Techniques for Identifying and Quantifying Microplastics Prior to or in Lieu of Spectroscopy

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Stages of Sample Preparation/Analysis
I. Morphology Key for Categorization

Fiber
Flexible, equal thickness, ends clean-cut, pointed or fraying

Fiber Bundle
≥20 fibers

Fragment
Rigid, variety of shapes

Sphere
Round, smooth surface, tend to be smaller (100-300 µm)

Pellet
Larger (3-5mm), often round or cylindrical

Film
Flat, thin, malleable

Foam
Soft, compressible

Adapted from Rochman et al. 2019
I. Fiber Bundles vs Fibers

**Fiber Bundle**
*Tightly-wound, consistent in appearance*

**Individual Fibers**
*Inconsistent appearance, loose*

Do NOT tease apart

Do tease apart
I. Clear Fibers

**Cellulosic**

- Tapered ends, rough surface texture, spiny projections off of main fiber body

**Synthetic**

- Surface texture appears smooth (may have bubble-like spheres), main body of fiber typically thick with few projections, tensile
II. Color Key for Categorization

Goals:

- Simplicity
- Consistency
- Harmonization with other studies
- Adapt colour categories when necessary
  - Subcategories for very common colours
  - Additional categories if necessary (e.g. multi-coloured)
II. Color Key for Categorization

For fibers, colour/clear combinations are common
- Bleaching may cause clear portions
- Assign colour based on dyed portion

For fragments (and other categories), multi-coloured particles or particles with images/text are possible
- Assign colour based on dominant colour (if possible)
III. Size Fractioning

Size fractioning is useful
- Reduces particle load
- Creates bins for data analysis
- Easier to focus on similarly sized particles

Consider the following:
- Hypothesis (e.g. effects sizes)
- Harmonization with other studies
- Methods (e.g. limitations for handling)
IV. Sorting and Picking

- Fine-tipped forceps
- Counting dish (with grid)
- Dissecting microscopes (3D view of particles)
V. Plating Samples

Covered, clearly-labelled, circled and numbered particles

Mounted on clear, adhesive surface with particles as flat as possible
VI. Subsampling

1 mm
500 µm
355 µm
125 µm
45 µm
25 µm

First 10 of each colour/morphology combination (each size fraction)

Recombine all size fractions after counting and picking
VII. Pictures and Measurements

Length and width measured
- Longest dimension and widest dimension perpendicular to length
- Do not measure frayed projections in fibers
- Use segmented lines when necessary
- Subsample for larger particle counts
VIII. Polymer Verification

- Plastic: 58%
- Anthropogenic: 31%
- Natural (inorganic): 3%
- Natural (cellulosic): 1%
- Unknown: 7%

Munno et al. Unpublished

\[ N_{MP} = 153 \]
IIX. Staining Methods

Rose Bengal stains organic matter

- Used to colour organic matter so it is distinguishable from synthetic polymers (Davison & Asch 2011)
  - Does not stain minerals or chitin
  - Faintly colours clear/white particles
IIIX. Staining Methods

Nile Red stains neutral lipids and highly hydrophobic microplastic (Greenspan & Fowler 1985)

- Fluorescent in hydrophobic environment
- Stains natural organic material

- Not recommended to use NR-staining alone for identifying MP (Shim et al. 2016)
IX. Limitations for Identifying & Quantifying Microplastics

Visual identification is not enough
- Feel of the particles is a contributor to identification

Bright colours exist in nature
- Colour of particles used as indicator of anthropogenic origin

Not all dyed materials are plastic
- Cellulosic fibers (e.g. cotton) can be dyed
Thank you! Questions?