



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L203

Trilogy Laboratory Fluorometer

Standard Operating Procedure

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Author(s)	Marc Los Huertos
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Organization	EA Program, Pomona College

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1. Purpose

This procedure describes the operation, calibration, and maintenance of the Turner Designs Trilogy Laboratory Fluorometer, a compact, multifunctional instrument used for fluorescence, absorbance, and turbidity measurements with interchangeable snap-in optical modules. The Trilogy is the primary instrument in the EA Program laboratory for chlorophyll *a* determination and turbidity analysis of water samples (Arar & Collins, 1997; Turner Designs, 2014).

2. Scope

- **Applies to:** Turner Designs Trilogy Laboratory Fluorometer equipped with fluorescence, absorbance, and turbidity optical modules
- **Does not apply to:** Field fluorometers, in-vivo chlorophyll measurements with multiprobes (see [SOP-F200](#)), or benchtop spectrophotometers (Hach DR3900; see [SOP-L303](#))
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure calibration standards are current
Lab Manager	Maintain optical modules, replace light sources as needed
Trained Personnel	Follow this SOP, report malfunctions
New Students	Complete training before independent use

4. Definitions

- **Blank correction:** Subtraction of the fluorescence signal from a blank (solvent only, no sample) from sample readings to remove background fluorescence from the solvent and optical components.
- **Chlorophyll *a*:** The primary photosynthetic pigment in phytoplankton and algae; quantified by the Trilogy using a chlorophyll *a* module (excitation ~430 nm, emission ~685 nm); expressed in $\mu\text{g L}^{-1}$ (water) or $\mu\text{g g}^{-1}$ (sediment) (Arar & Collins, 1997).

- **Emission wavelength:** The wavelength at which the fluorophore emits light after excitation; detected by the fluorometer's photodetector.
- **Excitation wavelength:** The wavelength of light used to illuminate the sample; chosen to match the absorption maximum of the target fluorophore.
- **Fluorometer:** An instrument that measures fluorescence intensity emitted by a sample after excitation; more sensitive than spectrophotometry for low-concentration analytes such as chlorophyll *a* and CDOM (Turner Designs, 2014).
- **Linear dynamic range (LDR):** The concentration range over which instrument response is linear; for the Trilogy chlorophyll module, approximately 0–1000 µg/L (Turner Designs, 2014).
- **Module (Trilogy):** An interchangeable optical block that sets the excitation and emission wavelengths for a specific analyte; available modules include chlorophyll *a* (acidification and non-acidification), CDOM, rhodamine WT, and turbidity.
- **Pheophytin *a*:** A degradation product of chlorophyll *a* formed by loss of the central magnesium ion; contributes to total fluorescence and is distinguished from chlorophyll *a* by the acidification step of EPA Method 445.0 (Arar & Collins, 1997).
- **Quinine sulfate unit (QSU):** A fluorescence unit based on the fluorescence of a standard quinine sulfate solution; used for CDOM and some other modules.

5. Safety and Hazards



Hazards

- **Chemical:** Chlorophyll extraction uses 90% acetone, which is flammable and an irritant. Handle in a fume hood. Acidification with 0.1 N HCl is required for EPA 445.0. See [SOP-L003](#).
- **Physical:** Glass test tubes used for extracted chlorophyll measurements can break. Handle with care.

5.1. Required PPE

- ☐ Safety glasses
- ☐ Lab coat
- ☐ Nitrile gloves (especially when handling acetone or standards)

5.2. Emergency Procedures

For acetone spills, extinguish all ignition sources, ventilate the area, and clean up per [SOP-L003 Hazardous Materials Handling](#). For skin or eye contact with acetone or acid, flush with water for at least 15 minutes.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Turner Designs Trilogy Fluorometer	With color touch screen (Turner Designs, 2014)
Fluorescence optical module(s)	Application-specific (e.g., chlorophyll <i>a</i> , rhodamine WT)
Absorbance optical module	With interchangeable filter paddles (560/10, 600/10, 750/10 nm)
Turbidity optical module	Infrared LED at 850 nm (ISO 7027)
Solid secondary standard	For verifying instrument stability
Printer or PC connection	For data export (USB or serial)

6.2. Consumables

Item	Notes
Methacrylate cuvettes (10 × 10 mm)	Minimum 2 mL; for ammonium and general fluorescence
Polystyrene cuvettes (10 × 10 mm)	For turbidity measurements
Glass cuvettes (10 × 10 mm)	Minimum 1.5 mL; for absorbance
Glass test tubes (12 × 35 mm)	For extracted chlorophyll measurements

6.3. Reagents and Standards

Item	Notes
Chlorophyll <i>a</i> standard	Pure chlorophyll <i>a</i> in 90% acetone; Turner Designs or equivalent
90% acetone (reagent grade)	For chlorophyll extraction
0.1 N HCl	For acidification step (EPA 445.0) (Arar & Collins, 1997)
Formazine turbidity standards	4000 NTU primary standard; valid 4 weeks after preparation
Ultrapure water	For blanks

7. Procedure

7.1. Startup

1. Turn on the Trilogy using the power switch on the back of the instrument.

2. Allow the instrument to warm up for approximately 10 minutes before taking measurements (Turner Designs, 2014).
3. Connect the Trilogy to a printer or PC if data export is needed.

7.2. Installing an Optical Module

1. Select the appropriate optical module for the analysis:
 - **Fluorescence module** for chlorophyll *a*, rhodamine WT, or other fluorescent analytes
 - **Absorbance module** for colorimetric assays
 - **Turbidity module** for nephelometric turbidity measurements
2. Open the sample compartment lid.
3. Align the module with the slot and press firmly until it clicks into place.
4. The Trilogy will automatically detect the installed module and display the corresponding menu on the touch screen.
5. If using the absorbance module, install the appropriate filter paddle for the target wavelength.

7.3. Selecting the Measurement Mode

Mode	When to Use
Raw mode	Qualitative measurements; no calibration required; results in RFU, raw absorbance, or NTU
Direct Concentration mode	Quantitative measurements in concentration units; calibration required

7.4. Fluorescence Module: Chlorophyll *a* (EPA Method 445.0)

EPA Method 445.0 is the standard fluorometric method for measuring extracted chlorophyll *a* and pheophytin *a* in marine and freshwater algae (Arar & Collins, 1997).

1. Calibrate the fluorescence module in Direct Concentration mode using a known concentration of pure chlorophyll *a* in 90% acetone (see §7.3).
2. Insert the glass test tube containing the extracted sample.
3. Touch **Measure Fluorescence** to obtain the pre-acidification reading.
4. Add the prescribed volume of 0.1 N HCl, mix gently, and wait 90 seconds.
5. Measure again to obtain the post-acidification reading.
6. Calculate chlorophyll *a* and pheophytin *a* per EPA 445.0 (Arar & Collins, 1997).

Standard Stability

Recalibrate with a fresh chlorophyll *a* standard every few months. The standard degrades over time, especially if exposed to light or warmth (Turner Designs, 2014).

7.5. Single-Sample Measurements

1. Open the lid and insert the appropriate cuvette or test tube.
2. Close the lid.
3. Optionally, touch **Sample ID** to name the sample; touch **Save**.
4. Touch the **Measure** button corresponding to the installed module.
5. The Trilogy measures the sample for 6 seconds and reports the average reading.
6. Results for the most recent 20 measurements are displayed on the home screen.

Important

The Trilogy stores a maximum of 20 measurements at a time. Older readings are overwritten. Measurements are not retained between power cycles. Always export data to a printer or PC before turning off the instrument or exceeding 20 readings.

7.6. Shutdown

1. Export all data to a printer or PC.
2. Remove the cuvette or test tube from the sample compartment.
3. Clean the cuvette holder if any liquid was spilled (wipe with 70% ethanol and a lint-free tissue).
4. Close the sample compartment lid.
5. Turn off the instrument using the rear power switch.

8. Quality Control and Acceptance Criteria

8.1. When to Calibrate

1. Before running a new batch of samples
2. After a temperature change of ± 5 °C in the lab
3. After changing to a different optical module or analyte
4. When verification readings deviate unacceptably from the expected value

8.2. Calibration Overview

Calibration converts the raw instrument signal to concentration units. The Trilogy supports single-point and multi-point calibrations (up to five standards plus a blank). Multi-point calibrations provide superior accuracy across a wide concentration range (Turner Designs, 2014).

8.3. Direct Concentration Calibration Procedure

1. Turn on the Trilogy and confirm the correct module is installed.
2. Touch **Calibrate** on the home screen.
3. Select **Run New Calibration**.

4. Select the desired unit of measurement.
5. Insert the blank (ultrapure water or 90% acetone, as appropriate) and touch **OK**.
6. Enter the concentration of the first standard. For multi-point calibrations, begin with the lowest concentration.
7. Insert the standard and touch **OK**.
8. After the reading, either select **Proceed with Current Calibration** or **Enter More Standards**.
9. Optionally, save the calibration for future use.
10. Verify calibration by measuring the same standard used in step 6; the displayed concentration should match the known value within the acceptable tolerance (Turner Designs, 2014).

8.4. Verification

- After calibration, re-read a mid-range standard to confirm accuracy.
- Re-read the standard every few hours during extended sessions to detect drift.
- Optionally, read the solid secondary standard and record its value for instrument stability tracking.
- If verification readings drift beyond acceptable limits, recalibrate before continuing.

8.5. QC Acceptance Criteria

- Laboratory reagent blank (LRB) every 10–15 samples; must read below the lower detection limit.
- QC standards must recover within $\pm 20\%$ of the known value (Arar & Collins, 1997).
- Relative standard deviation (RSD) of replicate measurements must be within $\pm 20\%$.
- Field duplicates must agree within $\pm 20\%$ RPD.
- Samples, standards, LRBs, and QCs must all be at ambient room temperature; fluorescence is temperature-dependent (APHA et al., 2012).

9. Waste Disposal

Waste Type	Disposal Method
Acetone extracts	Collect in organic waste carboy in fume hood; EH&S pickup
Acidified samples (HCl)	Neutralize and dispose per SOP-L003
Used cuvettes (non-hazardous)	Standard trash (polystyrene, methacrylate) or recycle (glass)
Formazine standards	Collect in aqueous waste; EH&S pickup

10. References

- Turner Designs (2014)
- Arar & Collins (1997)
- APHA et al. (2012)

- APHA, AWWA, & WEF. (2012). *Standard methods for the examination of water and wastewater* (22nd ed., p. 1360). American Public Health Association.
- Arar, E. J., & Collins, G. B. (1997). *Method 445.0: In vitro determination of chlorophyll *textita* and pheophytin *textita* in marine and freshwater algae by fluorescence*. U.S. Environmental Protection Agency, Office of Research; Development, National Exposure Research Laboratory. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=309417
- Turner Designs. (2014). *Trilogy laboratory fluorometer: User's manual*. <https://www.turnerdesigns.com/trilogy-laboratory-fluorometer>

11. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required safety training
L003	Hazardous Materials	Acetone and HCl handling
L310	Chlorophyll Extraction	Primary application of this instrument

12. Training Record

Before operating the Trilogy Fluorometer independently, personnel must:

1. Read this SOP in full
2. Complete [SOP-L002 Laboratory Safety](#) training
3. Review the Turner Designs Trilogy User's Manual (Turner Designs, 2014)
4. Observe a trained operator perform module installation, calibration, and sample measurement
5. Perform a calibration and measure a set of standards with acceptable results under supervision
6. Be signed off by the PI or lab manager

13. Appendix A: Troubleshooting – Fluorescence

Symptom	Probable Cause	Corrective Action
“Bad Calibration” error	Blank brighter than standard	Compare blank and standard in Raw mode; ensure standard is more concentrated than blank
Erratic readings	Incorrect standard value entered	Review the standard value entered; must match actual concentration
Negative values	Sample near zero	Normal if near blank; blank value is automatically subtracted

Symptom	Probable Cause	Corrective Action
Low readings	Wrong optical module	Verify that module excitation/emission wavelengths match the analyte
High background signal	Contaminated cuvette holder	Clean holder with 70% ethanol and a lint-free tissue

14. Appendix B: Troubleshooting – Turbidity

Symptom	Probable Cause	Corrective Action
Readings disagree with other meters	Different light sources (visible vs IR)	Calibrate with the same standard; color interference affects visible-light meters but not the Trilogy
Readings vary between replicates	Particle movement in sample	Normal; particles shift and scatter light differently each time
Readings change after recalibration	Formazine standard has aged	Use fresh formazine (valid for 4 weeks per ISO 7027)