



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L308

Microplastics in Water – Extraction and Enumeration

Standard Operating Procedure

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1. Purpose

This SOP provides methods for extracting, staining, and enumerating microplastics (particles less than 5 mm) from water samples. Microplastics are stained with Nile Red dye, vacuum-filtered onto glass-fiber filters, and counted under fluorescence using either a Crime-Lite forensic light source with goggles or the Echo Revolve microscope (Erni-Cassola et al., 2017; Maes et al., 2017).

This SOP is intended as an internal teaching and research protocol. Where EA Program data will be submitted to external programs or compared with regulatory monitoring datasets, researchers should consult the emerging standardized methods described in Appendix B and adapt procedures accordingly (California State Water Resources Control Board, 2022; Thornton Hampton et al., 2025).

2. Scope

- **Applies to:** Extraction and enumeration of microplastics from drinking water, surface water, stormwater, and other aqueous samples
- **Does not apply to:** Microplastics in soil, sediment, or biological tissue (see SCCWRP Technical Report #1410.A (Thornton Hampton et al., 2025)); chemical identification of polymer type (requires FTIR or Raman spectroscopy)
- **Regulatory alignment:** This SOP provides a Nile Red fluorescence screening method suitable for research and teaching. It does not replace the FTIR- or Raman-based methods required for California regulatory drinking water monitoring (California State Water Resources Control Board, 2022). Consult Appendix B and the PI before submitting data to SWAMP, CEDEN, or other regulatory programs.
- **Location:** EA Program laboratory, SGM 133 and SGM 134

3. Acknowledgements

This document builds on the work of Kyle Wees (PO '20), Baile (PO '22), and Jon Gunasti (PO '20). Clare Flynn developed the first SOP, further modified by Marc Los Huertos and Sidarth (PO '23).

4. Responsibilities

Role	Responsibility
Lab PI	Approve methods, review QC data
Trained Personnel	Prepare samples, perform extraction and enumeration
New Students	Complete training before independent use

5. Definitions

- **FTIR (Fourier Transform Infrared Spectroscopy):** An analytical technique that identifies polymer type by comparing a sample's infrared absorption spectrum to reference spectra; the gold standard for confirmatory microplastic characterization (California State Water Resources Control Board, 2022).
- **Microplastic (MP):** A synthetic polymer particle or fiber with at least one dimension between 1 μm and 5 mm; includes fragments, fibers, films, beads, and foam; classified as primary (manufactured at micro-scale) or secondary (degradation products of larger plastic items) (Maes et al., 2017).
- **Nanoplastic:** A plastic particle smaller than 1 μm ; not quantifiable by standard visual methods; an emerging concern for ecological and human health research.
- **Nile Red:** A fluorescent dye that selectively stains hydrophobic plastic surfaces, enabling detection under blue-light excitation; used as a screening tool to distinguish plastic particles from organic debris (Erni-Cassola et al., 2017; Maes et al., 2017).
- **Procedural blank:** A sample of laboratory-grade water carried through the complete analytical procedure alongside field samples; used to quantify airborne and procedural contamination; required for every analytical batch.
- **Raman spectroscopy:** A spectroscopic technique that identifies molecular composition from inelastic light scattering; complementary to FTIR; particularly useful for particles smaller than 10 μm (California State Water Resources Control Board, 2022).
- **Visual sorting:** The initial enumeration step in which particles on a filter are examined under a stereomicroscope or the Revolve fluorescence microscope and candidate plastic particles are identified by color, shape, and texture before any confirmatory chemical analysis.

6. Safety and Hazards

Hazards

- **Chemical:** Acetone is flammable (flash point $-20\text{ }^{\circ}\text{C}$) and potentially toxic by inhalation. All Nile Red solution preparation and sample staining must be performed in a fume hood. Review the SDS for acetone before use.
- **Optical:** The Crime-Lite 2BG2735 is a high-intensity 450–510 nm light source. Never shine it into anyone's eyes. Always wear the orange filter goggles when the Crime-Lite is on.

6.1. Required PPE

- ☐ Safety glasses
- ☐ Laboratory coat (cotton preferred – minimize synthetic fibers)
- ☐ Nitrile gloves
- ☐ Orange filter goggles (Crime-Lite enumeration only)

6.2. 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, flush with water for at least 15 minutes.

7. Required Materials

7.1. Equipment

Item	Location	Notes
Vacuum filtration apparatus	SGM 134	Glass funnel and fritted base
Vacuum pump	SGM 134	
Drying oven	SGM 134	Set to 60 °C for filter drying
Crime-Lite 2BG2735 (450–510 nm)	SGM 133	Store power supply and goggles together
Orange filter goggles	SGM 133	Must be used with Crime-Lite
Echo Labs Revolve microscope	SGM 133	See SOP-L211
Dissecting scope with 550 nm filter	SGM 133	Alternative to Revolve
Watch glasses	SGM 134	For filter drying in oven
Fume hood	SGM 134	Required for all Nile Red/acetone work

7.2. Reagents and Consumables

Item	Notes
Nile Red	<i>TBD catalog number</i>
Acetone (ACS grade)	Stored in flammables cabinet
Whatman 934-AH glass-fiber filters	42.5 mm, 1.5 µm pore
Glass sample bottles (500 mL)	Pre-cleaned; avoid plastic
Glass pipettes or syringes	For Nile Red injection

Glassware Decontamination

A standard protocol for removing residual micro- and nanoplastics from glassware before use has not yet been finalized. This is a critical gap that must be addressed before results can be

considered quantitative (see Appendix A).

8. Reagent Preparation

8.1. Nile Red Stock Solution

1. Working in a fume hood, dissolve Nile Red in acetone to 1 mg/mL.
2. Store in a dark amber glass bottle at room temperature.
3. Label with concentration, date, and analyst.

8.2. Working Concentration

The working concentration is 10 µg/mL, achieved by a 100:1 dilution of the stock. For a 500 mL water sample, add 0.5 mL of the 1 mg/mL stock solution (Maes et al., 2017).

9. Estimated Time

Task	Time
Nile Red staining and incubation	30 minutes
Vacuum filtration (per sample)	10–15 minutes
Filter drying (oven at 60 °C)	10 minutes
Enumeration (per sample, 3–10 fields)	30–60 minutes
Total per sample (excluding wait times)	Approximately 90–150 minutes

10. Procedure

10.1. Sample Preparation and Staining

1. Minimize synthetic fiber contamination: wear a cotton lab coat, keep samples covered, and work in a clean area.
2. Prepare samples in 500 mL volumes in glass bottles.
3. Working in a fume hood, inject the appropriate volume of Nile Red stock solution (e.g., 0.5 mL for a 500 mL sample) to achieve 10 µg/mL. Cap immediately (Maes et al., 2017).
4. Incubate with the injected dye for at least 30 minutes at room temperature.

10.2. Vacuum Filtration

5. Place a Whatman 934-AH glass-fiber filter (42.5 mm, 1.5 µm pore) on the fritted base of the vacuum filtration apparatus.
6. Vacuum-filter each sample through the filter.
7. Dry the filter on a watch glass in the oven at 60 °C for 10 minutes.

10.3. Enumeration Option A: Crime-Lite with Goggles

Laser Safety

Do not shine the Crime-Lite into anyone's eyes. Never observe illuminated samples without the orange filter goggles.

8. Place the dried filter on a dark, non-reflective surface.
9. Put on the orange filter goggles.
10. Illuminate the filter with the Crime-Lite (450–510 nm).
11. Count fluorescing particles through the goggles, or mount the filter under the dissecting scope with a 550 nm emission filter.
12. Record particle counts and morphology (fiber, fragment, bead, film).

10.4. Enumeration Option B: Echo Revolve Microscope (Fluorescence)

13. Mount the dried filter on a glass slide per [SOP-L211](#).
14. Use the fluorescence channel (excitation ~470 nm, emission ~550 nm long-pass).
15. Photograph and count fluorescent particles in at least 3 randomly selected fields of view.
16. Import images into the Revolve app; use the annotation tools to mark and count particles.

11. Quality Control

- Include at least one procedural blank per analytical batch; blank particle counts inform the detection threshold for field samples (Erni-Cassola et al., 2017; Maes et al., 2017).
- Analyze at least 3 (preferably 10) randomly selected fields of view per filter; report mean, standard deviation, and coefficient of variation.
- Include at least one spiked filter (known number of plastic particles added before staining) per batch to verify Nile Red staining performance.

Contamination Controls Are Essential

Every batch must include procedural blanks processed identically to samples. Without blanks, microplastic counts cannot be meaningfully interpreted (Maes et al., 2017).

12. Troubleshooting

Problem	Probable Cause	Corrective Action
High blank counts	Airborne contamination; dirty glassware	Improve lab cleanliness; decontaminate glassware
No fluorescence on spiked samples	Nile Red concentration too low; degraded dye	Verify stock; prepare fresh solution

Problem	Probable Cause	Corrective Action
Fluorescence fades during counting	Photobleaching	Minimize illumination time; work quickly
Organic matter fluoresces	Nile Red binding to natural organics	Consider H ₂ O ₂ digestion pre-treatment

13. Waste Disposal

Waste Type	Disposal Method
Acetone waste (Nile Red solution)	Collect in halogenated/organic waste; EH&S pickup
Used glass-fiber filters	Standard trash
Sample water (post-filtration)	Drain to sink

14. References

- Maes et al. (2017)
- Erni-Cassola et al. (2017)
- California State Water Resources Control Board (2022)
- Thornton Hampton et al. (2025)
- 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.

California State Water Resources Control Board. (2022). *Policy handbook establishing a standard method of testing and reporting of microplastics in drinking water*. California State Water Resources Control Board. https://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/microplastics.html

Erni-Cassola, G., Gibson, M. I., Thompson, R. C., & Christie-Oleza, J. A. (2017). Lost, but found with Nile Red: A novel method for detecting and quantifying small microplastics and nanoplastics in environmental samples. *Environmental Science & Technology*, 51(23), 13641–13648. <https://doi.org/10.1021/acs.est.7b04512>

Maes, T., Jessop, R., Wellner, N., Haupt, K., & Mayes, A. G. (2017). A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. *Scientific Reports*, 7, 44501. <https://doi.org/10.1038/srep44501>

Thornton Hampton, L. M., Mehinto, A. C., & Weisberg, S. B. (2025). *Standard operating procedures for the collection of samples for microplastics analysis part 1: Surface sediment and aquatic biota* (Technical Report #1410.A). Southern California Coastal Water Research Project (SCCWRP). <https://www.sccwrp.org/>

15. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required safety training
L003	Hazardous Materials	Acetone waste disposal
L211	Revolve Microscope	Fluorescence enumeration
L114	Vacuum Filtration	Filtration system operation

16. Training Record

Before performing microplastics analysis independently, personnel must:

1. Read this SOP in full
2. Complete [SOP-L002](#) and [SOP-L003](#) training
3. Review the SDS for acetone and Nile Red
4. Complete [SOP-L211 Revolve Microscope](#) training (if using the Revolve for enumeration)
5. Demonstrate safe Crime-Lite operation under supervision
6. Process at least one procedural blank and one spiked sample under supervision with acceptable results
7. Be signed off by the PI or lab manager

17. Appendix A: Open Questions and Development Needs

Item	Status	Notes
Glassware decontamination protocol	<i>TBD</i>	Critical for quantitative results
Organic matter interference assessment	<i>TBD</i>	Needed for high-organic samples
Polymer identity confirmation (FTIR access)	<i>TBD</i>	Lab does not currently have FTIR
Microplastic size class definition	<i>TBD</i>	Original SOP noted “DEFINE SIZE”
Nile Red and acetone catalog numbers	<i>TBD</i>	Add to materials table
Hydrogen peroxide digestion protocol	<i>TBD</i>	For removing organic matter before staining

18. Appendix B: Regulatory and Standards Context

In 2022, the California State Water Resources Control Board approved a policy requiring drinking water agencies to monitor microplastics, specifying FTIR and Raman spectroscopy as the two approved analytical methods (California State Water Resources Control Board, 2022). Standardized collection procedures for aquatic biota and sediment are described in SCCWRP Technical Report #1410.A (Thornton Hampton et al., 2025).

The Nile Red fluorescence screening method in this SOP is appropriate for teaching and exploratory research but does not meet California regulatory requirements (California State Water Resources Control Board, 2022). For data intended for submission to SWAMP or CEDEN:

1. Use microplastics-analysis-grade water for all blanks and rinses
2. Adopt SCCWRP contamination control procedures
3. Confirm polymer identity on a subset of particles by FTIR or Raman
4. Consult the PI before beginning any study intended for regulatory submission