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

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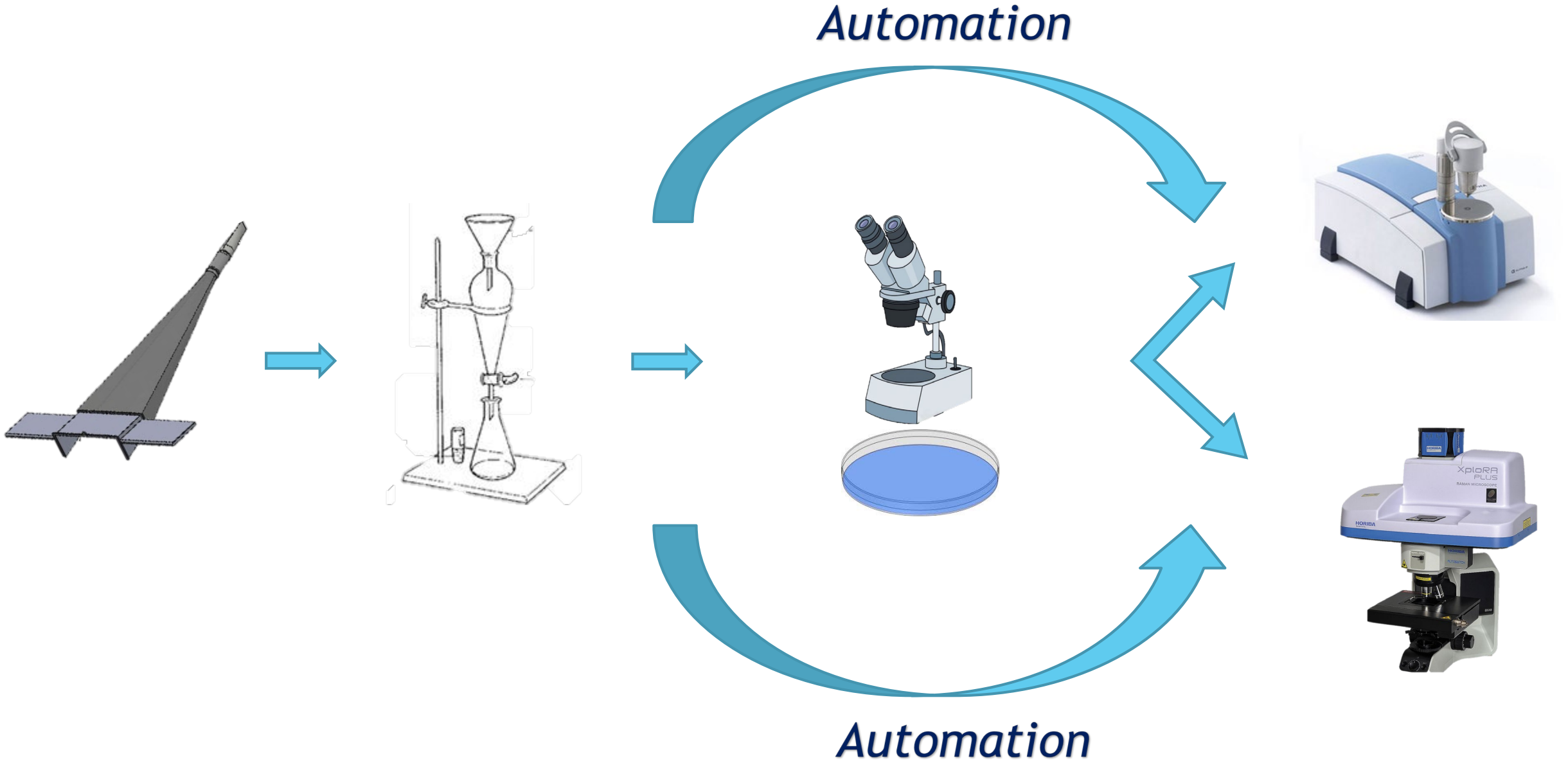
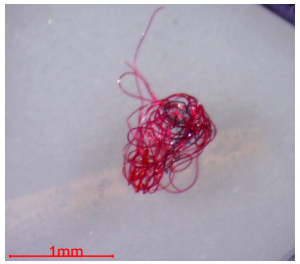
Stages of Sample Preparation/Analysis

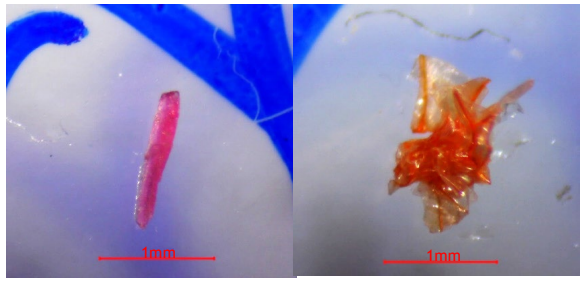


I. Morphology Key for Categorization

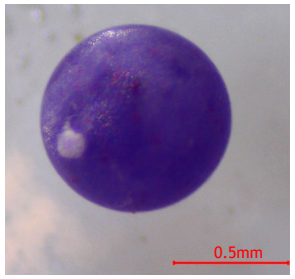
Fiber
Flexible, equal thickness, ends clean-cut, pointed or fraying



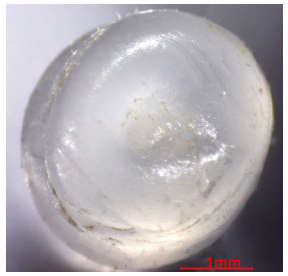
Fiber Bundle
≥20 fibers



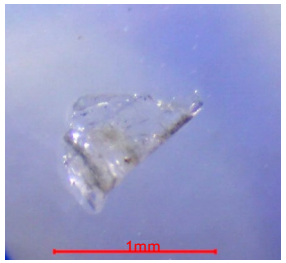
Fragment
Rigid, variety of shapes



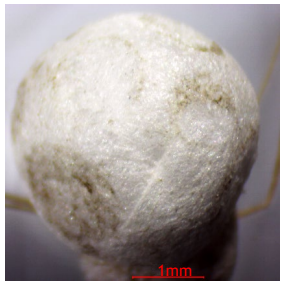
Sphere
Round, smooth surface, tend to be smaller (100-300 µm)



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



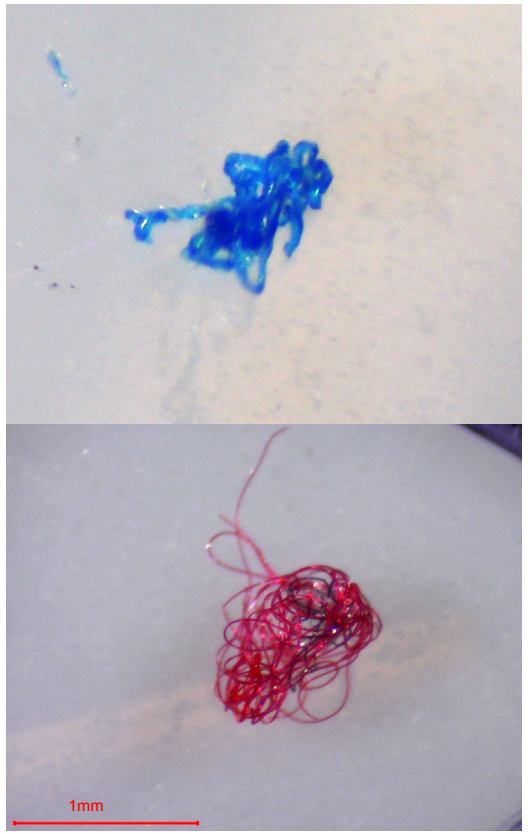
Film
Flat, thin, malleable



Foam
Soft, compressible

Adapted from Rochman et al. 2019

I. Fiber Bundles vs Fibers



Fiber Bundle

Tightly-wound, consistent in appearance

Do NOT tease apart

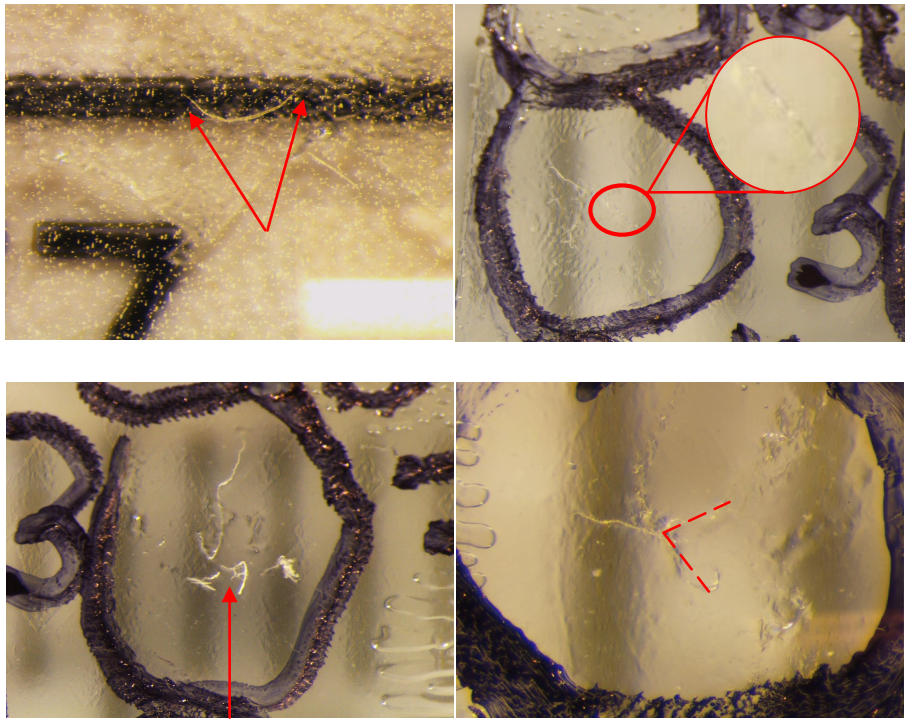
Individual Fibers

Inconsistent appearance, loose

Do tease apart

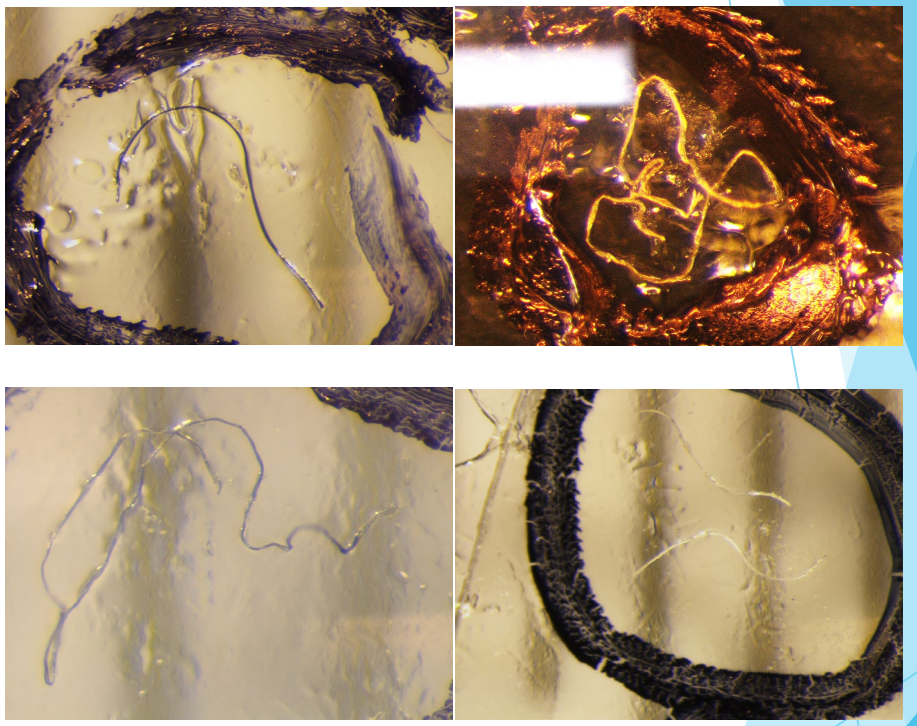
I. Clear Fibers

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

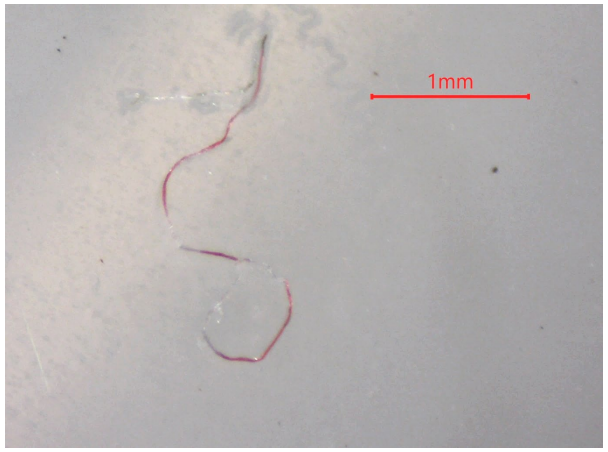
Goals:

- Simplicity
- Consistency
- Harmonization with other studies

Adapt colour categories when necessary

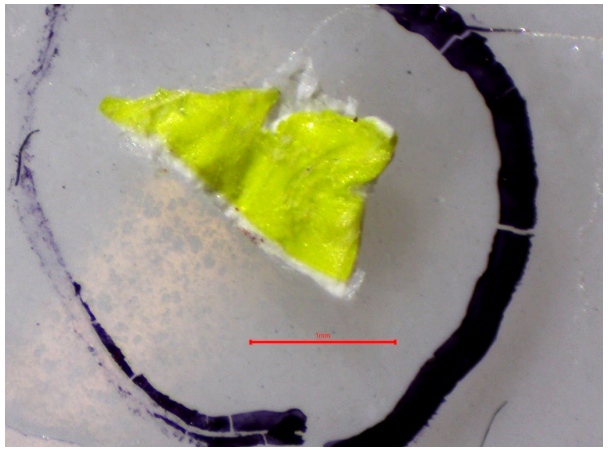
- Subcategories for very common colours
- Additional categories if necessary (e.g. multi-coloured)

II. Color Key for Categorization



For fibers, colour/clear combinations are common

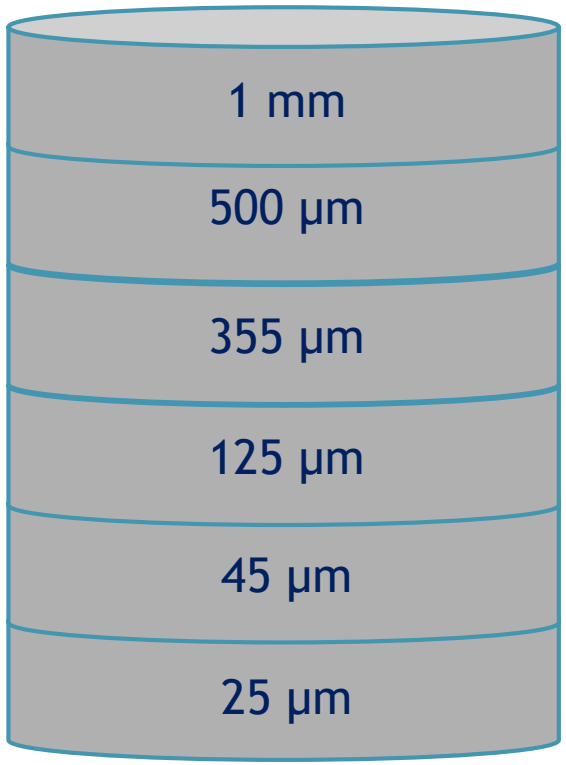
- Bleaching may cause clear portions
- Assign colour based on dyed portion



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

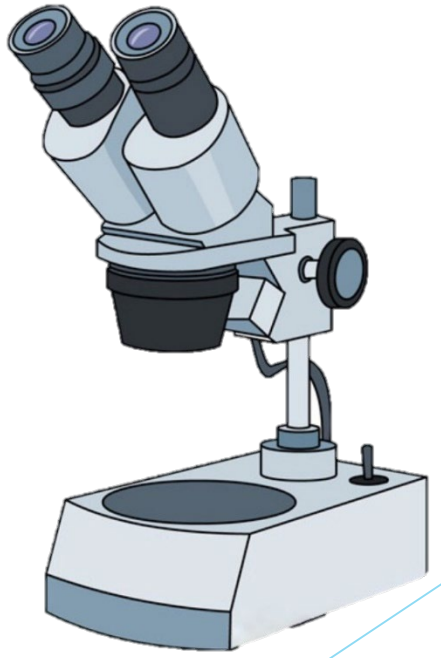
- Reduces particle load
- Creates bins for data analysis
- Easier to focus on similarly sized particles

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)

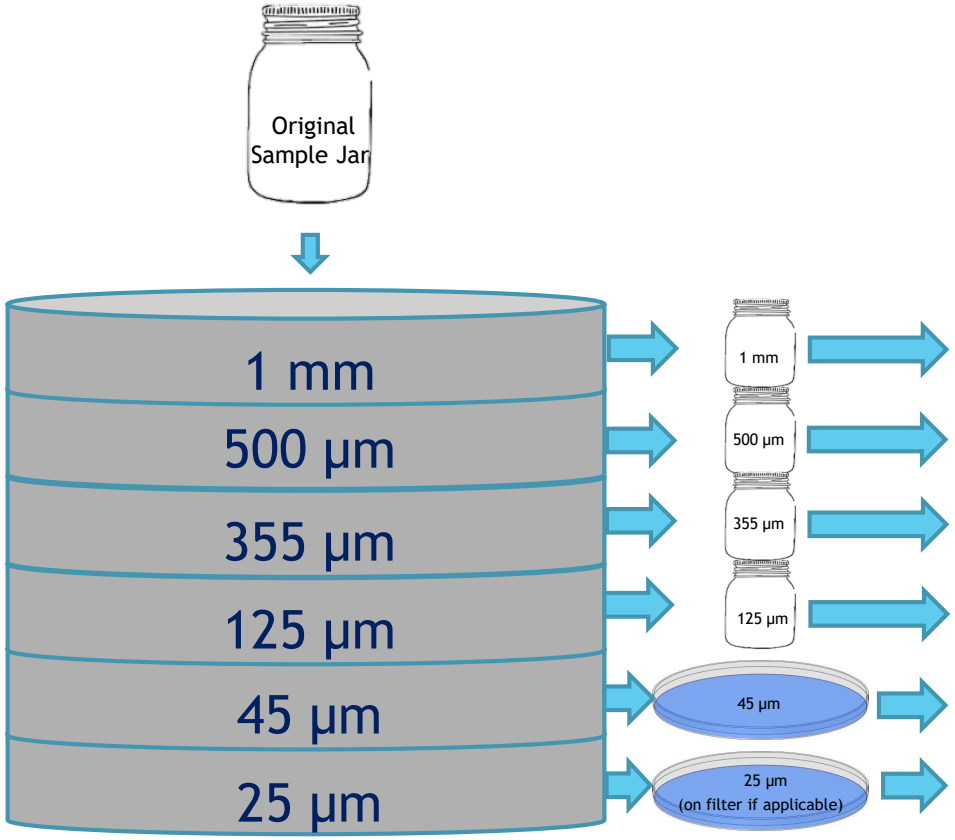


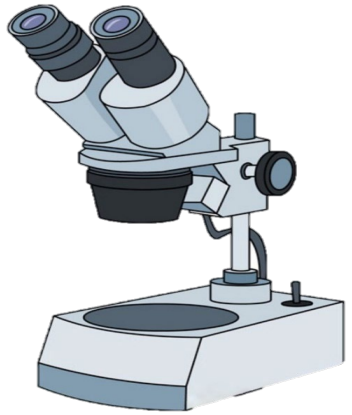
V. Plating Samples

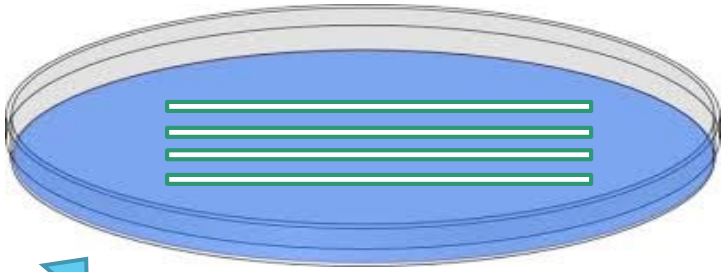
Covered, clearly-labelled, circled and numbered particles

Mounted on clear, adhesive surface with particles as flat as possible

VI. Subsampling







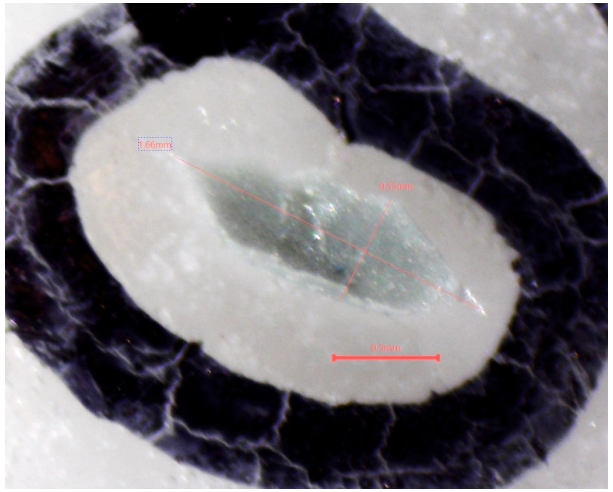
First 10 of each colour/morphology combination (each size fraction)

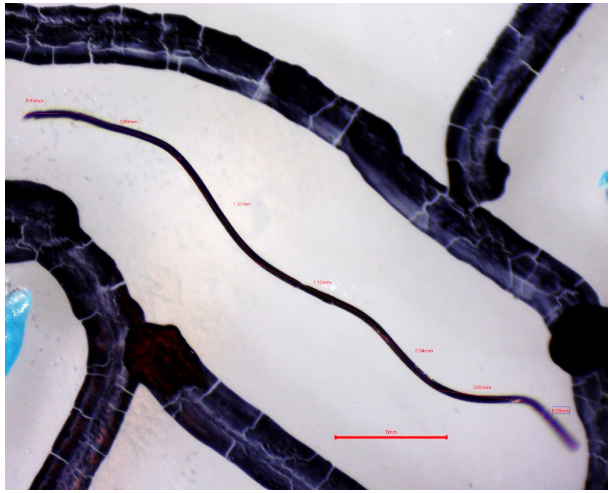
counting dish

Recombine all size fractions after counting and picking

Original Sample Jar

VII. Pictures and Measurements



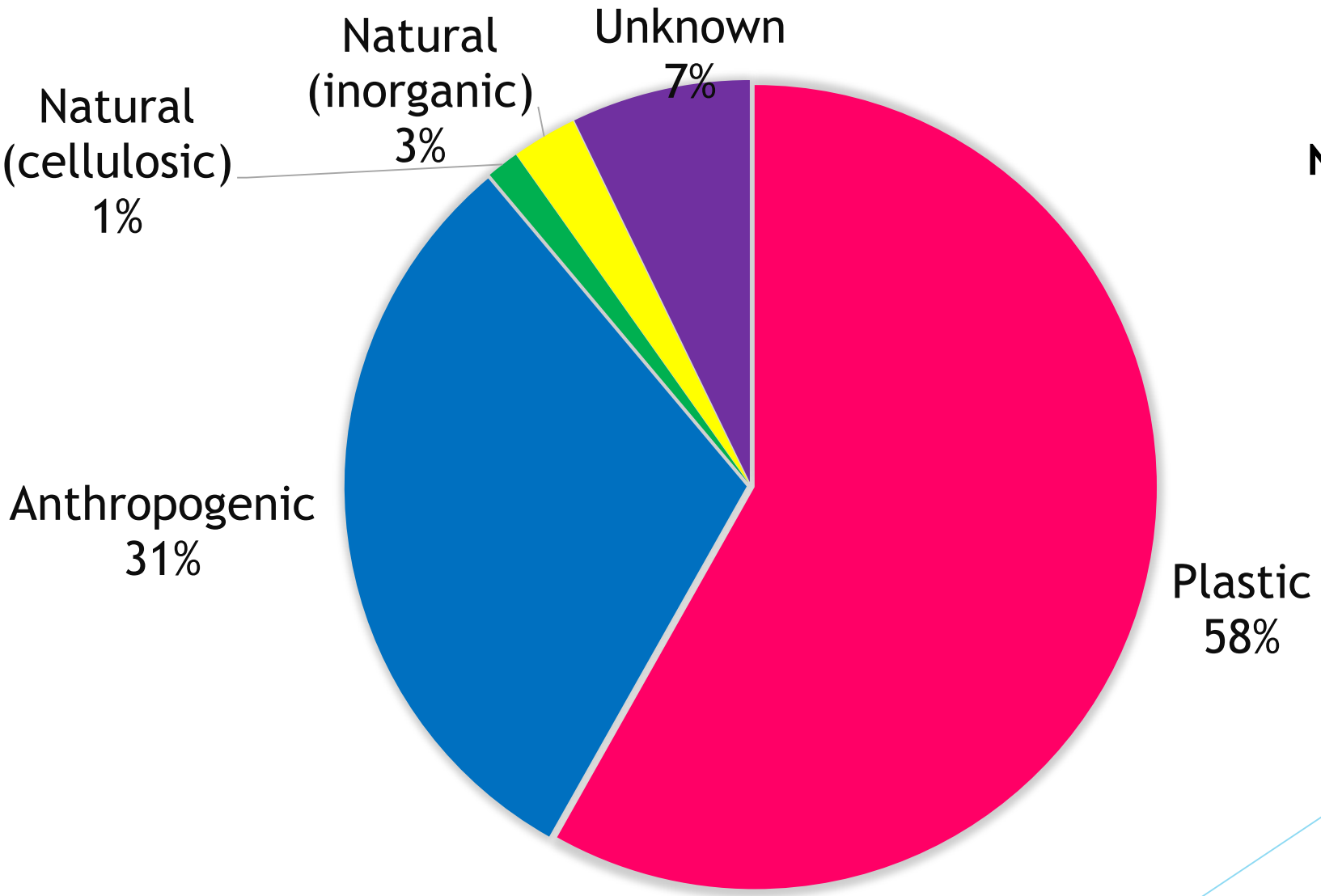


Length and width measured

- 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



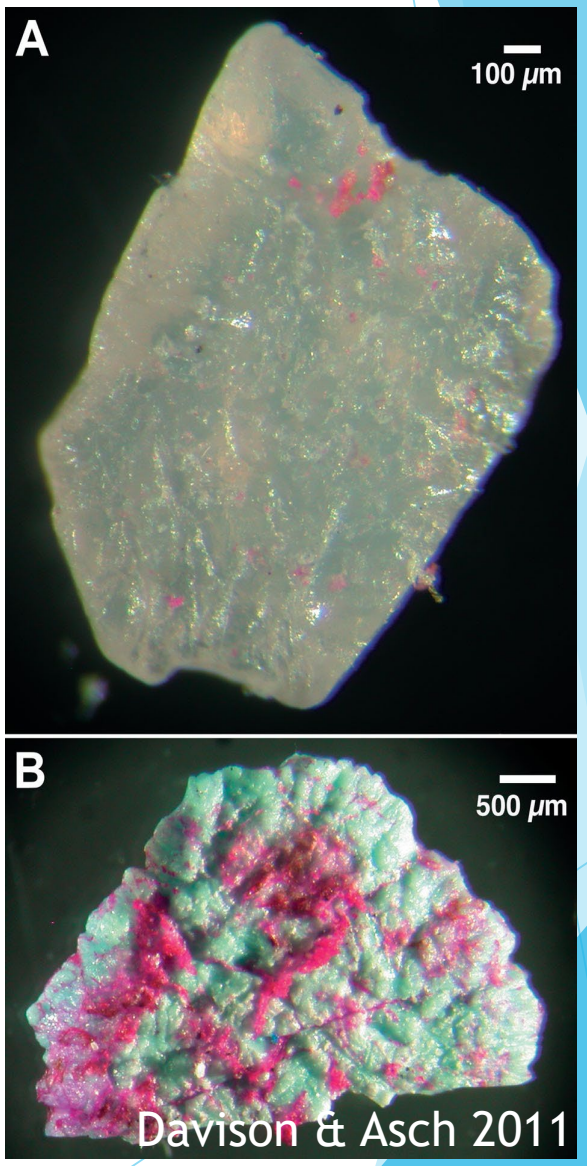
N_{MP}=153

Munno et al. *Unpublished*

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

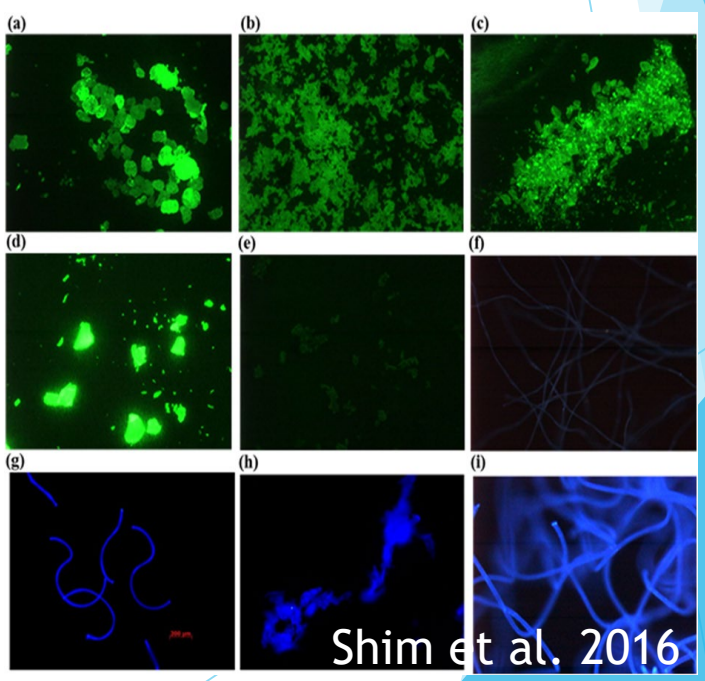


IIX. Staining Methods

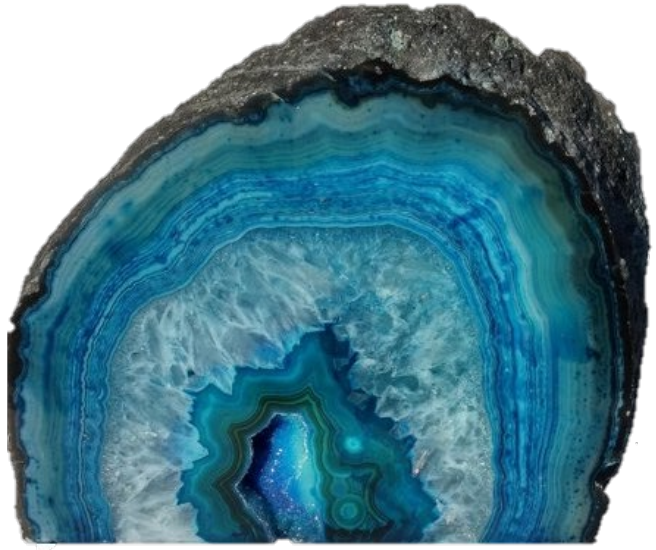
Nile Red stains neutral lipids and highly hydrophobic microplastic (Greenspan & Fowler 1985)

- 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?

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