FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 68200
Scriptwriter Name: Poornima G
Project Page Link: https://review.jove.com/account/file-uploader?src=20801048

Title: Multimodal Analysis of Microplastics in Drinking Water using a Silicon Nanomembrane Analysis Pipeline

Authors and Affiliations:
Teagan Horan[1], Samantha Romanick[2], Gregory Madejski[3], Ahmet Gurcan[2], James McGrath[2], James Roussie[1], Kathryn Neville[1]

[1]SiMPore Inc.
[2]Department of Biomedical Engineering, University of Rochester
[3]URnano, University of Rochester

Corresponding Authors:

Kathryn Neville	kneville@simpore.com

Email Addresses for All Authors:

Teagan Horan	thoran@simpore.com
Samantha Romanick	sromanic@ur.rochester.edu
Gregory Madejski	g.madejski@rochester.edu
Ahmet Gurcan	agurcan@u.rochester.edu
James McGrath	james.mcgrath@rochester.edu
James Roussie	jroussie@simpore.com
Kathryn Neville	kneville@simpore.com

Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. Filming location: Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 23
Number of Shots: 51

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Kathryn Neville:** Our research goals are to improve the analysis of microplastics present in multiple sample types, as well as improve the data generated from those particles of interest.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.6.2*

What are the current experimental challenges?

1.2. **Kathryn Neville:** Current microplastics analytical methods are prone to the introduction of extrinsic contamination. The methods we describe here eliminate transfer steps and alleviate this contamination problem.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.1*

What research gap are you addressing with your protocol?

1.3. **Teagan Horan:** We are providing a protocol utilizing a Silicon Nanomembrane that allows researchers to conduct Multimodal analyses of their particles of interest with increased efficiency and less contamination.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.1.1*

What advantage does your protocol offer compared to other techniques?

1.4. **Teagan Horan:** The analysis of microplastics is only as effective as the methods utilized; combining optical, electron, and spectroscopic imaging techniques allow for the fullest picture. Silicon nanomembranes enable these multiple analyses.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.3.2*

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. Quality Control Procedures and Ultrapure Solution Preparations

Demonstrator: Teagan Horan

2.1. To begin, don 100 percent cotton laboratory coat and nitrile gloves **[1]**.

2.1.1. WIDE: Talent putting on a 100 percent cotton laboratory coat and donning nitrile gloves.

2.2. Using 99 percent isopropyl alcohol, spray the nitrile gloves **[1]**. Rub the hands together thoroughly **[2]** and rinse with approximately 18 megaohm, 0.22 micrometer-filtered water **[3-TXT]**.

2.2.1. Talent spraying nitrile gloves thoroughly with 99 percent isopropyl alcohol. **Videographer's NOTE**: 2.2.1 to 2.2.3 were merged into one shot with multiple takes and angles

2.2.2. Talent rubbing gloved hands together to spread the isopropyl alcohol evenly.

2.2.3. Talent rinsing gloved hands under a stream of filtered water. **TXT: Repeat this process 3x; Use this filtered water throughout**

2.3. Fold a natural fiber delicate task wipe into quarters **[1]**, then spray with 70% isopropyl alcohol **[2]**. Wipe the hood surface from back to front in long strokes, re-folding the delicate task wipe to an unused surface every two strokes. **[3-TXT]**.

2.3.1. Talent folding a delicate task wipe into quarters. **Videographer's NOTE**: 2.3.1 to 2.3.3 were merged into one shot with multiple takes and angles

2.3.2. Talent spraying the folded wipe with 70 percent isopropyl alcohol.

2.3.3. Talent wiping the laminar flow hood surface from back to front using long, even strokes, and re-folding the wipe. **TXT: Repeat the wiping process if required**

2.4. Now, roll a silicone mat across the surface of the hood to pick up any remaining particles **[1]**. Spray the silicone roller with 99 percent isopropyl alcohol and scrub using a gloved hand **[2]**. Rinse the roller with filtered water **[3]**. After repeating the cleaning process three times, allow the roller to air-dry inside the hood **[4-TXT]**.

2.4.1. Talent rolling the mat across the hood surface to pick up debris. **Videographer's NOTE**: 2.4.1 AND 2.4.2 were merged into one shot

2.4.2. Talent spraying the silicone roller with 99 percent isopropyl alcohol and scrubbing with a gloved hand.

2.4.3. Talent rinsing the silicone roller under filtered water.

2.4.4. Talent setting the roller aside in the hood to air-dry. **TXT: Re-rinse the nitrile gloves as demonstrated earlier**

2.5. To generate ultrapure water and isopropyl alcohol, fill a 1-liter beaker with the 18 megaohm water under the hood **[1]**. Prime a 60-milliliter syringe and attached 0.22-micrometer cut-off syringe filter by pushing at least 200 milliliters of filtered water through the syringe and filter assembly **[2]**.

2.5.1. Talent filling a 1-liter beaker with filtered water under the hood.

2.5.2. Talent adding/drawing the filtered water to a syringe attached to a filter.

2.6. Then, rinse a glass screw-cap container three times with filtered water **[1]** and fill the container with syringe-filtered 18 megaohm, 0.22 micrometer-filtered water **[2]**.

2.6.1. Talent rinsing a glass screw-cap container three times with filtered water.

2.6.2. Talent filling the rinsed container with syringe-filtered water.

2.7. Repeat the beaker filling, syringe priming, and container rinsing steps using the desired percentage concentration of isopropyl alcohol instead of water to generate ultrapure isopropyl alcohol **[1]**.

2.7.1. Talent displaying final isopropyl alcohol obtained after processing.

3. Particle Capture from Liquid Samples via Filtration

3.1. Don personal protective equipment and nitrile gloves **[1]**. Spray a silicone gasket with ultrapure 99 percent isopropyl alcohol **[2]** and scrub the gasket with gloved fingers **[3]**. Then, rinse the gasket with ultrapure water **[4-TXT]**.

3.1.1. Talent putting on a PPE and nitrile gloves.

3.1.2. Talent spraying a silicone gasket thoroughly with ultrapure 99 percent isopropyl alcohol.

3.1.3. Talent scrubbing the silicone gasket with gloved fingers.

3.1.4. Talent rinsing the silicone gasket with ultrapure water. **TXT: Repeat the IPA wash 3x for each gasket**

3.2. First, generate the process blank. Utilizing the primed syringe, uptake 30 milliliters of ultrapure water and 30 milliliters of air into the 60-milliliter syringe **[1]**. Screw on a syringe filter **[2]**. Shake the syringe vigorously **[3]** and dispense the liquid and air through the filter **[4]**.

3.2.1. Talent drawing 30 milliliters of ultrapure water and 30 milliliters of air into the 60 milliliter syringe.

3.2.2. Talent screwing a syringe filter onto the syringe.

3.2.3. Talent shaking the syringe vigorously to mix.

3.2.4. Talent dispensing the contents through the syringe filter.

3.3. After rinsing 3 times, assemble the filtration apparatus according to the visual assembly graphic **[1] [2]**.

3.3.1. Talent assembling the filtration apparatus carefully.

3.3.2. LAB MEDIA: Figure 1B *Video Editor: Please use split screen to show both the shots of this step*

3.4. Turn on the vacuum to the filtration apparatus to create a negative flow through the filter disk stack **[1]**.

3.4.1. Talent turning on the vacuum and confirming negative flow through the stack.

3.5. To measure the background contamination of the process blank, dispense 50 milliliters of ultrapure water slowly over the nanomembrane in the center of the top disk using the rinsed syringe **[1]**. Allow the ultrapure water to filter through **[2-TXT]**. Once the sample is dry, turn off the vacuum **[3]**.

3.5.1. Talent dispensing 50 milliliters of ultrapure water over the nanomembrane using the rinsed syringe.

3.5.2. Shot of the ultrapure water filtering through the nanomembrane. **TXT: After filtration, keep the vacuum on for 1 min**

3.5.3. Talent turning off the vacuum once the sample is fully dry. **Videographer's NOTE**: along with other Vacuum shots, this was not that captivating so I shot various angles and tried methods to make the shot somewhat interesting.

3.6. Using clean tweezers, carefully remove the filter disks from the gaskets **[1]** and place them into a clean, labeled container such as a glass Petri dish or a darkened box **[2]**. Image the filter disks under microscopy for optical analysis and particle counting **[3]**.

3.6.1. Talent removing filter disks carefully with tweezers.

3.6.2. Talent placing the filter disks into a clean and labeled container.

3.6.3. Talent placing the filter disks under a microscope.

3.7. For experimental liquid samples, repeat the syringe rinsing process with an additional cleaned gasket and syringe-filter unit **[1]**. Next, uptake the desired amount of the new sample **[2]** and dispense the sample slowly over the nanomembrane in the center of the top disk **[3]**.

3.7.1. Talent rinsing the syringe. **Videographer's NOTE**: A pipette was used here

3.7.2. Talent drawing the desired amount of sample into the syringe.

3.7.3. Talent dispensing the sample slowly over the nanomembrane.

3.8. Once sample filtration is complete, rinse the membrane three times with 1 milliliter of ultrapure water **[4-TXT]**.

3.8.1. Talent adding the membrane with 1 milliliter of ultrapure water each time. **TXT: Vacuum and dry the sample**

4. Fluorescent Dye Preparation, Staining and Data Acquisition

4.1. Rinse two glass screw cap containers three times with ultrapure water **[1]**. Prepare a 0.1 milligram per milliliter solution of Nile Red in ultrapure 99 percent isopropyl alcohol in a clean glass container **[2]**. Gently invert the container ten times to mix the solution **[3]**. Filter the Nile Red solution into the second glass screw cap container **[4]**.

4.1.1. Talent adding ultrapure water to two glass screw cap containers.

4.1.2. Talent adding Nile Red to a clean glass container.

4.1.3. Talent gently inverting the glass container.

4.1.4. Talent adding the prepared Nile Red solution into the filtration unit placed in a second clean glass screw cap container.

4.2. Place the filter disk to be stained onto the support frit of the vacuum collection flask **[1]** and pipette 20 microliters of the 0.1 milligram per milliliter Nile Red solution onto the nanomembrane at the center of the filter disk **[2]**.

4.2.1. Talent placing the filter disk carefully onto the support frit of the vacuum collection flask.

4.2.2. Talent pipetting and dispensing 20 microliters of Nile Red solution precisely onto the center of the nanomembrane.

4.3. Incubate the stain on the nanomembrane for 5 minutes **[1]** and then vacuum filter the stain **[2]**.

4.3.1. Talent placing the sample aside.

4.3.2. Talent initiating vacuum filtration to remove the stained solution.

4.4. Rinse the filter disk three times with 1 milliliter of ultrapure 99 percent isopropyl alcohol to remove excess Nile Red stain **[1]**.

4.4.1. Talent adding the filter disk with 1 milliliter of ultrapure 99 percent isopropyl alcohol each time.

4.5. Allow the filter disk to sit on the support frit with the vacuum on for 2 minutes to filter and dry any residual liquid **[1]**. If it still does not dry after 2 minutes, transfer it to a 70-degree Celsius oven for 2 to 5 minutes using a clean glass Petri dish **[2]**.

4.5.1. Shot of the filter disk on the frit under vacuum.

4.5.2. Talent transferring the filter disk into a 70-degree Celsius oven.

4.6. For particle quantification, immobilize the filter disk on a microscope slide using a silicone gasket **[1]** and move it onto the microscope stage **[2]**.

4.6.1. Talent placing the filter disk onto a microscope slide using a silicone gasket.

4.6.2. Talent transferring the sample to the microscope stage.

4.7. Image the nanomembrane using brightfield illumination so that the maximum detected counts are approximately 90% of the detector camera's maximum range **[1]**.

4.7.1. Shot of the talent working at the microscope to capture brightfield images of the nanomembrane.

4.8. Image the nanomembrane using fluorescent illumination so that the maximum pixel intensities are around 25% of the detector camera's maximum range **[1]**.

4.8.1. Shot of the computer screen showing fluorescent images of the nanomembrane being captured. **Videographer's NOTE**: Filming screen was not possible as no real experiment was going on. I filmed various shots of talent at computer screens and scope to be able to utilize for 4.8.1 and 4.9.1

4.9. Finally, save the acquired images as a 16-bit composite TIFF file **[1]**.

4.9.1. Shot of the computer screen showing the images on the software interface being saved in 16-bit composite TIFF format. **Videographer's NOTE**: Filming screen was not possible as no real experiment was going on. I filmed various shots of talent at computer screens and scope to be able to utilize for 4.8.1 and 4.9.1

Results

5. Results

5.1. Bare silicon nitride and gold-coated silicon nitride nanomembranes were suitable for specific analysis types. Bare silicon nitride was suitable for transmission based optical techniques as well as spectroscopy **[1]**, while gold coated silicon nanomembranes were suitable for reflection-based techniques **[2]**.

5.1.1. LAB MEDIA: Figure 1. *Video editor – emphasize the panel for "Pipeline A"*

5.1.2. LAB MEDIA: Figure 1. *Video editor – emphasize both panels for "Pipeline B" and "Pipeline C"*

5.2. An ideal cascade of data is shown that was generated off a single silicon nanomembrane **[1]**. Suspected microplastic particles stained with Nile Red indicated that the tested tap water samples had a significantly higher count of particles greater than 20 microns compared to the 8 to 20-micron subfraction **[2]**.

5.2.1. LAB MEDIA: Figure 3

5.2.2. LAB MEDIA: Figure 3E. *Video editor: Emphasize the Dark red bars (20 micrometer)*.

5.3. Raman spectra collected with an 830-nanometer laser had a high correlation coefficient on the same particle **[1]** analysed with optical microscopy **[2]**. Spectra revealed that the particle was comprised of Polyethylene **[3]**.

5.3.1. LAB MEDIA: Figure 3C.

5.3.2. LAB MEDIA: Figure 3A.

5.3.3. LAB MEDIA: Figure 3C.

5.4. Scanning electron microscopy revealed detailed morphological features of particles captured on a silicon nanomembrane **[1]**.

5.4.1. LAB MEDIA: Figure 3D.

5.5. Energy-dispersive X-ray spectroscopy analysis showed that the main particle composition was primarily carbon and nitrogen. This, along with a Trypan-blue stain uptake, suggests that the particle is likely organic in origin **[1]**.

5.5.1. LAB MEDIA: Figure 3F. *Video editor: Highlight the C and N rows in the table*

5.6. Suboptimal sample preparation yielded unclear data. Improperly rinsed Nile Red stain makes particle identification difficult **[1]** and suboptimal Raman spectra with a low correlation coefficient was obtained, suggesting that the particle's chemical identity cannot be reliably confirmed **[2]**.

5.6.1. LAB MEDIA: Figure 5A.
5.6.2. LAB MEDIA: Figure 5B

Pronunciation Guide

---

1. Isopropyl

Pronunciation link:
https://www.merriam-webster.com/dictionary/isopropyl
IPA: /ˌaɪsəˈproʊpəl/
Phonetic Spelling: eye-suh-proh-puhl

---

2. Micrometer

Pronunciation link:
https://www.merriam-webster.com/dictionary/micrometer
IPA: /maɪˈkrɑːmɪtər/ (note: this is for the measurement instrument; used in context, it likely refers to a micrometric unit, pronounced /ˈmaɪkroʊˌmiːtər/)
Phonetic Spelling: my-kroh-mee-ter

---

3. Megaohm

Pronunciation link:
https://www.howtopronounce.com/megaohm
IPA: /ˈmɛgəˌoʊm/
Phonetic Spelling: meh-guh-ohm

---

4. Laminar

Pronunciation link:
https://www.merriam-webster.com/dictionary/laminar
IPA: /ˈlæmɪˌnɑːr/
Phonetic Spelling: la-muh-nar

5. Nanomembrane

Pronunciation link:
https://www.howtopronounce.com/nanomembrane
IPA: /ˈnænoʊˌmɛm.breɪn/
Phonetic Spelling: nan-oh-mem-brayn

6. Filtration

Pronunciation link:
https://www.merriam-webster.com/dictionary/filtration
IPA: /fɪlˈtreɪʃən/
Phonetic Spelling: fil-tray-shun

7. Syringe

Pronunciation link:
https://www.merriam-webster.com/dictionary/syringe
IPA: /səˈrɪndʒ/
Phonetic Spelling: suh-rinj

8. Petri

Pronunciation link:
https://www.merriam-webster.com/dictionary/Petri%20dish
IPA: /ˈpiːtri/
Phonetic Spelling: pee-tree

9. Spectroscopy

Pronunciation link:
https://www.merriam-webster.com/dictionary/spectroscopy
IPA: /spɛkˈtrɑːskəpi/
Phonetic Spelling: spek-trah-skuh-pee

10. Trypan-blue

Pronunciation link:
https://www.howtopronounce.com/trypan-blue
IPA: /ˈtrɪpæn bluː/
Phonetic Spelling: trip-an bloo

11. Polyethylene

Pronunciation link:
https://www.merriam-webster.com/dictionary/polyethylene
IPA: /ˌpɑːliˈɛθəˌliːn/
Phonetic Spelling: pah-lee-eth-uh-leen

12. Nile Red

Pronunciation link:
https://www.howtopronounce.com/nile-red
IPA: /naɪl rɛd/
Phonetic Spelling: nile red

13. Silicon

Pronunciation link:
https://www.merriam-webster.com/dictionary/silicon
IPA: /ˈsɪlɪˌkɑːn/
Phonetic Spelling: sil-ih-kahn

14. Raman

Pronunciation link:
https://www.howtopronounce.com/raman
IPA: /ˈrɑːmən/
Phonetic Spelling: rah-mun