially distributed as a liquid solution of known concentration. For most fluorescent dye field experiments performed in Tarfala a 20% solution of Rhodamine WT (e.g. distributed in a 5L container) is used. Use this 20% solution to:

1. prepare calibration solutions for fluorometers
2. prepare the tracer mass that is injected into the stream

2.3.2 Fluorescein

Fluorescein is a dark orangered powder with molecular formula $C_{20}H_{12}O_5$ that turns fluorescent greenish-yellow when dissolved in an alkaline solution. Uranine is the water soluble form of fluorescein that is used in water tracing applications. Fluorescein is turned into Uranine via a chemical process. Fluorescein has an absorption maximum at 494 nm and emission maximum of 521 nm in water. Fluorescein also has an isosbestic point (i.e. equal absorption for all pH values) at 460 nm. Uranine can be purchased in liquid form typically as 40% solution (e.g. Abbey Color Inc.) and can be detected with the Albilia FL30 field fluorometer.

Table 1: Temperature-correction coefficients for Rhodamine WT dye.

Temperature difference (T_a –T)[a]		Temperature-correction coefficient
°F	°C	
–20	–11.1	1.36
–15	–8.3	1.25
–10	–5.6	1.16
–8	–4.4	1.13
–6	–3.3	1.09
–5	–2.8	1.08
–4	–2.2	1.06
–3	–1.7	1.05
–2	–1.1	1.03
–1	–0.6	1.02
0	0	1.00
1	+0.6	0.99
2	1.1	0.97
3	1.7	0.96
4	2.2	0.94
5	2.8	0.93
6	3.3	0.91
8	4.4	0.89
10	5.6	0.86
15	8.3	0.80
20	11.1	0.74

[a] T_a is the standard cuvette-sample temperature and T is the cuvette-sample temperature at the time the sample was tested in the fluorometer.

3 Preparation of calibration solutions

In order to get optimal results using the FL30 for discharge measurements (i.e. accuracy ∼1%) it is necessary to apply the best calibration scheme. Other factors influencing the uncertainty of a discharge measurement using fluorescent dye is the choice of the UV-light, the use of an acidic pH resistant tracer, and homogeneous mixing in the stream. Calibration solutions can be prepared from commercial liquid or solid (i.e. powder) fluorescent dye products.

When using the FL30 it is recommended to prepare a calibration tracer solution that results in a concentration of 100 ppb (parts per billion) when diluted in 4.95 L of streamwater. The large amount of calibration solution (total of 5 L) is needed so that the entire fluorometer can be submersed into the calibration solution. Thus, plan to have a 10 L bucket handy when conducting the calibration in the field.

3.1 Preparing a calibration standard from powder:

Note! If Uranine and Rhodamine WT dyes are not available in liquid form please follow the steps outlined below.

Proceed in two steps:

1. Dissolve 1 g powder into 1 liter water. This will generate a solution with a concentration of 1000 ppm ($\rightarrow 10^{-3}$) (dilution by 100). This solution is called Stock 1.

2. Take 10 ml of this solution into 1 liter again. This will generate a solution of 10 ppm or 10'000 ppb. Prepare 50 ml of this solution for calibration of the FL30 in the field. A 1 mg-precision scale is required.

3.2 Preparing a calibration standard from liquid dye:

3.2.1 Using 20% commercial Rhodamine WT solution:

Proceed in two steps:

1. Pour 1 ml (= 1000 μl) of the liquid dye into a 1 L bottle and fill it up with water until 1 L is reached. This will generate a solution with a concentration of 1000 ppm ($\rightarrow 10^{-3}$) (dilution by 100). This solution is called Stock 1.

2. Take 10 ml (= 10'000 μl) of Stock 1 into 1 liter again. This will generate a solution of 10 ppm or 10'000 ppb. This solution is called Stock 2. Prepare 50 ml of this solution for calibration of the FL30 in the field.

3.2.2 Using 40% Uranine solution:

Proceed in two steps:

1. Pour 1.25 ml (= 1250 μL) of the liquid dye into a 1 L bottle and fill it up with water until 1 L is reached. This will generate a solution with a concentration of 1000 ppm ($\rightarrow 10^{-3}$) (dilution by 100). This solution is called Stock 1.

2. Take 10 ml (= 10'000 μL) of Stock 1 into 1 liter again. This will generate a solution of 10 ppm or 10'000 ppb. This solution is called Stock 2. Prepare 50 ml of this solution for calibration of the FL30 in the field.

3.3 Unit conversions

The following conversions apply for concentrations:

- 1 g l^{-1} = 1000 ppm
- 1 mg l^{-1} = 1000 μg l^{-1} = 1 ppm = 1000 ppb
- 1 μg l^{-1} = 1 ppb

The following conversions apply for volumes:

- 1 l = 1000 ml
- 1 ml = 0.001 l
- 1 ml = 1000 μl

3.4 Dilution equations

To make dilutions of a liquid solution:

$$\frac{goal\ conc. * goal\ volume}{conc.\ stock} = required\ volume\ of\ stock \tag{5}$$

Example: To make a 1 liter stock with a concentration of 10 ppm from a stock with a concentration of 1000 ppm, the dilution equation looks as followed:

$$\frac{10\ ppm * 1\ l}{1000\ ppm} = 0.01\ l\ or\ 10\ ml\ of\ the\ 1000\ ppm\ stock \tag{6}$$

Note! Use consistently the same units in the equation (e.g. all concentrations in ppm and volumes in l) to estimate accurate dilutions.

3.5 Tracer injection mass

The FL30 has a lower and upper detection limit. In between both limits the concentration range measured by the fluorometer is directly proportional to the concentration of the fluorophore in the sample. The upper limit of linearity for the FL30 is approximately 400 ppb (μg/L). Thus, injection of too little or too much dye during an experiment can result in erroneous measurements. In order to achieve a comfortable peak concentration of 50-70 ppb in the time-concentration curve the following rules of thumb apply:

1. per 1 m^3s^{-1} of discharge 5g of the fluorescent dye powder are needed
2. per 1 m^3s^{-1} of discharge approximately 5–10 ml of 20% Rhodamine WT solution is needed
3. per 1 m^3s^{-1} of discharge 1.5 ml of 40% fluorescein or Uranine solution is needed

Depending on the discharge a volume of up to 150 ml of the 20% Rhodamine WT dye is required to perform a successful discharge measurement (Table 2). Hence, prepare bottles with predefined volumes of undiluted liquid dye (e.g. 20% Rhodamine WT solution) or prepare a high-concentration solution (e.g. 30% solution) by dissolving a specified amount of e.g. rhodamine powder in 1 L of water. For example, in order to make a 30% solution of Uranine dissolve 300 g of fluorescein powder in 1 L of water.

4 Discharge measurements with the FL30

4.1 List of items to bring to the field

- microSD memory card for data logger
- Bottles with tracer to be injected into the stream (e.g. several bottles with various amounts of 20% Rhodamine WT solution)
- Bottles with 50 ml of the calibration solution (Stock 2)
- Sampling bucket to conduct calibration
- 2 radios for communication between injection and measurement point

Table 2: Amount of fluorescent dye needed to reach a peak concentration of 50–70 ppb during various discharge conditions. The amounts are listed for different types and concentrations of the dye tracers.

Discharge $m^3\ s^{-1}$	Tracer mass powder g	Tracer mass 20% solution ml	Tracer mass 40% solution ml
1	5	10	5
2	10	20	10
3	15	20	10
4	20	30	15
5	25	30	15
6	30	40	20
7	35	40	20
8	40	50	25
9	45	50	25
10	50	50	25
15	75	80	40
20	100	120	60

- Hiking pole or long stick to attach tracer bottles to for injection
- Duct tape
- Latex gloves
- Screw driver to open logger box
- Notebook
- Safety equipment such as harness, rope

The following items might need to be replaced or restocked once in a while:

- Replacement 12V battery if needed.
- Paper towels
- Latex gloves
- Silica gel for logger box
- Duct tape (to attach dye bottles to a stick or hiking pole)
- Calibration solution, dye for injection

4.2 In the field

1. Take out the FL30 (incl. cable, logger box and sonde), the bucket and measuring cylinder, 1 bottle of calibration solution and 1 bottle of the 20% dye solution from the transport box. Take the measuring cylinder, bucket, logger box, cable, calibration standard and sampling bucket to the measuring point.

2. Assemble the FL30 by connecting the cable to the sonde and the data logger (grey box). The cable ending with the rubber cuff needs to be connected to the logger box. Red points on the plug and socket indicate how the connection is established. Insert the microSD card into the logger.

Figure 1: Cable connection to the FL30 logger box.

3. Immerse the sonde slowly into the stream using a rope or the sonde cable until the sonde is approximately 30 cm below the water surface or resting on the streambed (in shallow streams only). Secure the sonde's location. Keep the sonde in the water for approximately 5–10 minutes for temperature equalization.

4. Take a 10 l bucket and pour in 50 ml of the standard solution with a concentration of 10'000 ppb (Stock 2).

5. Add water from the stream to reach 5 l exactly using the measuring cylinder. This means you need to add 4950 ml of streamwater. The resulting solution has a final concentration of 100 ppb.

6. Pull out the sonde from the stream, shake of excess water and immerse it into the bucket with the calibration solution.

7. Power on the instrument by connecting the logger to the 12V battery. Make sure to connect the red cable to the positive pole of the battery. Now the logger is booting. Check the battery voltage. If the battery voltage is below 12 V replace the battery. Start the data acquisition. Wiggle the sonde slightly in the bucket to allow flushing of the sonde with calibration solution. Always use a data acquisition rate of 2 sec when conducting the discharge measurements. Ensure that the rotating switches in the logger box show the following number sequence: 002010. If not adjust the switches (SR, ST, 1, 2, 3, 4) accordingly until the number is matched.

8. During the calibration, note the following information in a field notebook:
   - Date and location of conducted experiment
   - Local time when inserting the sonde into the bucket (e.g. from your watch)
   - Logger time when inserting the sonde into the bucket. The logger time typically does not match the real time. During data processing of the logger data the logger time is the only information you have to identify and distinguish your discharge measurement from other measurements that are stored on the memory card. Thus not the logger time!
   - Type and amount of calibration solution (e.g. RWT, 50 ml, 10'000 ppb)
   - Type and amount of injected dye (e.g. RWT, 10 ml, 20% solution)
   - Water temperature (°C) measured by the logger
   - Description of the location and distance of the injection point (e.g. 10 m upstream of flume inlet)
   - Peak concentration measured by the FL30
9. After 1-2 minutes, immerse the sonde back into the stream without interrupting the data acquisition (Do not disconnect the logger from the battery during this step!). Read the measured concentration when the sonde is in the stream and note the value. This is the baseline concentration to which the experiment needs to return after injecting the dye.
10. Contact the person that is injecting the dye further upstream via radio. Inject the dye. Keep an eye on the concentration displayed in the logger window. The concentration should increase and decrease while the tracer is passing the measuring point.
11. Wait until the measured concentration reaches the baseline concentration before stopping the data acquisition. After finishing the experiment disconnect the logger from the battery.
12. Retrieve the sonde from the stream, dry it up, disassemble and put it back into the storage box. Remove the microSD card from the logger and take it back to your computer for analysis. Store the fluorometer and ropes in the travel box. Rinse the calibration bucket several times with streamwater.
13. To protect the cable from moisture, put each cable plug into a bag of silica gel and close the plastic bag tightly around it. Change the desiccant if the silica gel is loosing color.

4.3 Data analysis in FLUO

4.3.1 Installation of FLUO

FLUO is a software that allows calibration of the FL30 fluorometer, read out of the micrSD memory card from the data logger, processing of measured tracer concentrations and set up of the GPRS modem while the data logger is connceted to a computer. Before starting the installation process make sure you have the microSD memory card from the data logger and USB-card reader at hand.

1. From the FLUO installation CD start the `setup.exe` in the Setup_28.0 folder. The installation does not require any user-specific settings or changes. Accept the default settings during the installation process.

2. After the installation locate the installation folder "FLUO" on your local harddisc (e.g. on a Windows computer one can find the program at C:\Program Files\FLUO.

3. Insert the microSD memory card into the USB card reader and copy the "cal.dat" and Calibrat.dat" files from the card into the FLUO folder. Overwrite the existing files. Both files contain the calibration definitions of your instrument.

4. In the FLUO folder, locate the "disc.e" file and open it in a text editor. This file contains information that is needed to perform stream gauging with the FL30. The ".e" suffix indicates that this file's content is written in English. A similar file, "disc.f" exists in French.

5. Look at the first line of the "disc.e" file. By default this line looks as followed:
   `0 <- 0:simple screen 1:gauging screen.`

   0 indicates that when starting the program FLUO the user is only presented with a simple data view, when using the "Process mV" menu.

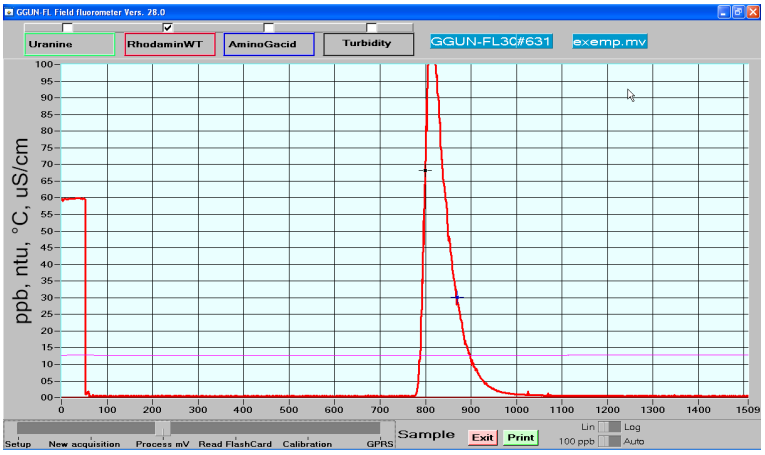


Figure 2: The "Process mV" menu when running the FLUO software in the simple mode.

   1 indicates that the user is presented with a more complex user input in the "Process mV" menu, to estimate discharge volumes based on measured tracer concentrations.

   Change the first number to 1 to use FLUO for stream gauging. Save and close the file.

6. Depending on the tracer used in the experiment the tracer type and "*volumetric mass*" of the tracer in the "disc.e" file needs to be changed. In case the experiment was performed using a tracer made from powder, the "*volumetric mass*" in the disc.e file has to be set to 1.0. If Rhodamine WT 20% solution is used, set the "*volumetric mass*" to 1.123.

7. The FLUO program is now ready to use. After starting the program go to the "Process mV" menu. If the program still shows the "simple screen" check whether you have writing permission on the FLUO folder and whether the value in the first line of the "disc.e" file is set to 1.

Note! Installation of FLUO on a Windows 7 or Windows 8 64-bit machine is requiring administrator permission in order to be able to write files on the c-drive and to start the program. On a

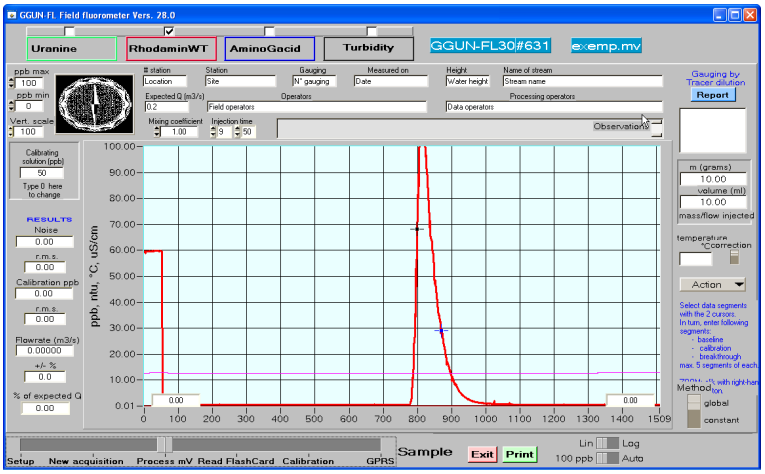


Figure 3: The "Process mV" menu when running the FLUO software in stream gauging mode.

Windows 7 or 8 computer the FLUO prgram should always be started as administrator (not as a normal user), which can be accomplished by right-clicking on the fluo.exe and selecting "run as administrator)".

4.3.2 Calculate discharge using FLUO

In order to calculate discharge from field sampled tracer data start the FLUO software. After start-up the user is in the "Setup" menu of the program depicted in Figure 4.

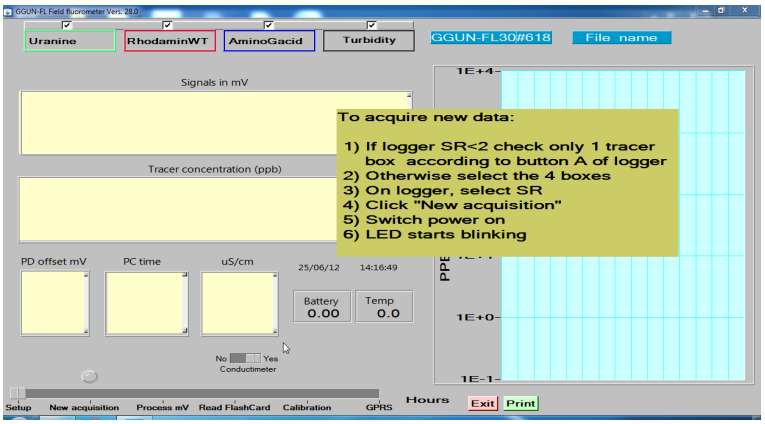


Figure 4: The start-up screen of the FLUO program.

To process the data follow these steps:

1. Insert the microSD memory card into the USB card reader and the computer.
2. Click the menu "Read Flashcard" at the bottom of the program. A new program window opens. Click "Extract last data from card". Locate the USB-drive with the microSD card

on your computer and point to the file F0xz.txt. (*xz* are the last two digits of the serial number of the fluorometer: e.g. 631, thus, the file name is F031.txt). The serial number of the fluorometer is printed on the data logger. If the file size of the F031.txt becomes too big you can open the file in a text editor and delete old data (e.g. several lines of logged data).

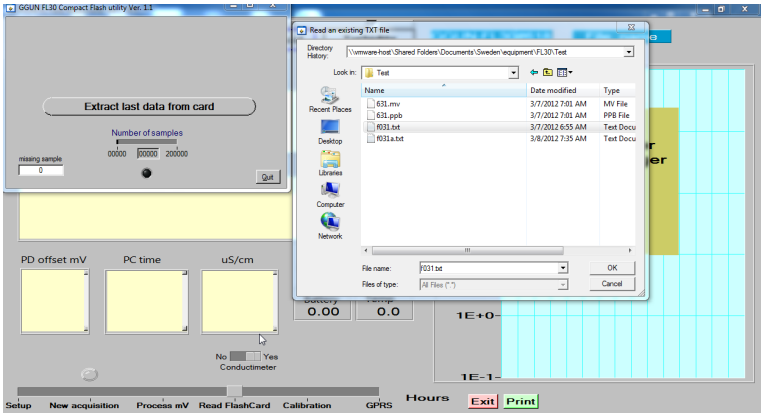


Figure 5: The "Read Flashcard" menu of the FLUO software with the input file selection window.

3. After the input F0xz.txt file is chosen specify a file name for saving data in the working folder. Make sure you are in the FLUO folder when creating this file. Quit the window.

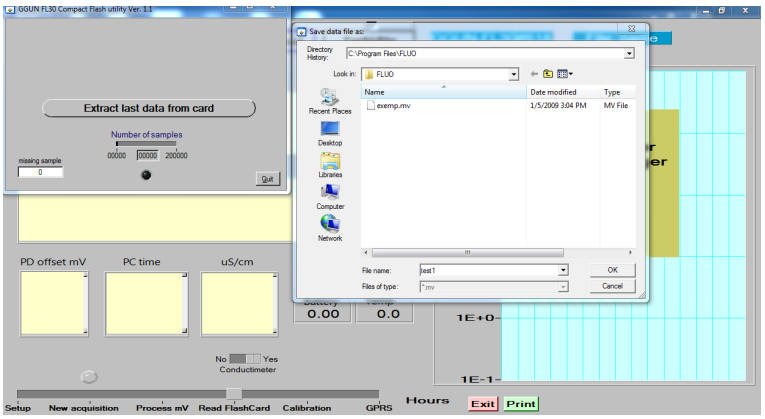


Figure 6: The "Read Flashcard" menu of the FLUO software with the "Save file as..." window.

4. Click the menu "Process mV" and read in the just created *.mv file. The FLUO software should show a window similar to the one shown in Figure 3.

   After this step the time-concentration curves appear. The FL30 can be calibrated to detect different tracers. The most common ones for discharge measurements are Uranine (equivalent to Fluorescein), Rhodamine. In addition, the FL30 measures can measure a third tracer as well as turbidity, which is used to correct the measured tracer concentrations. More information on the selection of tracers for which the FL30 can be calibrated can be

found at http://www.albillia.com/Tracers.html. The tracers are shown as green, red and blue time-concentration curves in the FLUO software, respectively. Turbidity is shown as a black line. Visibility of each tracer in the time-concentration curve can be switched on or off by checking boxes at the top of the "Process mV" window.

5. To estimate the discharge of one breakthrough curve, first enter the concentration of the "calibrating solution" (in ppb) in the window on the left of the time-concentration curve (Figure 7). This is needed to specify the concentration that was used to calibrate the FL30 in the bucket in the field. To change the value enter 0 in the field and press ENTER. A second window appears that shows four fields in which the user enters the stock volume used in each dilution step (dilution 1–4) and the final concentration of the "calibrating solution (ppb)" at the bottom. The final concentration of the calibrating solution is obtained by entering the stock volume in microliters (μ l) diluted in 1 liter during every step performed starting from Stock 1.

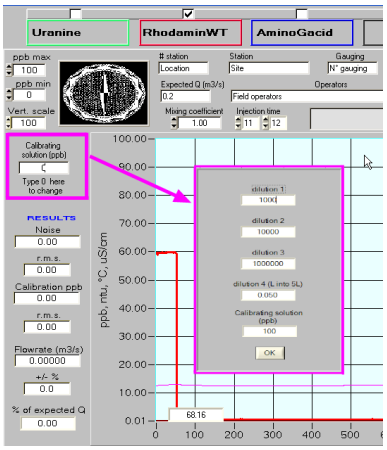


Figure 7: The "calibrating solution" settings in the FLUO software.

Example: If you followed the instructions in section 4.2 resulting in a 100 ppb calibration of the fluorometer in the field the following values should be entered:
dilution 1: 1'000 (equal to 1 ml = 1000 μL of Stock 1).
dilution 2: 10'000 (equal to 10 ml =10'000 μL of Stock 2).
dilution 3: 1'000'000 (this value is give and does not change).
dilution 4; 0.05 (volume in liter of Stock 2 that was diluted in 5 L of water in the field).
The final concentration of the "Calibrating solution (ppb)" should be 100 ppb. Press OK to close the window.

6. Enter the mass (g) or volume (ml) of injected tracer (e.g. of 20% Rhodamine WT solution) in the upper right corner of the window (magenta highlighted window in Figure 8). This should be for example 10 if 10 ml of 20% Rhodamine WT dye were injected. FLUO will automatically calculate the mass in grams after entering the value for the injected volume.

7. Start the breakthrough curve analysis. Figure 9 shows the time-concentration curve when loading the "exemp.mv" file. The measured Rhodamine WT concentration (time on the x-axis, concentration on the y-axis) shows a plateau at approximately 60 ppb at the beginning

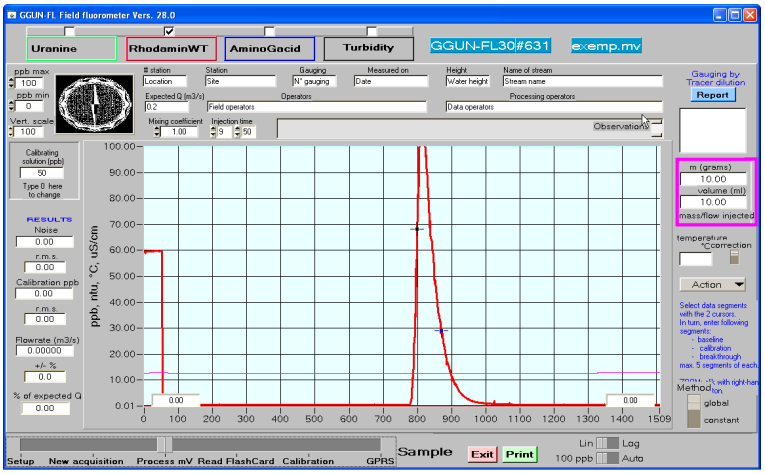


Figure 8: The "mass/flow injected" field in the FLUO software. Enter here the tracer mass (e.g. 10 ml of 20% Rhodamine WT solution) that was injected into the stream.

of the experiment and a typical breakthrough curve between 750 and 1100 seconds after measurement start.

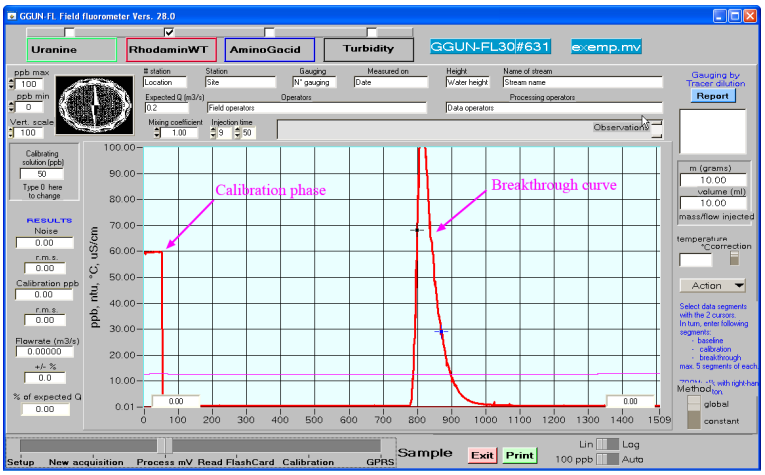
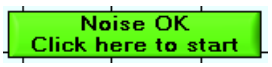


Figure 9: A typical time-concentration curve recorded with the FL30 showing the calibration plateau and the breakthrough curve after injecting the tracer upstream of the measurement point.

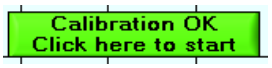
8. Locate the black and blue markers in the graph window. Select a segment of low concentrations (preferably between the calibration plateau and the breakthrough curve) in the graph to define the background signal or "Noise" in the measurement. This defines the natural concentration of a tracer dye in the stream before the tracer mass was injected.

9. Click on the pull-down menu on the right side of the screen and click "Action". Now a temperature (in °C) appears in the window above the pull-down menu, which shows the

water temperature measured with the FL30 during the selected period of the tracer test. This value is used for temperature correction of the measured tracer concentrations. Click again on the pull-down menu and click "Noise". Now a green window "Noise OK" appears on the screen. Click on this window to finish the baseline definition.



10. Move the black and blue markers to select a segment from the calibration plateau of the time-concentration curve. Right click on the area between the blue and black marker and you will zoom into the graph. Now a more precise placement of the markers can be performed. Note, if the measured concentration in the calibration phase varies a lot, try to select a segment with very little change (i.e. horizontal line). This makes the discharge calculations more accurate. Click on the pull-down menu and select "Calibration". A green "Calibration OK" window appears. Click on this window to finish the calibration definition.



11. Move the black and blue markers to select the breakthrough curve (one marker before and one after the curve). Right click on the area between the blue and black marker to zoom into the graph for a more precise placement of the markers. Click on the pull-down menu and select "Flowrate". Click OK on each of the appearing windows. Now, the estimated flowrate appears in the lower left side of the screen (in $m^3\ s^{-1}$). This is the final discharge value and should be noted for comparison with stage records.

Note! You can analyze several breakthrough curves with the same calibration settings. To do so, just move the black and blue markers to select a new breakthrough curve (one marker before and one after the curve). Right click on the area between the blue and black marker to zoom into the graph for a more precise placement of the markers. Click on the pull-down menu and select "Flowrate". Click OK on each of the appearing windows. Now, the newly estimated flowrate appears in the lower left side of the screen (in $m^3\ s^{-1}$). For each breakthrough curve analyzed a new value appears in a window on the upper right screen (under the blue report button). If five breakthrough curves have been analyzed the program has to be reset by clicking on the pull-down menu and selecting "Reset". This will clear the analyzed discharge values out of the memory but not reset the calibration settings. The user will have to restart the program if the general settings such as calibration concentration and baseline concentration need to be changed.