SOP for Microplastic Picking

PURPOSE
This SOP describes the procedure by which microplastics will be counted, and picked from wet samples (extracted) prior to photographs, measurements and chemical identification.

MATERIALS

<table>
<thead>
<tr>
<th>Item</th>
<th>Suggested Materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass Petri Dishes for wet picking</td>
<td>VWR Catalog no. 25354-069</td>
</tr>
<tr>
<td>Small Glass Petri Dishes for dry picking</td>
<td>VWR Catalog no. 25354-025 (For use with a 47mm diameter filter)</td>
</tr>
<tr>
<td>Petri Dishes for picked particles</td>
<td>Size and material not specified</td>
</tr>
<tr>
<td>Superfine-tip forceps</td>
<td>VWR Catalog no. 63042-688</td>
</tr>
<tr>
<td>Petri dish grid stickers</td>
<td>Amazon - “Diversified Biotech PetriStickers PSTK-1070 Square Grid Label for Petri Dish, 70 Square Grid (Pack of 36)”</td>
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<tr>
<td>Laboratory labeling tape</td>
<td>-</td>
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<tr>
<td>Aluminum foil</td>
<td>-</td>
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<tr>
<td>Double sided tape</td>
<td>Available from stationary stores</td>
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<tr>
<td>Clear projector paper</td>
<td>Available from stationary stores</td>
</tr>
<tr>
<td>Metal teaspoon</td>
<td>Amazon - “4.5” Stainless Steel Teaspoon,Set of 6”</td>
</tr>
<tr>
<td>Stereoscope</td>
<td>Interchangeable black and white base preferable for picking</td>
</tr>
</tbody>
</table>

Personal Protective Equipment (PPE)

The following PPE are mandatory for sample processing:
- Clean cotton lab coat
- Clean nitrile gloves

PROCEDURE

Procedural Blanks

- We recommend one field and/or laboratory blank per every ten samples which is run through the same protocol as the test samples; particles identified, quantified, and characterized.

Preparation
Before using any glassware or tools, wash with soap and water (surfactant helps to remove contaminant microplastics). Rinse three times with tap water, then three times with filtered/RO water.

When equipment/tools/labware are not being used, or when samples are not being analyzed, keep covered to prevent procedural contamination.

Label all petri dishes and beakers using laboratory tape.

Create an adhesive base for mounting picked particles (e.g., double-sided tape on transparent projector paper for Raman spectroscopy or SkinTac mounted on a glass slide for FTIR).

Use a gridded petri dish OR stick a grid sticker to the outside base of the glass petri dish.

Prepare one clean beaker or glass jar to pour the picked portions of the sample into.

Prepare one clean beaker filled with RO to store picking utensils when not in use.

Set up the microscope with the black base facing up to start.

**Setting up your picking station**

**Particle identification, characterization and picking**

*Wet Samples (e.g., drinking water, grab surface water sample)*

1. Bring your sample over to the microscope. If your sample is size-fractioned, bring one extracted size fraction over to the microscope at a time (it is advised to start from largest to smallest).

2. Take a small teaspoon of extracted sample and place contents into the counting dish. *Note: Do not be tempted to take a large spoonful. The more water there is in the counting dish, the more difficult it will be to see all the particles!*

3. Rinse the teaspoon into the counting dish using RO water to remove any particles from the sample that remain on the spoon.
4. Looking at the counting dish through the microscope, work methodically along each row, moving through the grid numbers. Pause in each grid square to check for suspected microplastic particles. Adjust the field of view to check for particles both within the water and floating on the surface. It is recommended that floating particles are picked first as floating airborne fiber contamination is common.

5. For each suspected microplastic particle identified, note its morphology and color on a datasheet. Make note of anything unusual in the comments.

6. Pick each suspected microplastic particle, or subsample – e.g., the first 10 particles identified from each colour/morphology category (e.g. blue fiber, black fragment), within each size fraction. Use the fine tipped forceps to pick a particle and mount it onto your surface, then circle and number the particle using a fine-tip sharpie. The number of the particle should correspond to the particle description on the data sheet.

   Note: If you are subsampling do not forget to tally all suspected microplastic particles. E.g., after 10 particles of each color/morphology have been picked, no longer pick from that colour/morphology category, but continue to count and characterize all other particles you find and record them on your datasheet.

7. Once you reach the last number in the grid, check around the inside edge of the petri dish for particles.

8. Cover the petri dish, remove from the microscope base, turn the base over to the white side and repeat steps 3-6 again.

9. Once the spoonful has been checked using both the black and white microscope base beneath, and all suspected microplastics have been identified/picked, empty the contents of the counting dish into the prepared and labelled clean beaker and cover with foil.

10. Repeat steps 2-8 until the entire sample has been analyzed under the microscope.

11. Once the jar is empty, triple rinse the jar trying to minimize the amount of RO water used, and pour the rinsed contents into the counting dish, repeat steps 3-8.

12. If you are saving your sample, pour the picked sample into a clean and labelled jar for storage if it is not in a jar already.

13. Clean and rise all materials, and repeat process for the next size fraction.

**Dry samples (on a filter)**

1. Bring the chosen extracted size fraction over to the microscope (it is advised to start from largest to smallest). Put the filter into a counting dish with a grid sticker or add a grid sticker to the bottom of the existing petri dish (recommended). Take caution to move the filter minimally and carefully so particles are not lost.

2. Looking at the counting dish through the microscope, work methodically along each row, moving through the grid numbers. Pause in each grid square to check for suspected microplastic particles.

14. For each suspected microplastic particle identified, note down its morphology and color on a datasheet. Make note of anything unusual in the comments.

15. Pick all, or subsample (see above), all suspected microplastic particles. Use the fine tipped forceps to pick a particle and place it onto the double-sided tape, then circle and number the particle using a fine-tip sharpie. The number of the particle should correspond to the particle description on the data sheet.

   Note: Remember to record the colour/morphology of all particles, whether they are picked or left on the filter.
Once you reach the last number in the grid, check around the inside edge of the petri dish for any missed particles.

16. Cover the petri dish, remove from the microscope base, turn the base over to the white side and repeat steps 3-6 again.

17. Once the sample has been checked using both the black and white microscope base beneath, and all suspected microplastics have been identified/picked, empty the contents of the counting dish into the prepared and labelled clean beaker and cover with foil.

18. Repeat steps 2-8 until the entire sample has been analyzed under the microscope.

19. Check along the edges of the inside of the petri dish for particles that may have moved from the filter during transfer to the inside microscope.

Figure 1. Flow chart demonstrating the process for counting, picking (including subsampling) and storing particles from each size fraction. Note that four size fractions are given as an example here, but this is dependent on the research in question. Samples may be counted wet or dry (on a filter) depending on the laboratory procedure and sample type.