Guide to Microplastics Identification

A Comprehensive Methods Guide for Microplastics Identification and Quantification in the Laboratory

Shaw Institute
30 YEARS OF ENVIRONMENTAL IMPACT RESEARCH

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"Improving human and ecological health through innovative science and global partnerships"

The Shaw Institute is a 501(c)3 nonprofit scientific research organization based in Maine and New York. Founded in 1990 by environmental health scientist Dr. Susan Shaw, its mission is to discover and expose environmental threats to people and wildlife through innovative science and to engage in global partnerships to improve human and ecological health. Over three decades, our research on plastics, ocean pollution, flame retardants, and climate change has informed public opinion and fueled policy, impacting millions of people in the U.S. and around the world.

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Introduction

Millions of tons of plastic enter the world's oceans every year; it is estimated that ocean plastic will outweigh fish by 2050 (World Economic Forum 2016). Microplastics enter the oceans from the breakdown of larger plastic pieces and from wastewater streams containing polyester fibers and smaller microbeads used in consumer products such as cosmetics. Plastics contain and adsorb thousands of toxic chemicals and act as a vector for pathogenic organisms, posing a health threat to marine species and human consumers.

The Shaw Institute has been a key driver and thought leader on microplastics since 2011, when our findings revealed that microplastics are pervasive in Gulf of Maine waters and bivalves (mussels, oysters), with worrying implications for human health. Every liter of water we sampled contained, on average, 17 microplastic fragments; one sample alone contained 450 pieces of microplastic. Our subsequent feeding study revealed that blue mussels may reduce their intake of algae, their normal food, by up to 50% in the presence of plastic particles (Woods et al. 2018). Over time, this could result in starvation and an inability to reproduce or adhere to coastal substrate. This study drew national media attention and exposed the reality of human intake of plastics.

Today, plastic pollution is a global crisis – plastic waste has pervaded the biosphere and micro- and nanoplastics are entering our bodies (Schwabl et al. 2018). The Shaw Institute is confronting the crisis as the U.S. Partner and Science Liaison to the Plastic Health Coalition, an alliance of global partners formed to accelerate research on the human health effects of plastic and create a science-based platform for action (https://www.plastichealthcoalition.org).

Microplastics research in aquatic environments has expanded rapidly over the past decade, but standardized methods are lacking, which impedes comparison of findings across studies. Meaningful comparisons require standard methods for sampling, processing, and analysis. To address this need, Shaw Institute researchers have created a comprehensive laboratory **Guide to Microplastics Identification** containing detailed methods for the microscopic identification and quantification of microplastics in aquatic media. By clarifying and improving techniques tested by our scientists and others, this Guide provides a valid, useful methodology for the visual detection of organic and synthetic materials in water, tips for microscope efficiency, and color photos of the most common shapes and sizes of microplastic fragments. Use of the Guide, along with recommended field sampling techniques (Barrows et al. 2017), provides the basis for a standardized, replicable microplastics research program.

The advent of new technologies has led to the development of new laboratory techniques for extracting, quantifying and identifying microplastics in a variety of matrices. See Section VIII, Appendix B for an overview of methods.

Microplastic Characteristics

NOTE: *Microplastics are extremely diverse and variable. During identification, use your best judgment to identify as many plastic characteristics as possible. If you are unsure about a fragment, do not count it as plastic. While counts should be as accurate as possible, it is better to have a conservative estimate (also see References).*

I. What do microplastics look like? (Hidalgo-Ruz et al., 2012)

1. Small size (largest dimension ≤5mm)
2. No cellular or organic structures visible
3. Fibers should be equally thick throughout their entire length
4. Particles should exhibit clear and homogeneous color throughout (see Section IV for rule variations)

II. Equipment you will need

1. Dissecting microscope, compound microscope, slides, cover slips, filters (ideally gridded)
2. Petri dishes (ideally glass)
3. Tweezers and probe for prodding fragments
4. Clean working area with no contamination (See Section VI)
5. Data sheets (See Appendix)

III. How to read a filter

1. Examine the fragments within the filtration perimeter. The perimeter will be defined by the shape of your filtration fragment and by varying intensities of coloration (i.e., brown, yellow, green, or slightly discolored white). Some are more visible to the naked eye than others. Underneath the dissecting microscope, even light colored perimeters are easily distinguishable.

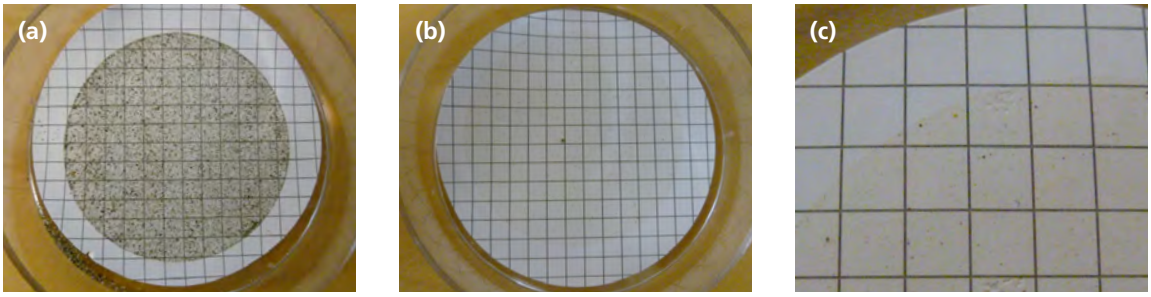


Figure 1: Different colored filters: Brown (a); Yellow (b, c).

2. If filters are curved and too difficult to examine under the microscope, glue them directly to the petri dish using two or three very small drops of glue on the filter edges.

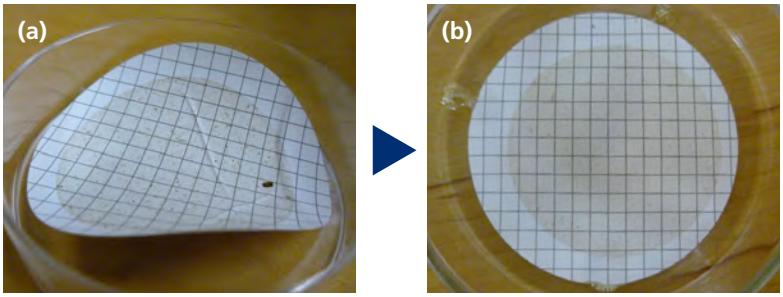


Figure 2: Glue procedure: Curved filter (a); Glued filter (b).

3. Read each filter from left to right, then move down one row, and read from right to left. A grid is helpful to ensure fragments are not counted twice.

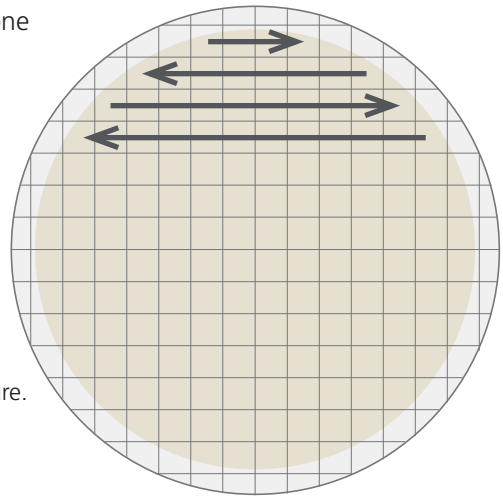


Figure 3:
Filter reading procedure.

IV. Identifying microplastics

A. Microscope inspection

Inspect filters under a dissecting microscope at 4.5x magnification. Filters should be dry, as wet filters reflect the light of the microscope. Typically, covered filters will dry after 24 hours at room temperature, but depending on the moisture of the filter and temperature in the lab, it may take longer.

B. Prodding fragments

Most plastic fragments are somewhat flexible and will not break when prodded. Tweezers and probes will allow you to prod individual fragments. Plastic fragments will often bounce or spring when prodded. If a fragment breaks when touched, do not count it as plastic.

C. Examining filters with high debris loads

Detritus and salt may cover or inhibit views of underlying microplastic fragments. Carefully pick through and move aside debris in order to locate all microplastics on your filter.

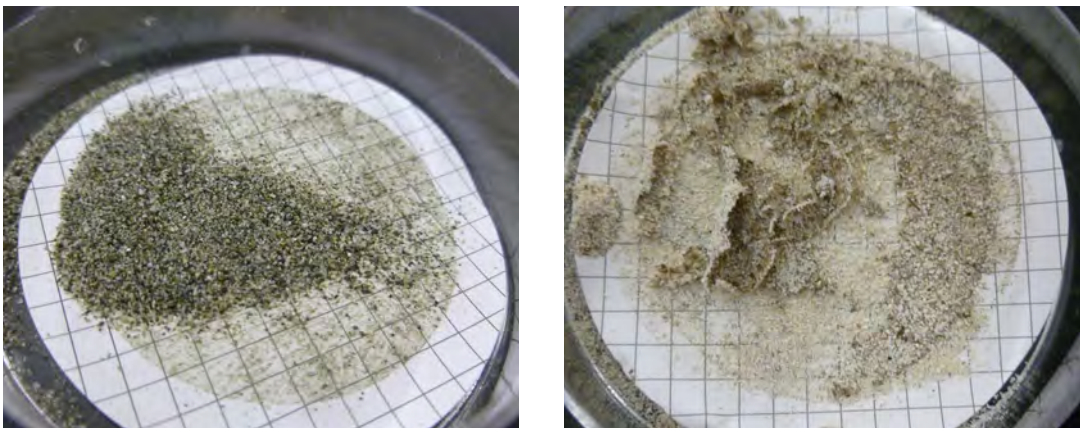


Figure 4: Detritus on filters.

D. Alternative identification methods

i. The hot needle test (based on De Witte et al., 2014)

This test is useful when plastic fragments and organic matter are indistinguishable. In the presence of a very hot needle, plastic fragments will melt or curl. Biological and other non-plastic materials will not. The hot needle test works well when fragments are spread apart. When many fragments are in close proximity, this test can be difficult to conduct. When using this technique, be sure your needle is very hot and held as close as possible to the fragment in question (without blocking your view). If the needle is not hot enough, you will see no movement, even if the fragment is plastic. This test should be used in conjunction with other characteristics of plastic fragments. If you find yourself unsure, remove the fragment in question and inspect it under a compound microscope.

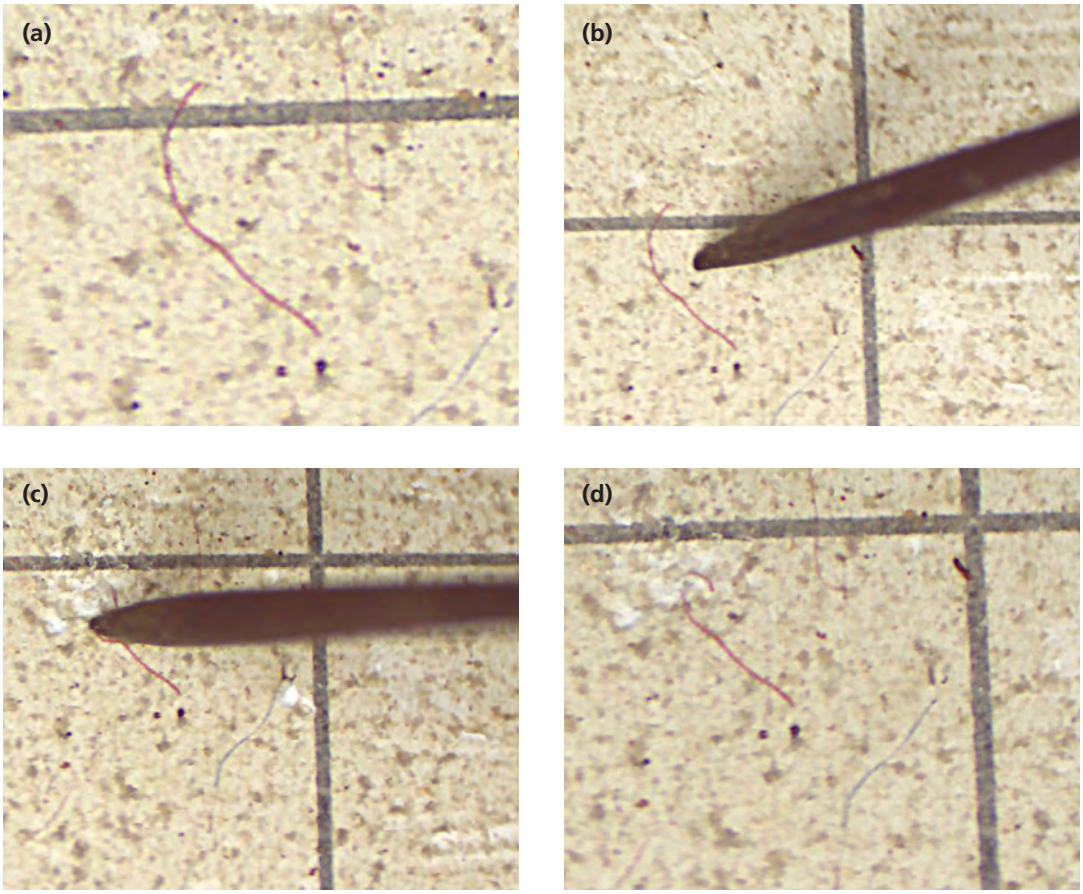


Figure 5: Hot needle test on red filament: Pre-test (a); Approach of hot needle (b); Hot needle in close proximity (c); Final result – filament reacted and curled due to heat (d).

ii. Compound microscope inspection

If the composition of a fragment is questionable, carefully remove and inspect it under a compound microscope where biological structures are more readily visible. It is beneficial while training to examine filtered algae under the microscope so you can confidently differentiate between algal fragments and plastic fragments with biofouling (i.e., growth of algae on plastic surface).

E. Variations to look for when using the Hidalgo-Ruz rules

The Hidalgo-Ruz et al. (2012) rules will help you identify most microplastics. However, you may come across fragments that vary from these rules. Identify as many characteristics as possible for accurate categorization.

i. Rule 1: *No cellular or organic structures visible*

Variation: Microplastics will never have cellular or organic structures. However, biofouling can alter the appearance of a fragment of plastic. Organic material may be visible just on the surface of the plastic. Carefully note if organic structures are present throughout a fragment, in just one portion, or on the surface.

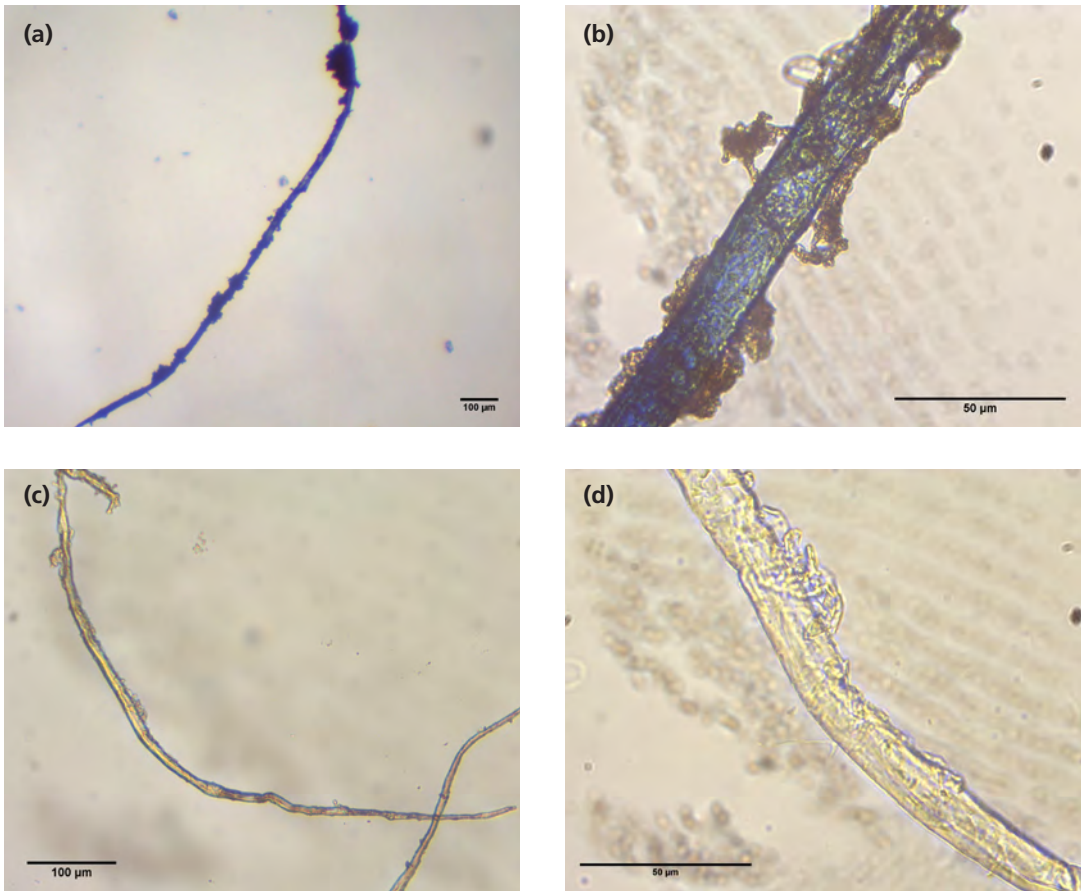


Figure 6: Biofouled microplastic fibers: Blue biofouled fiber (a, b); Translucent biofouled fiber (c, d).

ii. Rule 2: *Fibers should be equally thick throughout their entire length*

Variation: Generally always true for fibers. However, sometimes splitting or fraying is seen.

Figure 7: Frayed microplastic fiber.

iii. Rule 3: *Particles should exhibit homogeneous color throughout*

Variation: Some plastics are not homogenous in color. You may find patterns or stripes. Additionally, biofouling can potentially disguise color, or part of the fiber may be bleached.

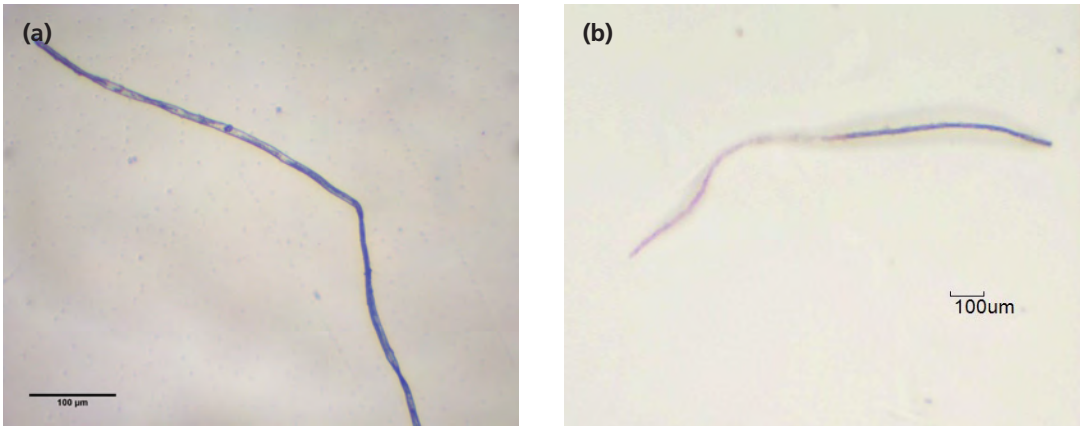


Figure 8: Partially bleached blue fiber (a); Red, white and blue fiber (b).

F. Other materials you may find on your filter

i. Algae

Algae, especially when translucent, can sometimes be difficult to distinguish from plastic underneath the dissecting microscope. Even when prodded, these biological fragments may not break. If you are unsure, remove the fragment in question and inspect it under a compound microscope. Look for cellular structures throughout the fragment. If there are cellular or organic structures just on one portion, or just on the surface, it may be due to biofouling (See Section E, part i).

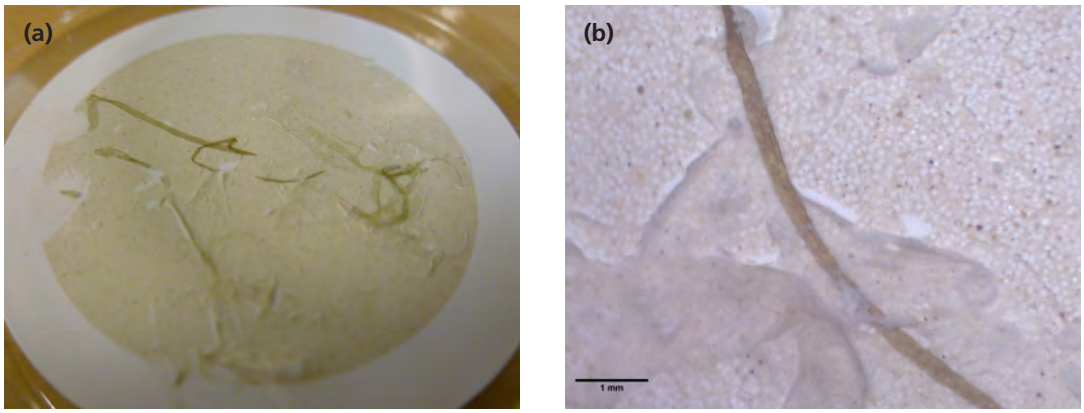


Figure 9: Algae examples: *Ulva* algae on a filter (a); The translucent film from the algae may look like plastic, but is very brittle and breaks apart when prodded (b).

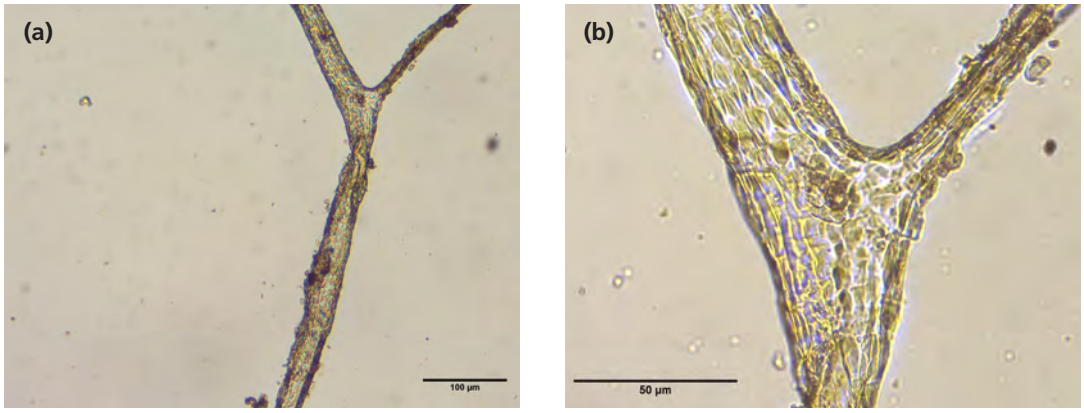


Figure 10: A fragment of algae. The forking seen here is usually indicative of algae. Notice the cellular structure seen throughout the fragment.

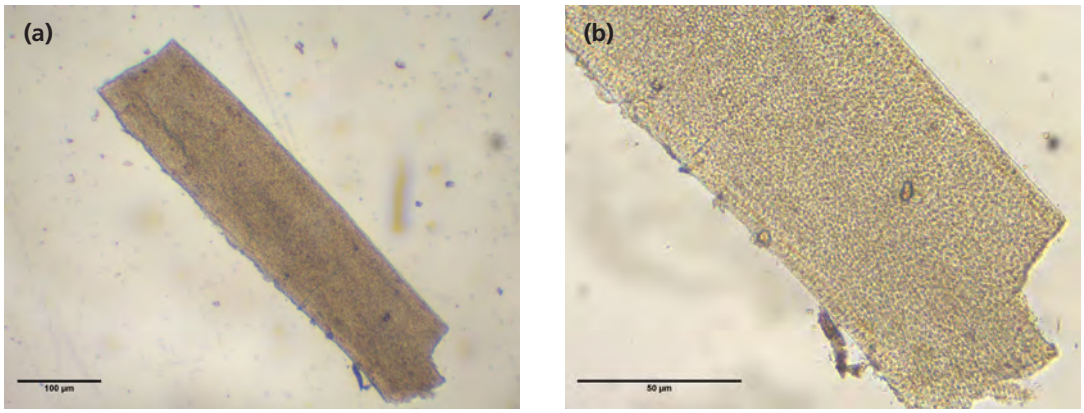


Figure 11: A fragment of algae without forking. Notice cellular structure seen throughout.

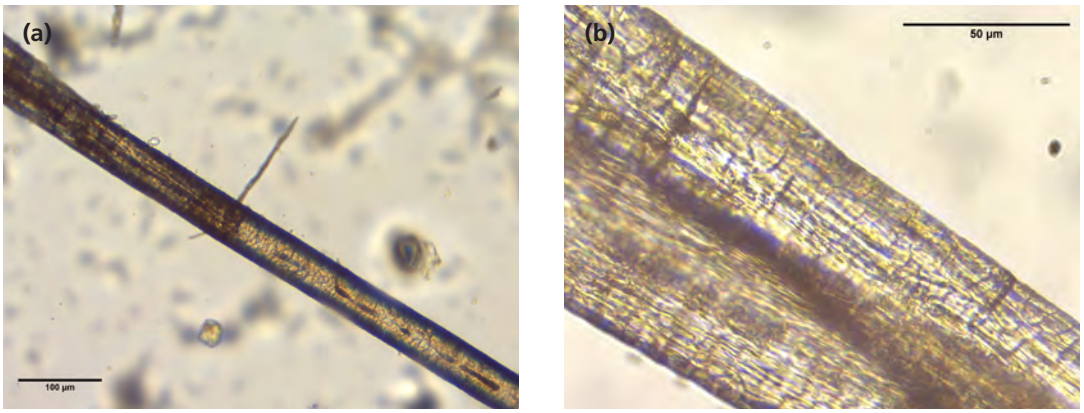


Figure 12: A fragment of algae. Notice cellular structure seen throughout.

ii. Salt crystals and sand

Salt and sand fragments may also sometimes look similar to plastic. However, when prodded, salt crystals and sand will break apart and sand will sound like breaking glass. If you are having difficulty distinguishing sand from microplastics, consider using the hot needle test.

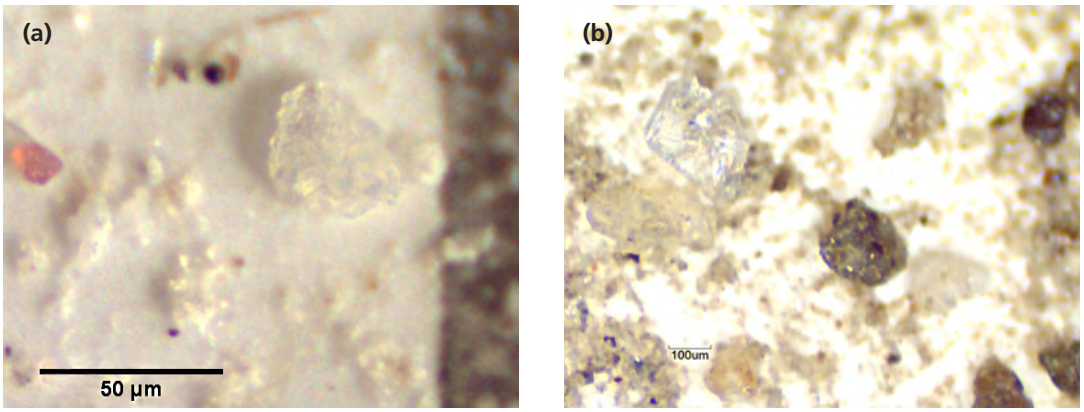


Figure 13: Non-plastic examples: Salt crystal (a); Sand grain (b).

iii. Animals, animal parts and shells

You may find parts of animals, shells, phytoplankton, zooplankton, etc., in your sample. These will usually break apart or lose much of their structural integrity when prodded.

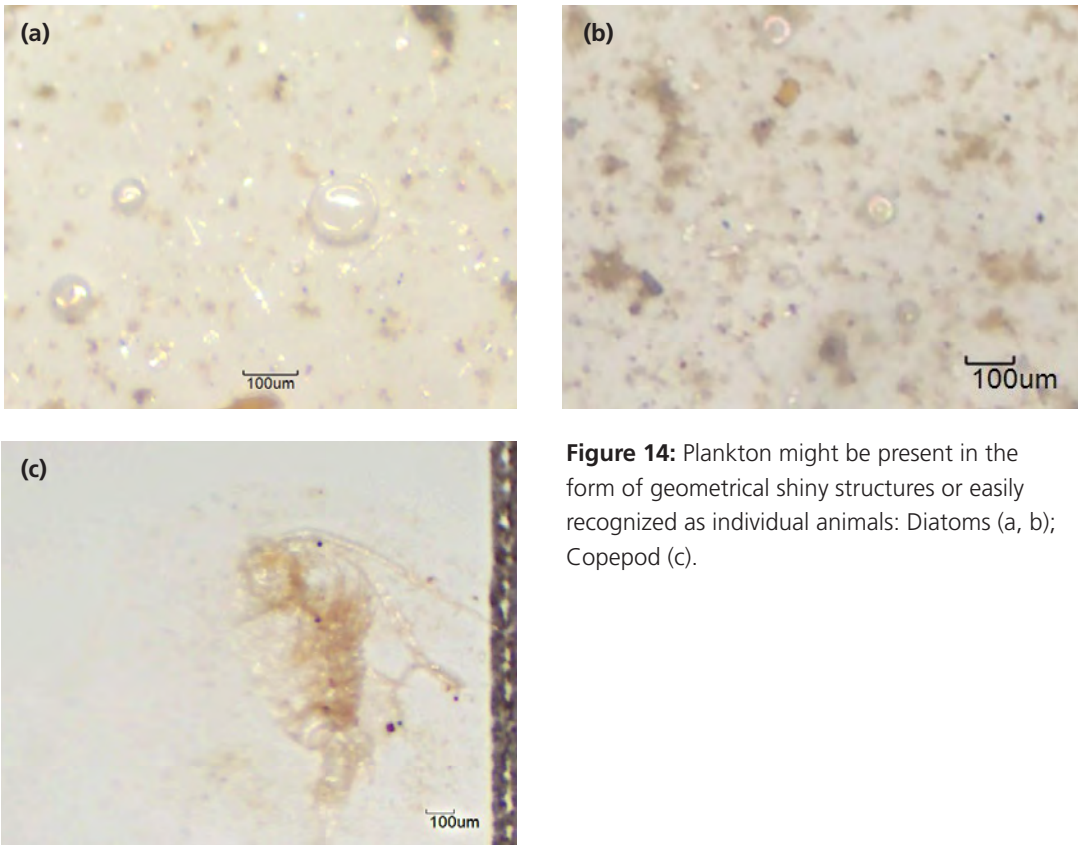


Figure 14: Plankton might be present in the form of geometrical shiny structures or easily recognized as individual animals: Diatoms (a, b); Copepod (c).

Figure 15: Trichome. Looks like plastic, and will spring right off of the filter when prodded.

iv. Metal paint and aluminum foil

You may see pieces of metal paint or aluminum foil in your samples. Metal paint can scrape off a boat. Aluminum pieces can flake off of foil lined sample lids or equipment. You can identify these pieces due to their intense reflectivity and shininess. They will not be flexible like plastic and will not respond to the hot needle test.

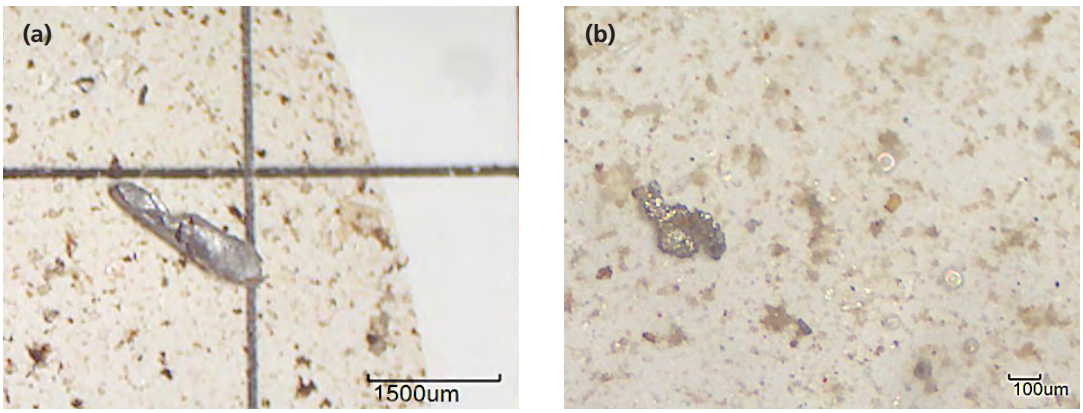
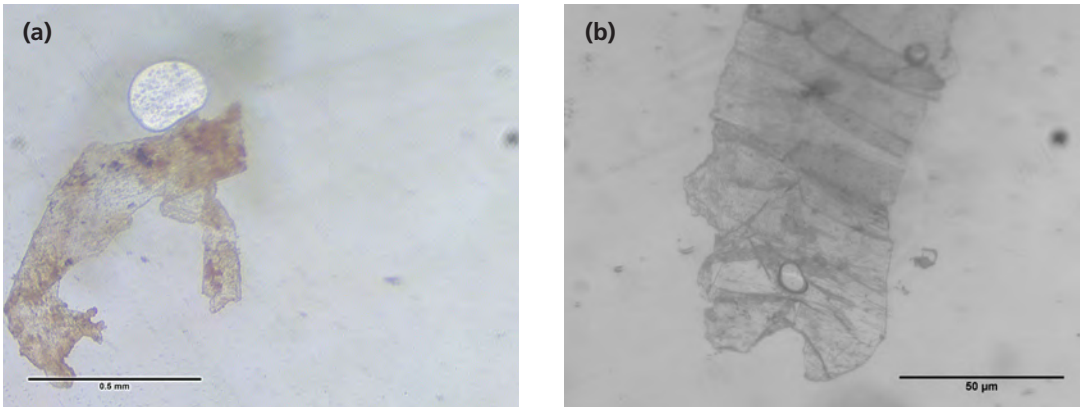


Figure 16: Examples of aluminum fragments: Large fragment (a); Smaller fragment in detail (b).

V. Categories of Microplastics

Microplastic fragments can be categorized based on color and shape. If possible, it is also beneficial to measure the length or area of each fragment. Software programs such as Image J and MoticCam both have this capability. Make sure that your equipment is calibrated for accurate measurements. Rough estimates of plastic size (e.g., length equals # squares) can also strengthen your analysis.



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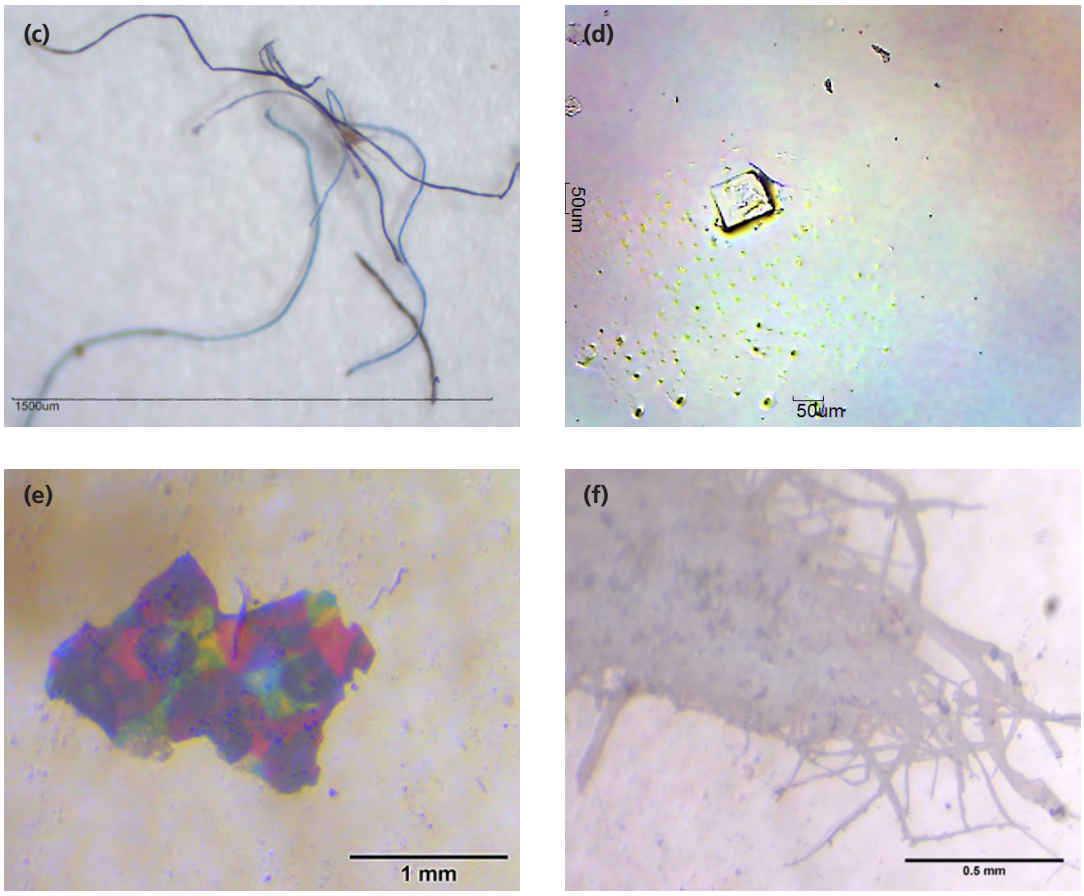


Figure 17: Different shapes and colors of microplastics: Translucent fragment (a, b); Filaments (c); Angular (d); Multi-colored fragment (e); Clump of white fibers (f).

A. Categories used to describe microplastics

Some studies further categorize fragments, as seen in the chart below. Without FT-IR analysis (i.e., plastic polymer identification), it is extremely difficult to confidently pinpoint the source of microplastics in your sample. Source: Hidalgo-Ruiz et al., 2012

Categories	Examples
Sources	■ Consumer product fragments (e.g., fishing net) ■ Raw industrial pellets
Type	■ Plastic fragments, pellets, filaments, plastic films, foamed plastic, granules, styrofoam
Shape	■ For pellets: cylindrical, disks, flat, ovoid, spheroids ■ For fragments: rounded, subrounded, subangular, angular
Erosion	■ Fresh, unweathered, incipient alteration, and level of crazing (conchoidal fractures), weathered, irregular surface, jagged, linear fractures, subparallel ridges, very degraded
Color	■ Transparent, crystalline, white, clear-white-cream, red, orange, blue, opaque, black, grey, brown, green, pink, tan, yellow and pigmentation

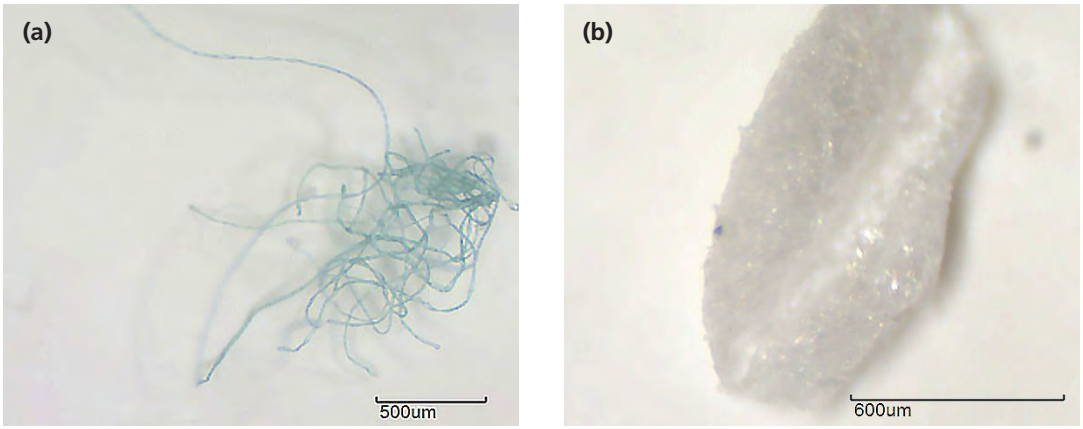
VI. Contamination considerations

Sample contamination from airborne fibers is a problem for many studies. It is necessary to be aware of this and to take precautions to reduce it.

A. Methods to reduce contamination

1. Keep your filter covered whenever possible. If you are not looking at it under the microscope, it should be covered.
2. Store filters in glass petri dishes. Plastic petri dishes will work, but glass is better to reduce possible contamination from the dish itself.
3. Wipe down all surfaces before inspecting each sample. A brightly colored sponge is recommended. Any fragments sourced from the sponge will be more easily identified as contamination if it is a unique color.
4. Rinse all tweezers, probes, and your hands three times under a heavy stream of water before opening a petri dish for inspection.
5. Wear cotton or natural fiber clothes. Avoid bringing any synthetic materials into lab.
6. Minimize traffic in your lab or working space, if possible. The smaller the number of people in and out of your workspace, the less chance of contamination.
7. If possible, process samples in a clean-flow cabinet to reduce particles in the surrounding air.
8. Always use a control blank. This is a filter that undergoes all the same protocols of a sample (i.e., filtration, drying, examination, etc.) except it is known to contain no microplastics. Examining blanks alongside samples can help estimate the amount of microplastics in your sample potentially caused by air-born contamination.

B. Contamination examples



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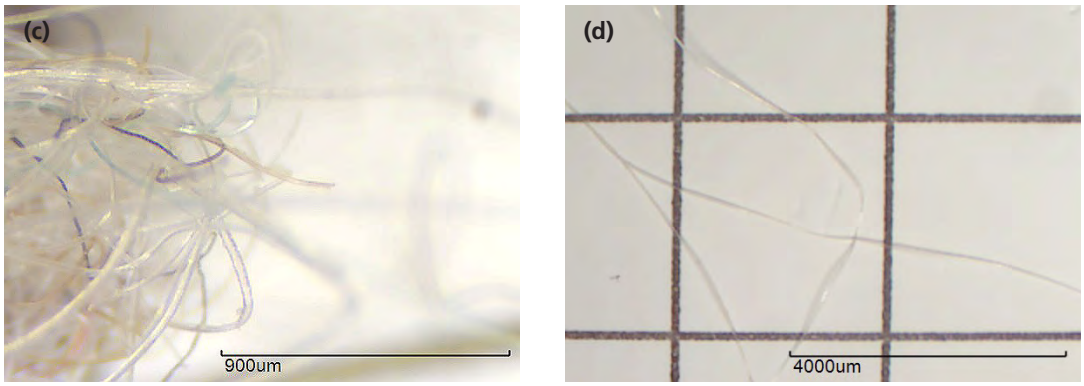

Figure 18: Examples of different types of lab contamination: Cotton clothing fibers (a); Styrofoam fragment (b); Wool clothing fibers (c, d).

VII. References

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VIII. Appendices

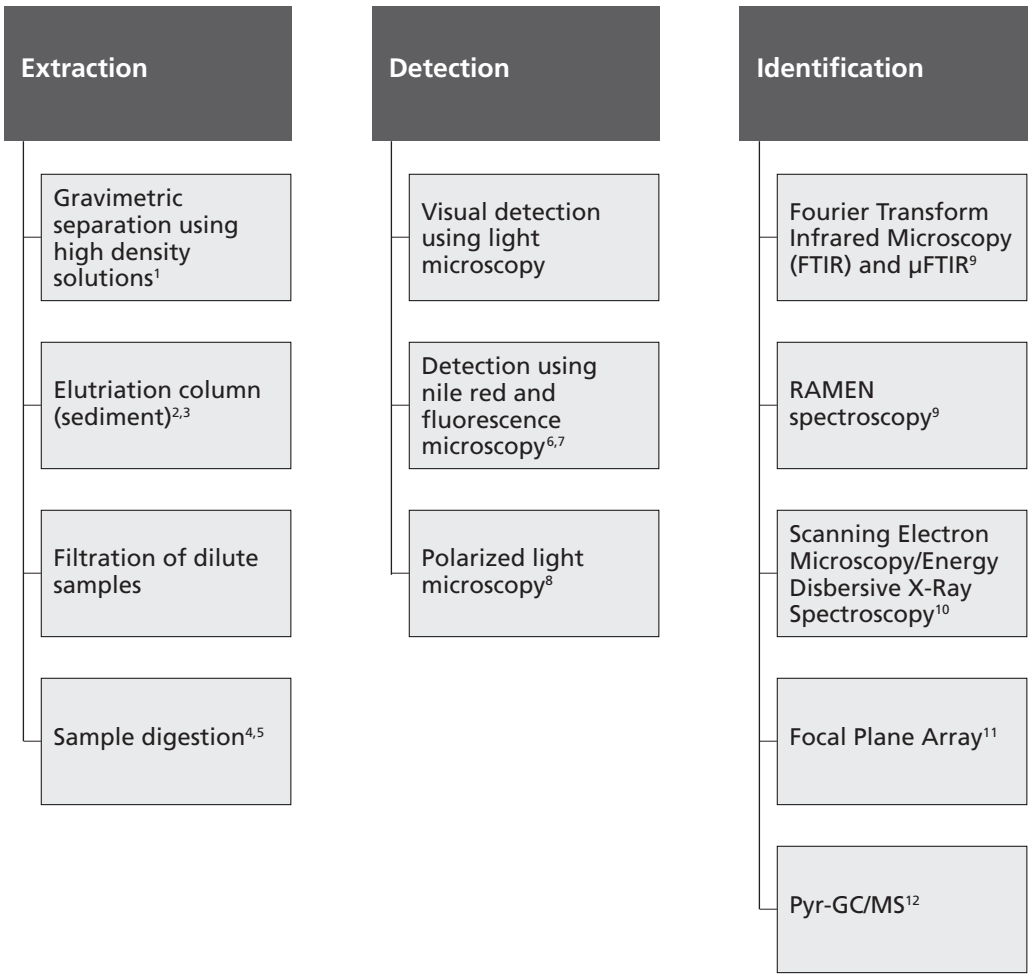
A. Microplastics Data Sheet

This is an example of a datasheet for use while counting. Make tick marks in the boxes for each color/shape combination encountered.

		Color							
		Blue	Red	Transparent /White	Black	Green	Other Colors	**Total per Filter**	**Fragments /Liter**
Shape	Round								
	Filament								
	Angular								
	Other Shape								
	Total								

B. Laboratory Methods for the Analysis of Microplastics in Various Media

The advent of new technologies has led to the development of laboratory techniques for extracting, detecting and identifying microplastics in a variety of matrices. Current-use methods shown in the diagram are detailed in the publications listed below.



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