Microplastics in the Environment

Optimizing membrane filter selection for analytical methods used to characterize microplastics

Lindsay D. Lozeau, Maricar Dube, Kevin Sydlowski, Ranjani Muralidharan
05 AUGUST 2024

MilliporeSigma is the U.S. and Canada Life Science business of Merck KGaA, Darmstadt, Germany.

Science & Lab Solutions - Biology

Filtration and Sample Preparation across Environmental Workflows

Sample Collection | **Sample Preparation** | **Sample Analysis**

Accessories

Sample Prep

Solvents & Reagents

Columns

Reference Standards

Analytical Sample Preparation

Nonsterile Millex® syringe filters

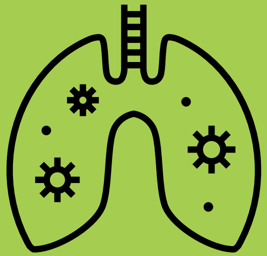
Cut Disc Filter Membranes

Analytical Equipment and Hardware

General Filter Holders & Supporting Equipment

Specialized Hardware

EMERGING CONTAMINANTS



2015

2018

2020

2023

MICROPLASTICS

Analytical Methods
Published and In Process

Name	Date	Portion of Workflow	Matrix	Sample Prep	Analytical Method(s)
NOAA NOS-OR&R-48	JUL 2015	Entire workflow	Seawater, sediment, bed samples	Sieve, density settle & digest	Microscopy
ASTM D8332-20	AUG 2020	Sampling	Drinking water, surface water, wastewater influent, effluent, marine waters	Sieve	Py-GC/MS, IR or Raman Spectroscopy, Microscopy
ASTM D8333-20	AUG 2020	Sampling	Drinking water, surface water, wastewater influent, effluent, marine waters	Sieve, wet peroxide oxidation	Py-GC/MS, IR or Raman Spectroscopy, Microscopy
SWRCB	**NOV 2021** AUG 2022	Entire workflow	Drinking water	Sieve, filtration, microscopy	Microscopy, IR or Raman Spectroscopy
prEN ISO 16094	**Draft**	3 parts, sampling through analysis	Drinking water, ground water, precipitation water, surface water, water post-treatment	Sieve, filtration, microscopy	Spectroscopy, Py-GC/MS
ISO/CD 5667	**Draft**, started 2021	Sampling	Drinking water, surface water, freshwater, seawater, wastewater & effluents		Spectroscopy, Py-GC/MS
prEN ISO 4484	Part 1 Final **draft** 2023	3 parts related to measurement	Textiles in water		
ASTM DXXXX	**Working group**	Entire workflow	Influent, Effluent, wastewater, ambient water, drinking water, bottled water	Sieve, filtration, microscopy	Microscopy, IR Spectroscopy
ASTM DXXXX	**Working group**	Entire workflow	Drinking water, wastewater, surface water, ground water, marine waters	Sieve, filtration, microscopy	Microscopy, Py-GC-MS
ASTM 67563	**Draft**	Sampling	Sewage, wastewater effluent	Sieve	N/A

Published Method

In development

Updated as of May 2024

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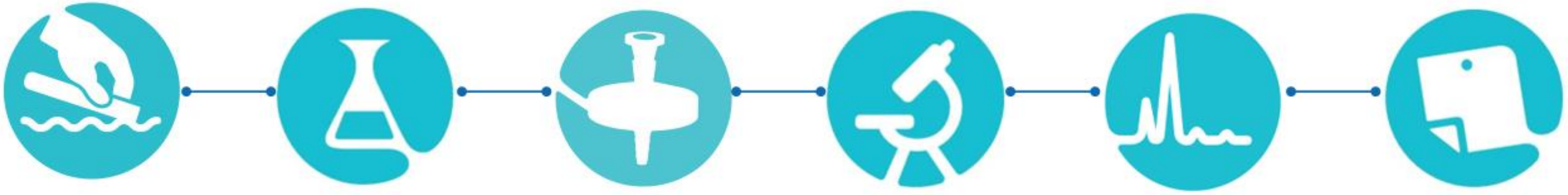
The Microplastics Workflow is **Diverse**



Sample Collection

Sample Preparation

Filtration

- Digestion
- Sieving/filtration
- Density/oil separation

Visual Identification

- Microscopy
- Fluorescence
- Electron Microscopy

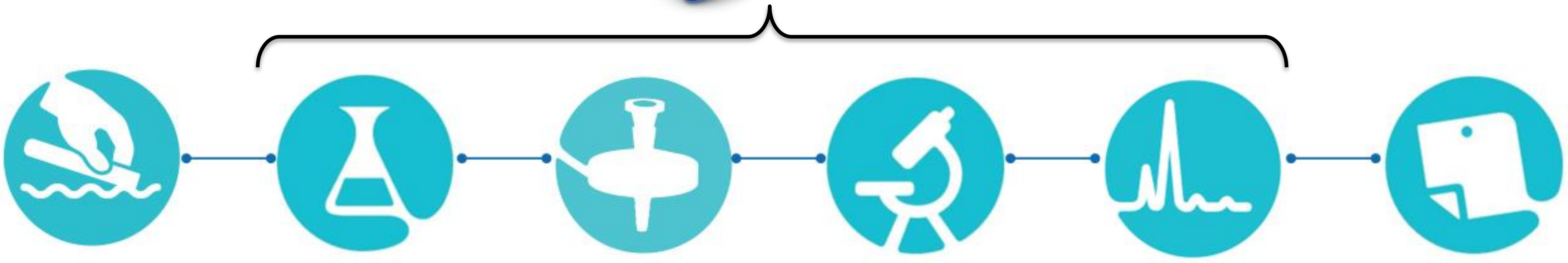
Spectral or Chromatographic Analysis

- FTIR spectroscopy
- Raman spectroscopy
- Pyrolysis-GC-MS

Data Analysis & Reporting

MilliporeSigma, **2024**. *Membrane filters for microplastics analysis*.

The Microplastics Workflow is Diverse
Filtration Involved in Majority of Workflows



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One Membrane to Rule them All?

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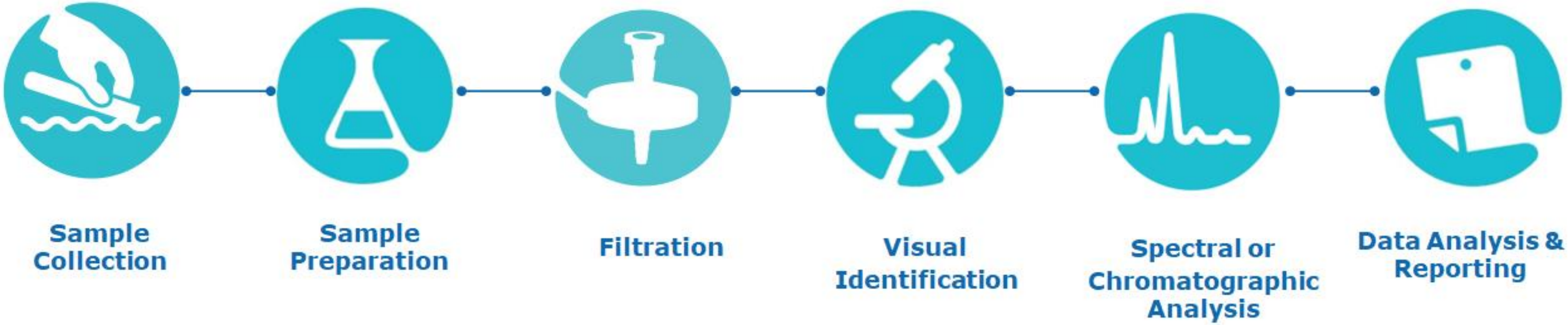

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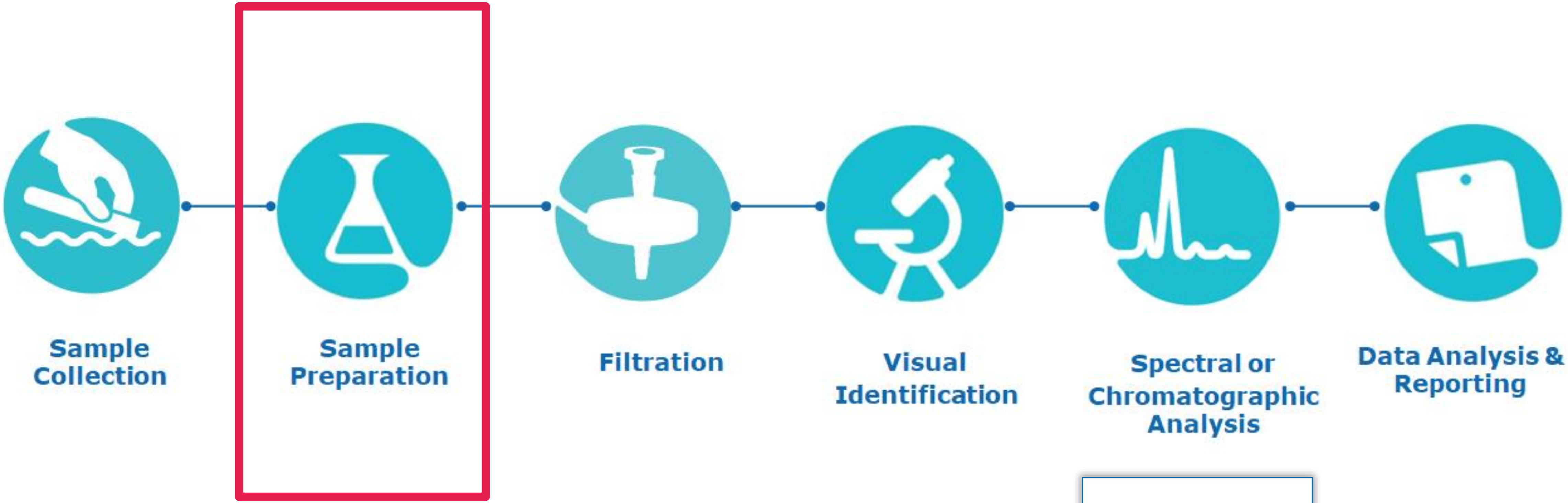
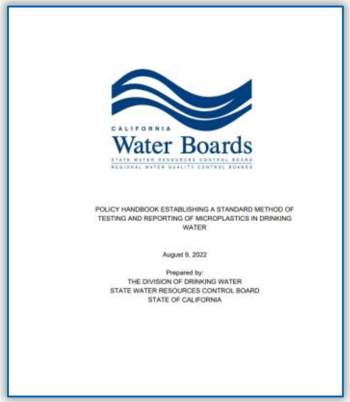


MAG Water – Microplastics Analysis Grade water


CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS

POLICY HANDBOOK ESTABLISHING A STANDARD METHOD OF TESTING AND REPORTING OF MICROPLASTICS IN DRINKING WATER

August 9, 2022

Prepared by:
THE DIVISION OF DRINKING WATER
STATE WATER RESOURCES CONTROL BOARD
STATE OF CALIFORNIA

Division of drinking water, *State water resources control board*, State of California, **2022**. Policy handbook establishing a standard method of testing and reporting of microplastics in drinking water.

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CALIFORNIA
Water Boards
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“High purity water filtered through a filter with pore-size 1 µm or smaller…”

Swinnex

Methods: *Flow labeled bead solutions of different diameters through filters assembled in Swinnex, determine retention spectrophotometrically in filtrate compared to standard curve.*

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California Water Boards
State Water Resources Control Board
Regional Water Quality Control Boards

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Material	Retention (µm)	Cat. No.	Average % Particle Retention			Recommended for making MAG Water? (Free of 1.0 µm particles?)
			5.09 µm	0.96 µm	0.784 µm	
Glass fiber	0.7	APFF04700	99.9±0.02	9.99±0.014	100±0.05	Yes
	1.0	APFB04700	99.9±0.05	99.97±0.018	100±0.00	Yes
	1.2	APFC04700	99.9±0.19	99.99±0.011	99.9±0.07	No
Quartz fiber	--	AQFA04700	99.9±0.04	100.0±0.009	99.9±0.05	Yes
Polycarbonate	0.8	ATTP04700	99.9±0.12	99.36±0.035	62.1±4.30	Yes
	1.2	RTTP04700	100±0.05	18.05±2.735	7.70±0.97	No
	2.0	TTTP04700	99.9±0.04	5.733±1.101	3.76±0.67	No
Mixed cellulose ester (MCE)	0.8	AABP04700	99.8±0.27	99.99±0.015	100±0.01	Yes
	1.2	RAWP04700	100±0.05	100.0±0.017	100.0±0.01	No

Division of drinking water, *State water resources control board*, State of California, **2022**. Policy handbook establishing a standard method of testing and reporting of microplastics in drinking water.

"High purity water filtered through a filter with pore-size 1 µm or smaller…"

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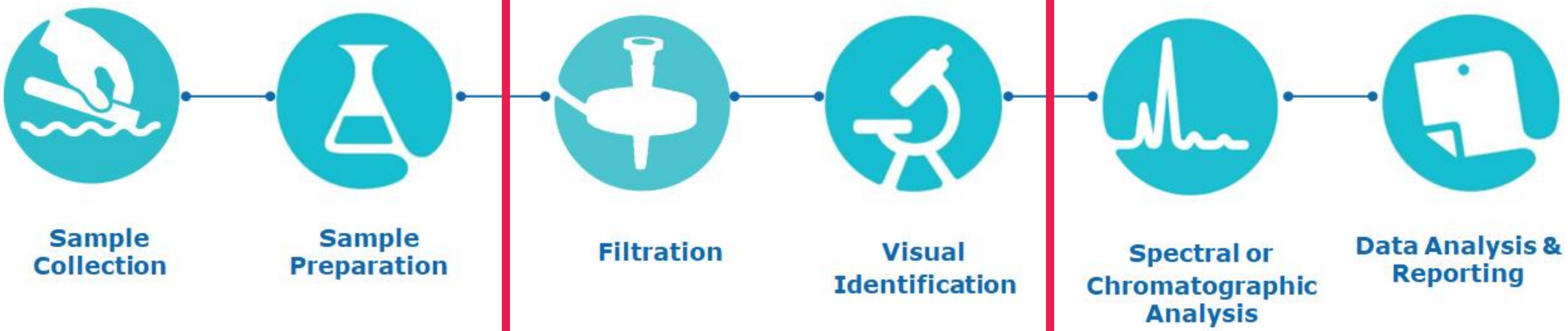
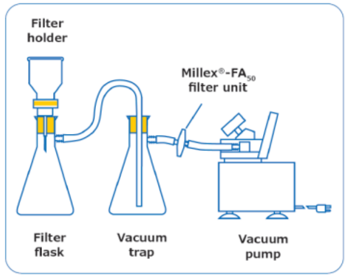
Filtration Involved in Majority of Workflows



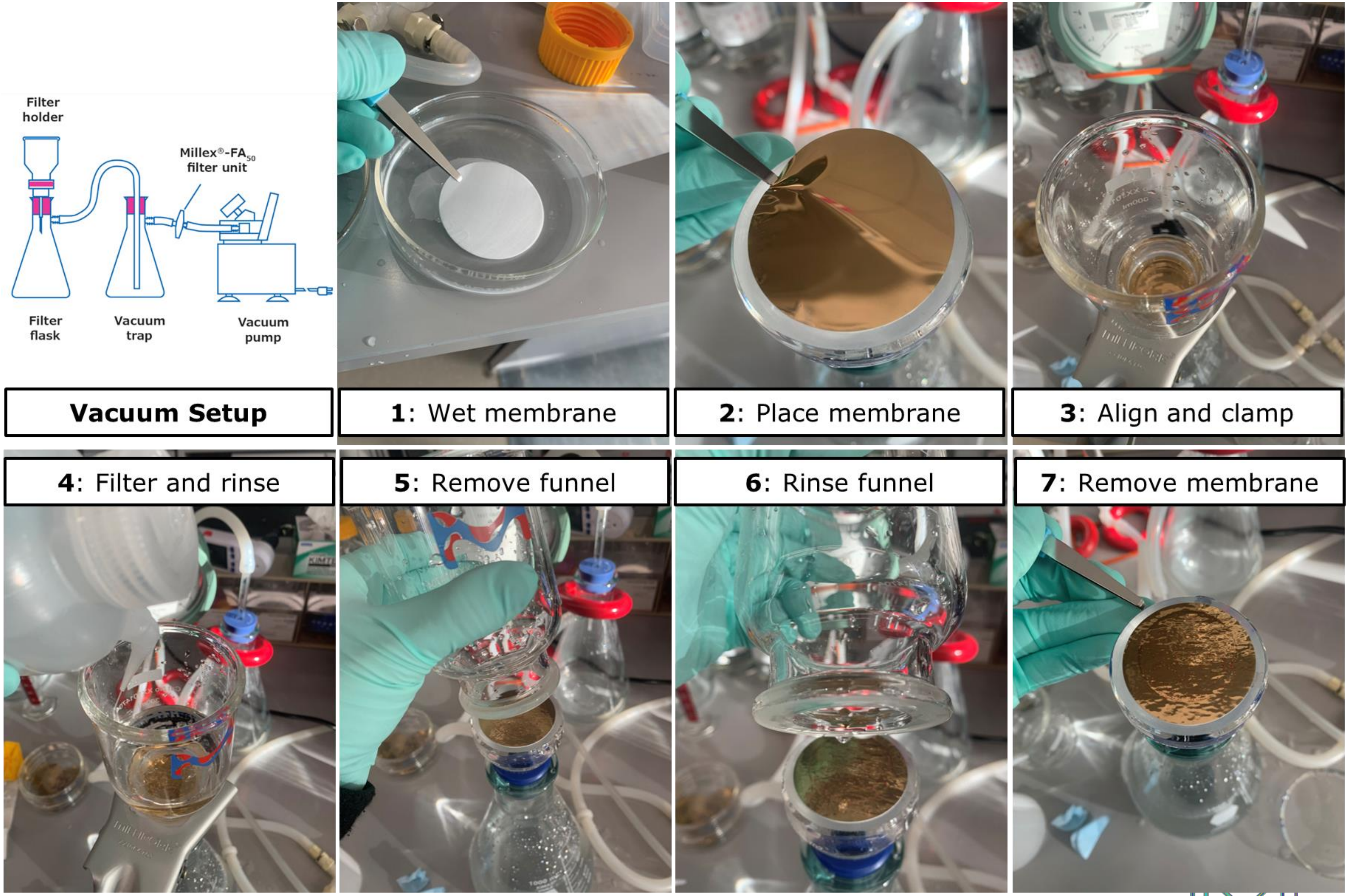


Vacuum Workflow

Recommended practices, drying, particle recovery, light microscopy

MilliporeSigma, **2024**. *Membrane filters for microplastics analysis*.

Establishing the Vacuum Workflow to Isolate Microplastics



Ways to reduce particle loss →

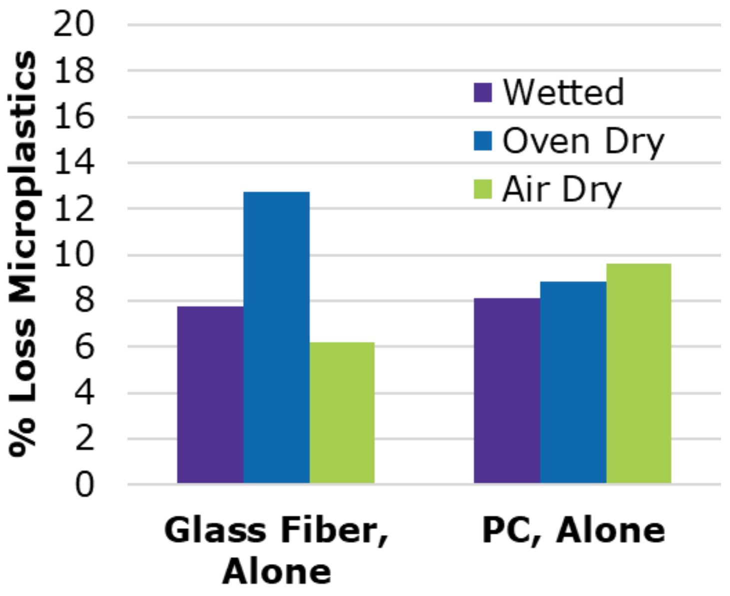
K. Sydlowski (**2023**)

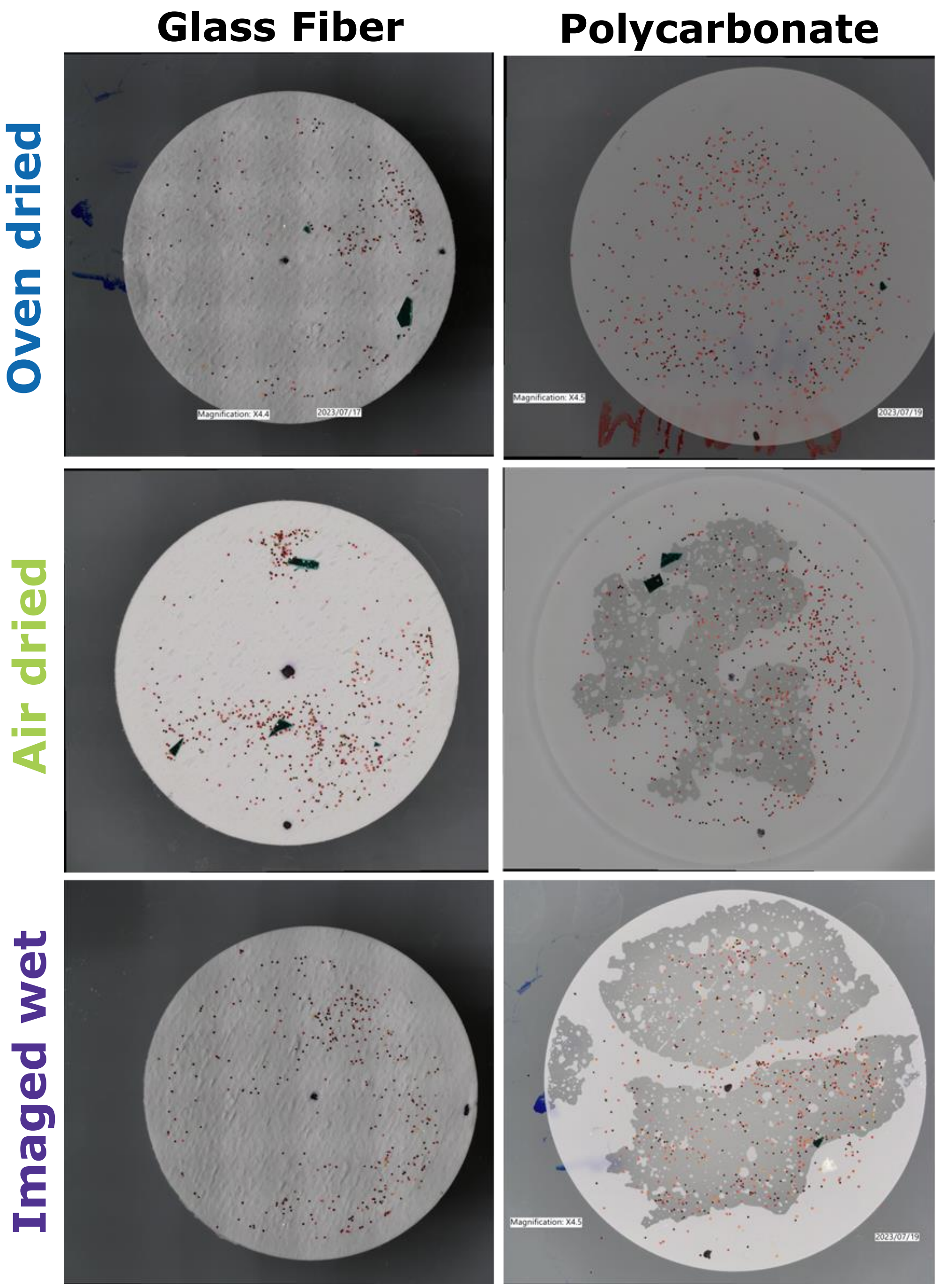
Establishing the Vacuum Workflow
Counting particles using microscopy

Method
Step 1: Spike (glitter)
Step 2: Filter/Rinse
Step 3: Drying procedures
Adomat, Y., *et al.* (**2021**), *Sci. Total. Environ.*
Step 4: Image/Observe
Step 5: Count

	Handling	Recovery	Image quality
Oven	red	yellow	green
Air	yellow	light green	yellow
Kept Wet	green	light green	red

Percent Loss Particles





Data: K Sydlowski (**2023**)
4.4-4.5x MAG, Image stitched over full 47mm disc

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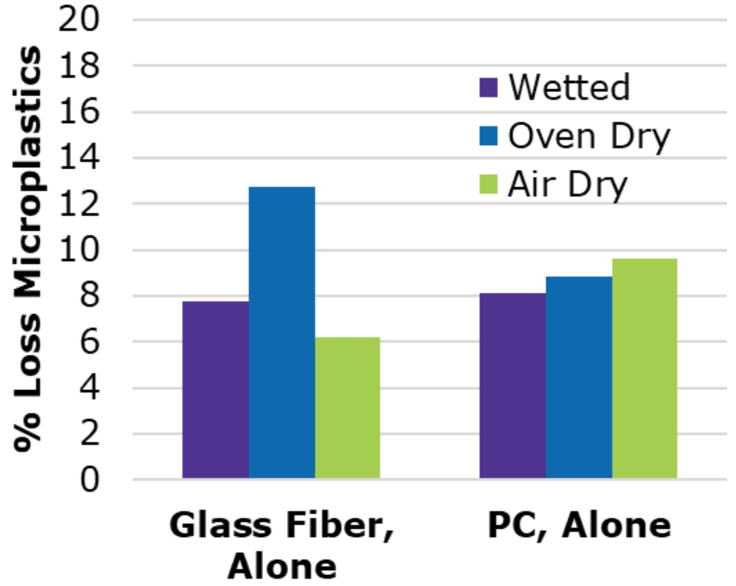

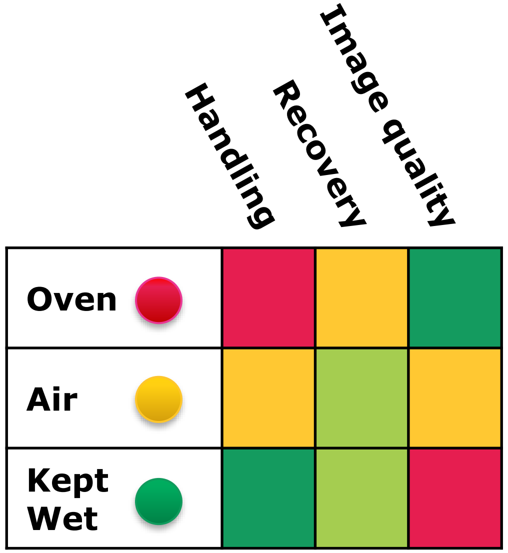

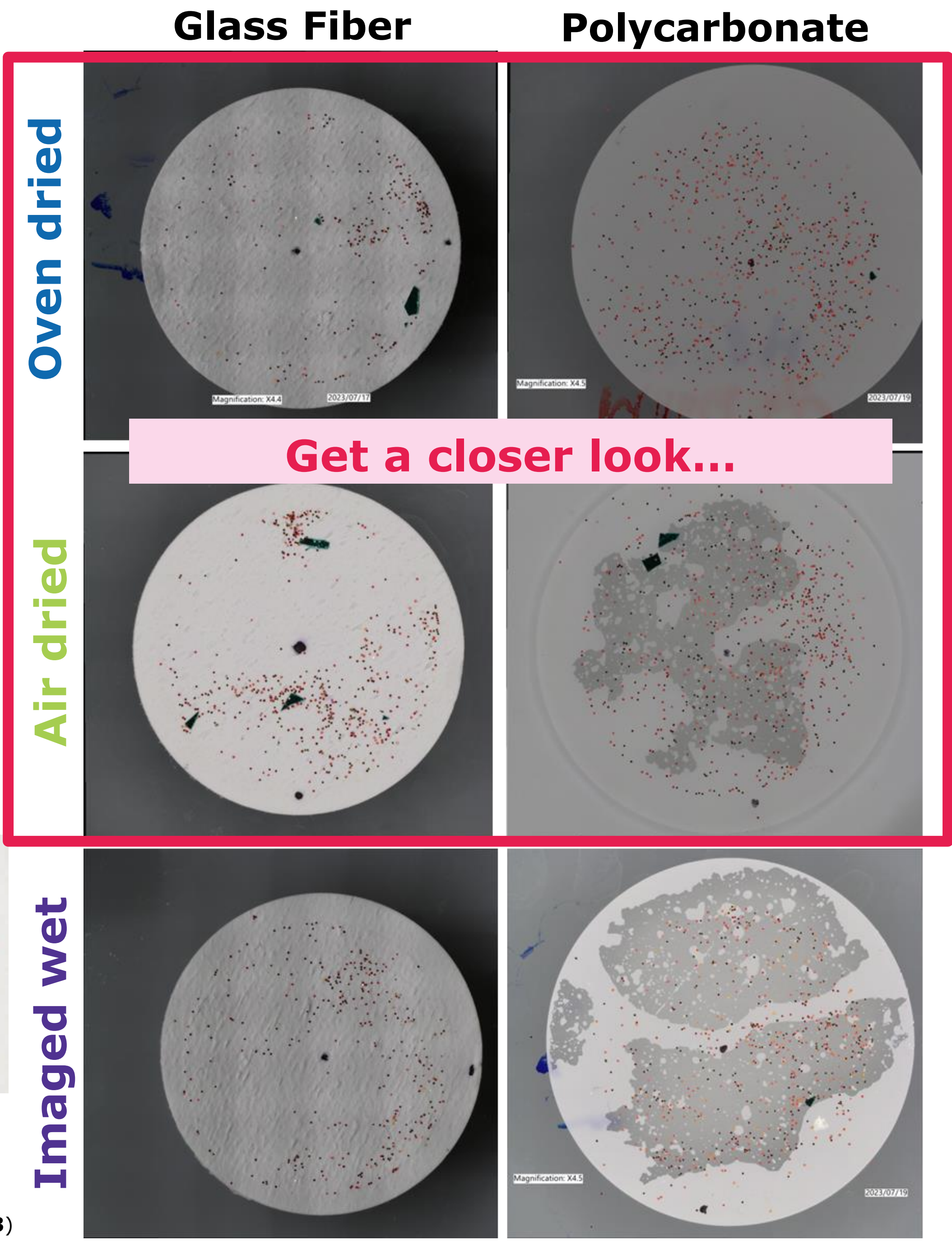

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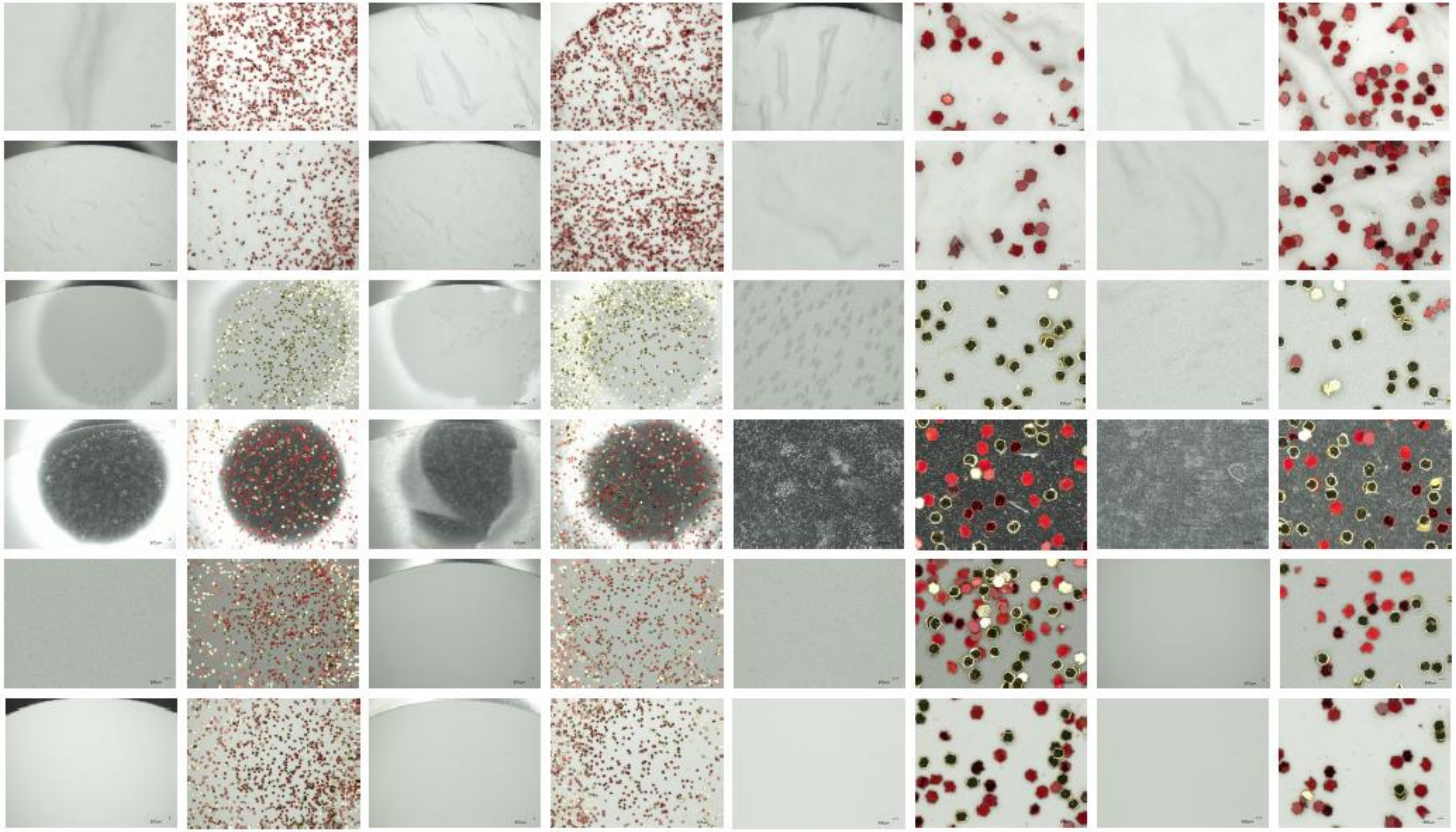
MilliporeSigma, **2024**. *Membrane filters for microplastics analysis*.

		20x Magnification				100x Magnification			
		Membrane wetted with PBS, air died	Filtered microplastics, air dried	Membrane wetted with PBS, dried at 65 °C	Filtered microplastics, dried at 65 °C	Membrane wetted with PBS, air died	Filtered microplastics, air dried	Membrane wetted with PBS, dried at 65 °C	Filtered microplastics, dried at 65 °C

Good for MAG water, fluorescence, sturdy filters: 1.0 μm Glass fiber; Quartz fiber

Good FTIR analysis & SEM/TEM; wide retention: 0.8 μm polycarbonate; 8.0 μm polycarbonate

Gridded option, good for counting: 0.8 μm black mixed cellulose ester (MCE); 1.2 μm black (MCE)



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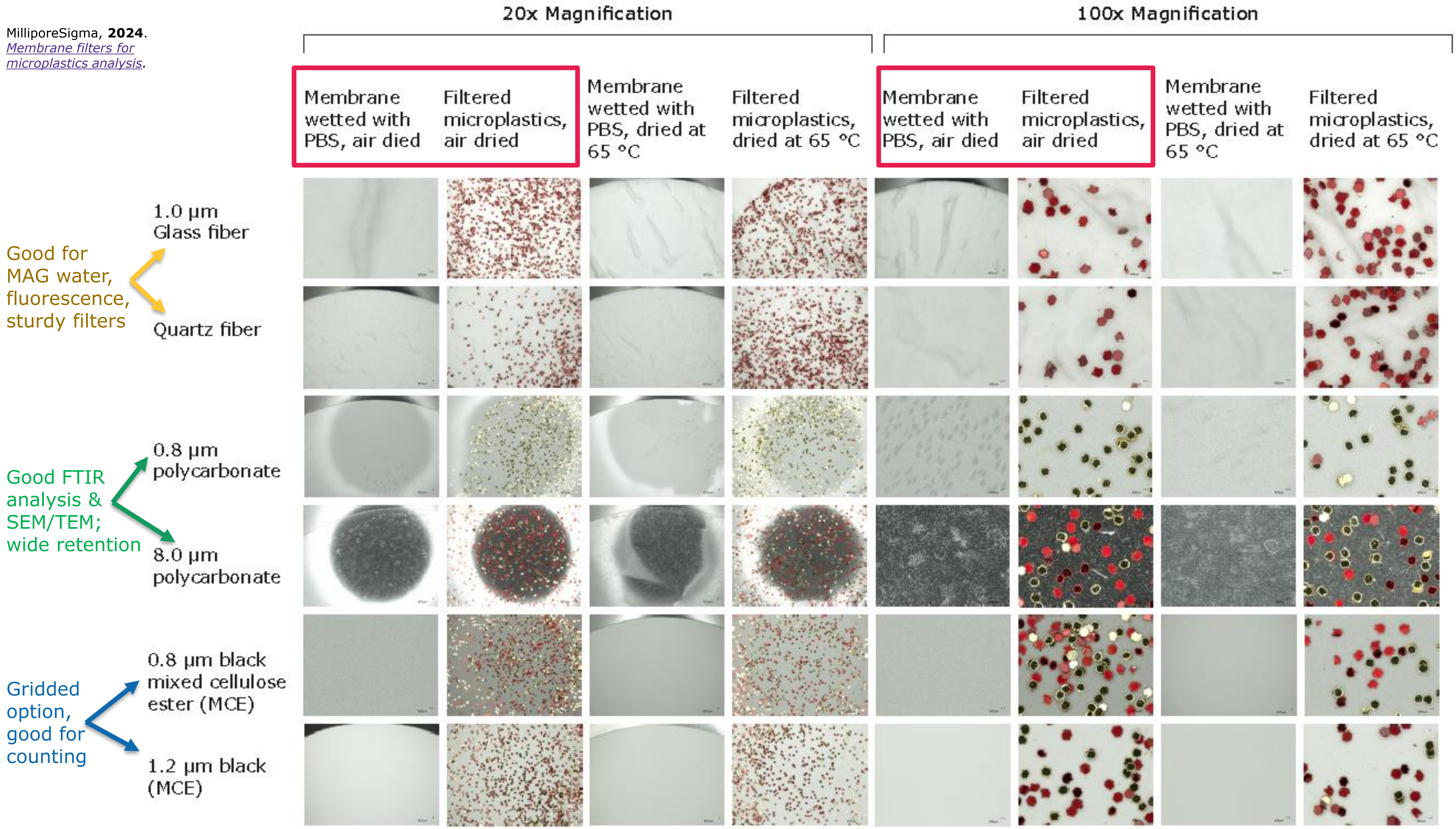

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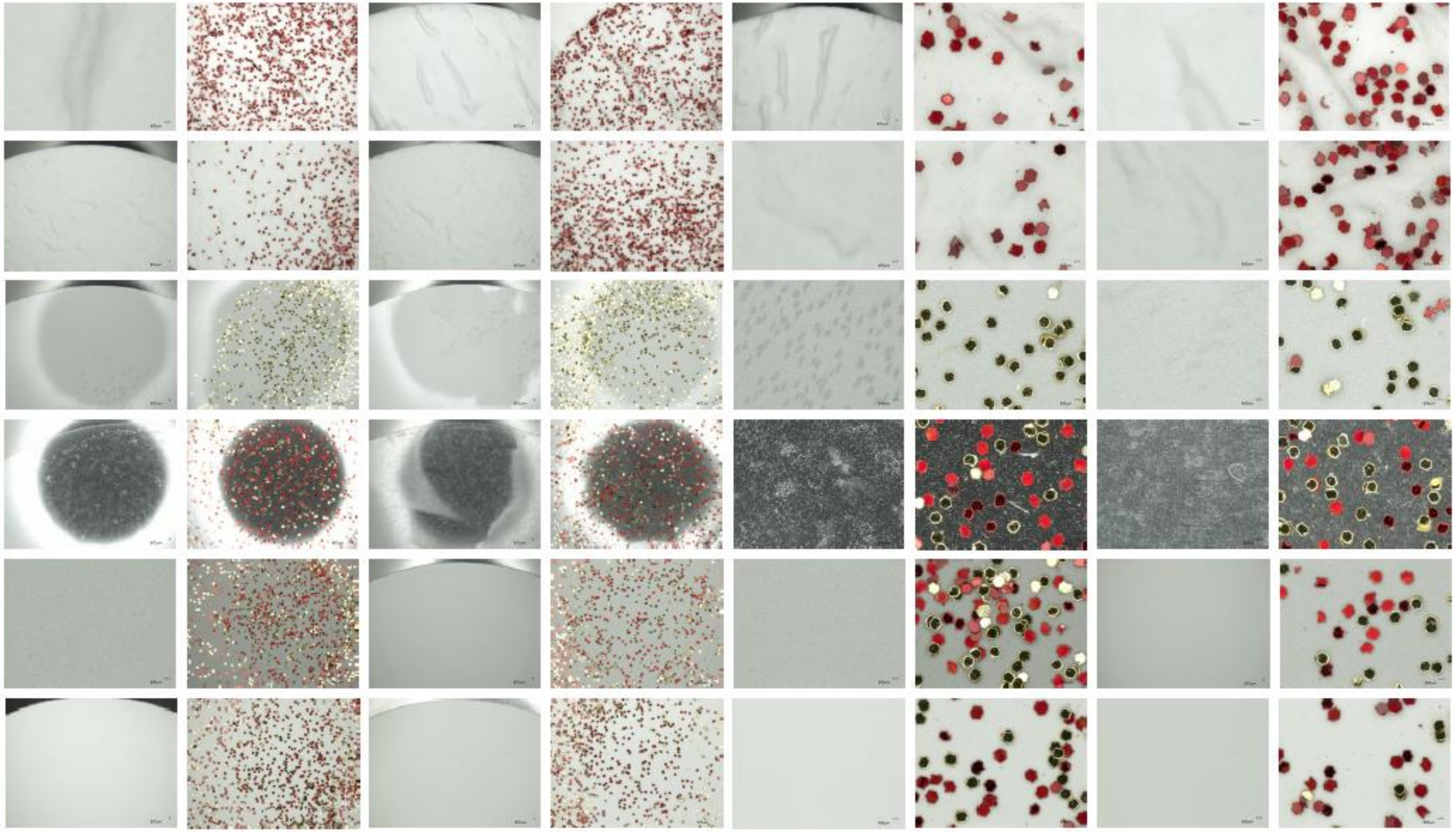

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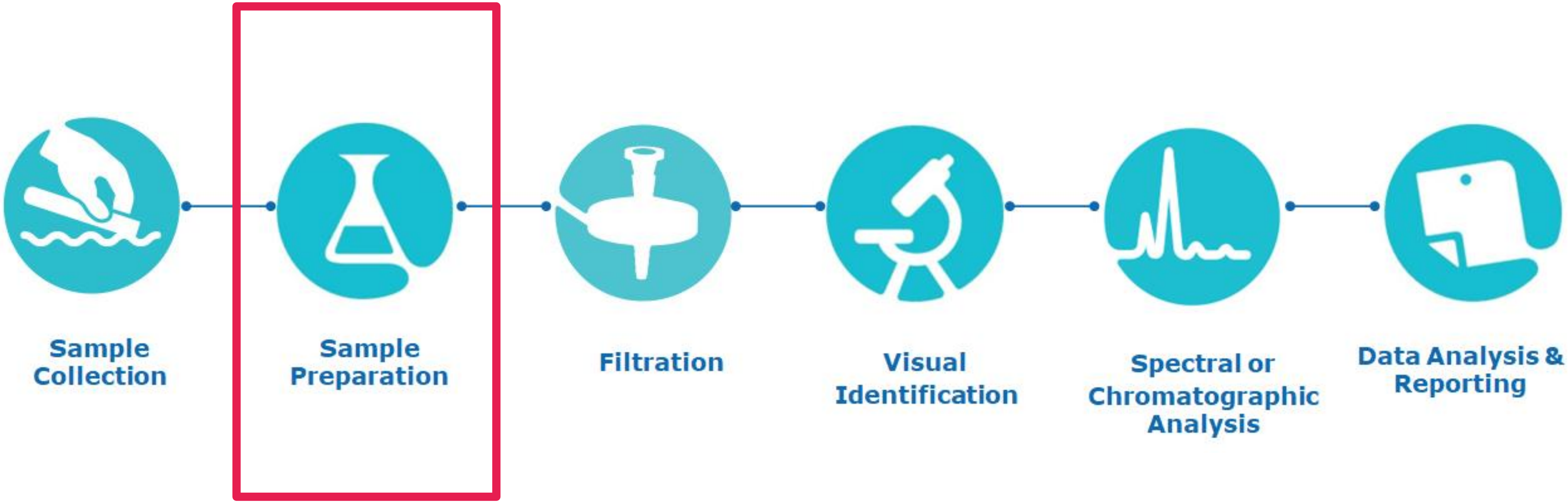
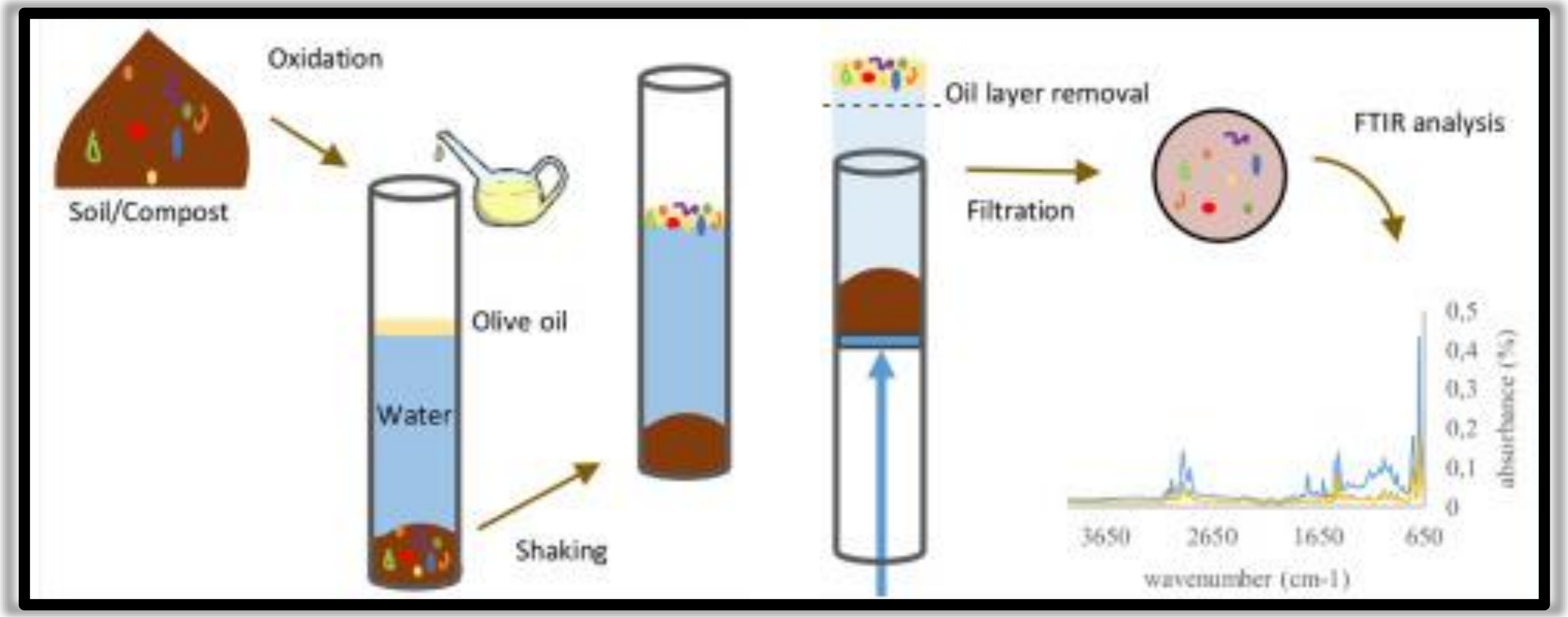


High Particulate Samples

Separation methods, chemical digestion

MilliporeSigma, **2024**. *Membrane filters for microplastics analysis*.

Common Microplastics Extraction Methods
Salt Separation, Digestion and Oil Flotation



Method: Determine throughput of various microfilter types, 0.2 µm pore size, of 160 mL, 5% (v/v) olive oil solution in hypersaline water (34 g/L NaCl) with vacuum filtration workflow.

Scopetani, Costanza, *et al. Sci. Total Environ.*, **2020**, *733*: 139338.

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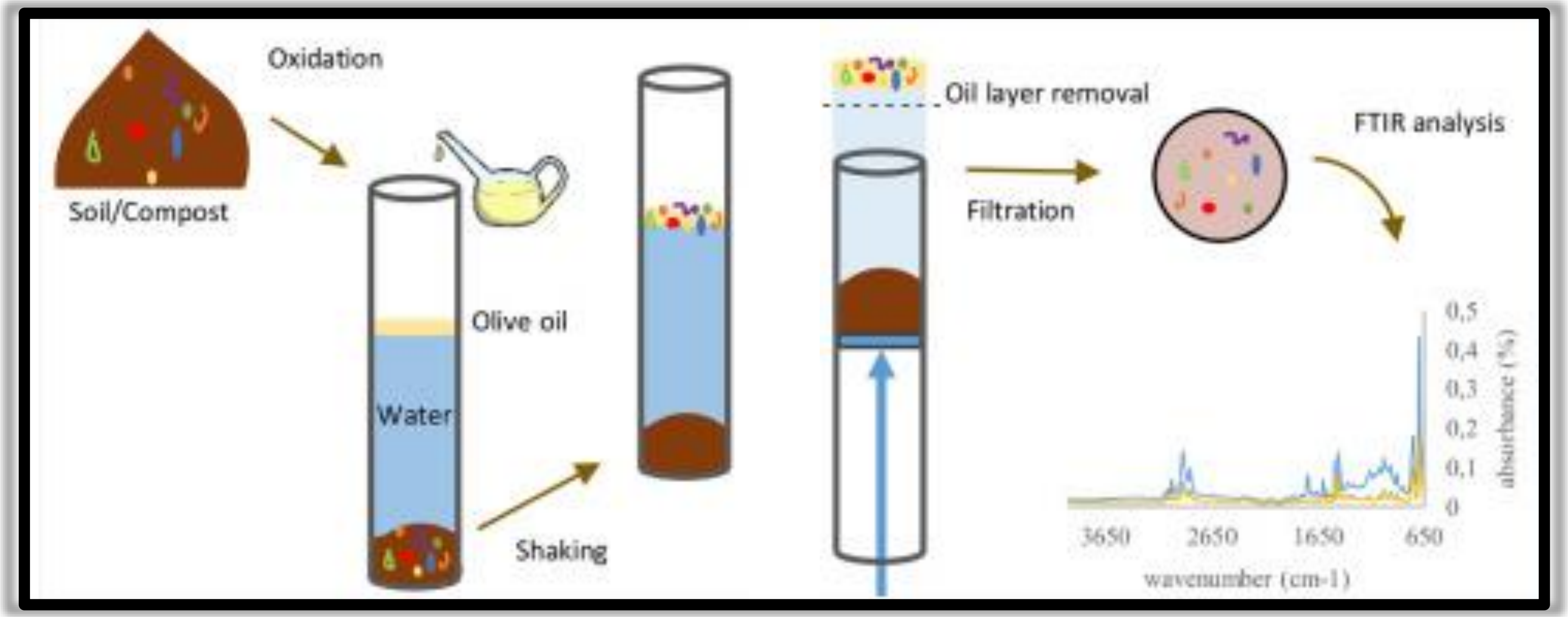

= unfilterable category…

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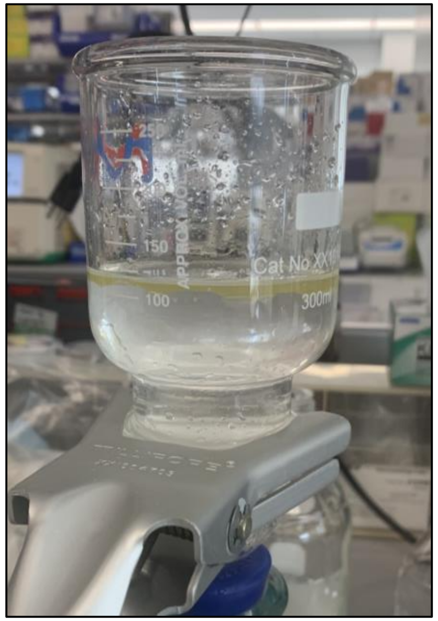

Filtering an unfilterable: Oil Flotation

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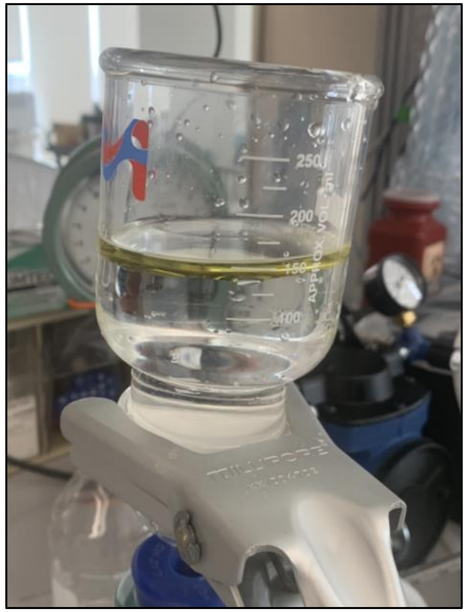

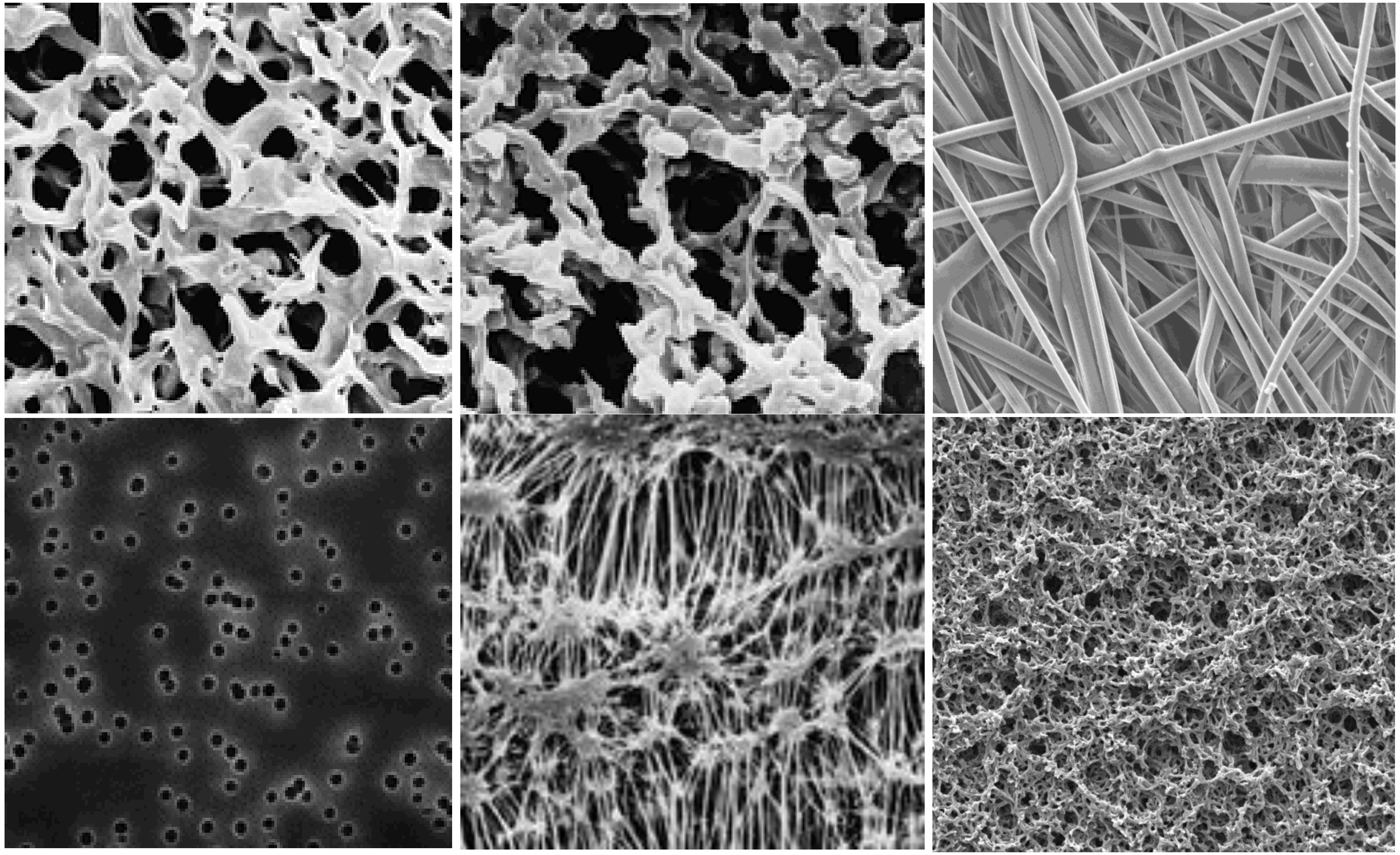

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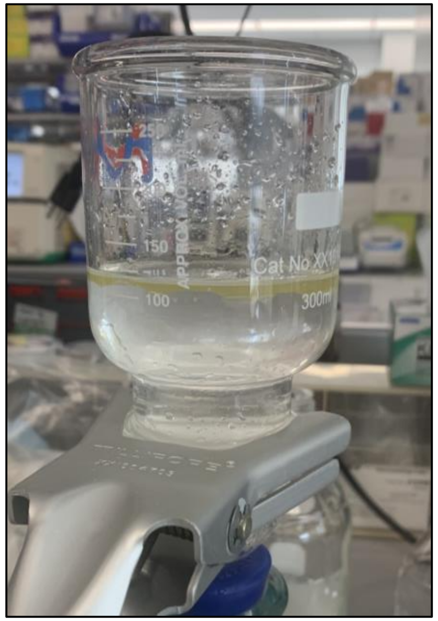

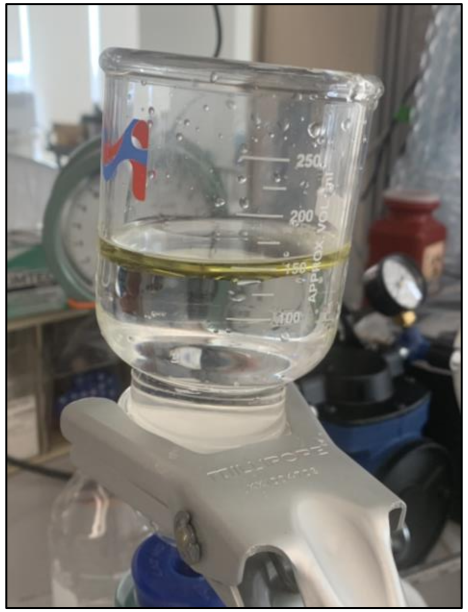

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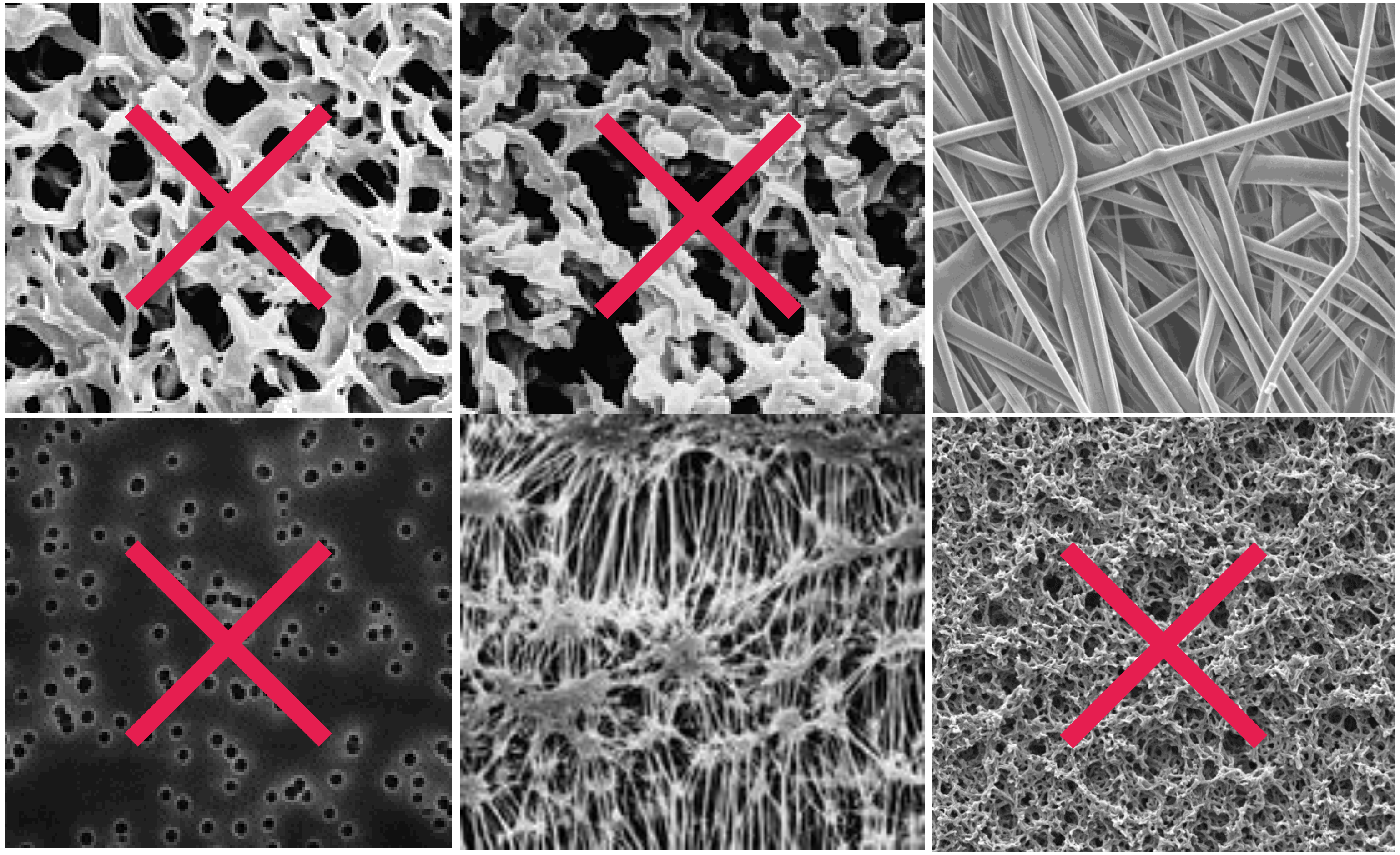


Chemical Digestion & Salt Separation
Membrane Compatibility

→ **Salt solutions (density separation)** typically omitted from compatibility charts (NaCl, NaI, $ZnBr_2$, etc. [6])

Fenton Reaction (→ Hydrogen peroxide, Iron sulfate heptahydrate)

Filter Type	Hydrogen peroxide (30% v/v)	Iron sulfate heptahydrate (0.05 M)	Potassium Hydroxide, Alkaline (10% v/v)
Glass Fiber	• **Recommended**[1] • *Difficult to find broad datasets*	• **No data**	• **Not recommended** (3-6N)[1,2]
Quartz Fiber	• **No data**	• **No data**	• **No data**
Polycarbonate	• **Recommended**[1,3,4] • *Varying percentages (3-90%)*	• **No data**	• **Not Recommended/Poor** (3-6N)[1,3,4]
Mixed Cellulose Ester (MCE) white	• **CONFLICTING DATA**[1,3] • *Both recommended and not recommended*	• **No data**	• **CONFLICTING DATA**[1-5] • *Primarily Not Recommended but some claim resistant, 3-6N*
Mixed Cellulose Ester (MCE) black	• **No data**	• **No data**	• **CONFLICTING DATA**[1-5] • *Primarily Not Recommended but some claim resistant, 3-6N*
Aluminum Oxide	• **No data**	• **No data**	• **No data**

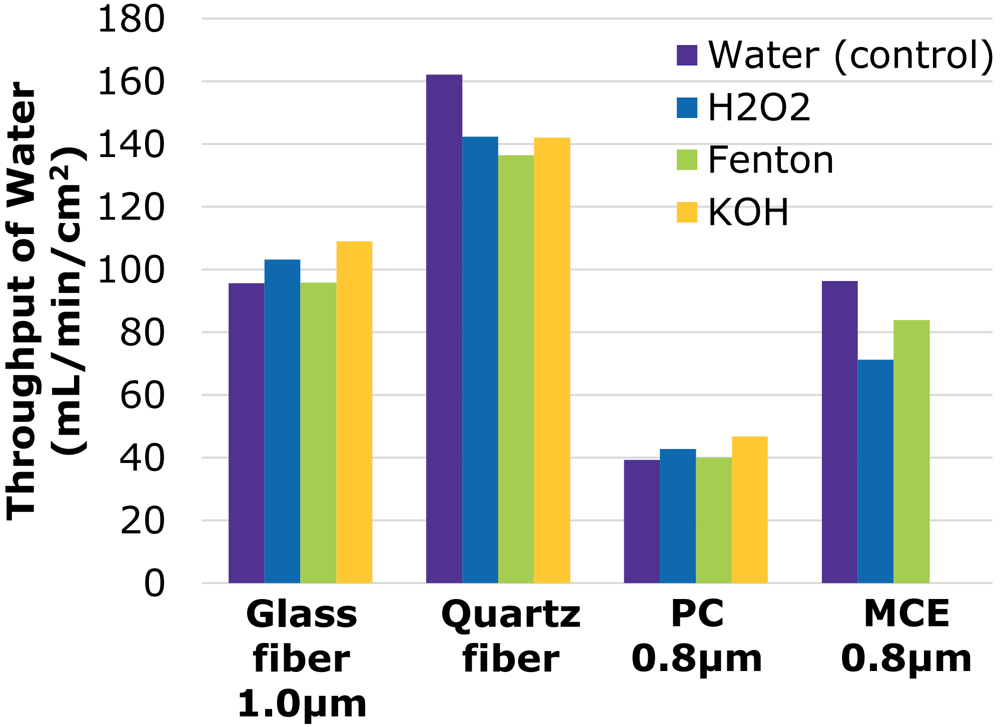
Goal: Determine which membrane is most suitable for common digestion methods and determine chemical compatibility for microplastics separation. (*"Suitable": Exposure, handleability, drying, function and images*)

[1] Sterlitech. https://www.sterlitech.com/chemical-compatibility-chart. [2] Pall. https://www.pall.com/content/dam/pall/laboratory/literature-library/non-gated/chemical-compatibility-chart.pdf. [3] MilliporeSigma. https://www.emdmillipore.com/Web-CA-Site/en_CA/-/CAD/ShowDocument-Pronet?id=201510.399&usg=AOvVaw3h0KMcgRcLW-ZMsoV9AlbV. [4] Cole Parmer. https://www.coleparmer.com/chemical-resistance. [5] Membrane Solutions. https://www.membrane-solutions.com/News_81.htm. [6] Prata, J.C., *et al. TrAC Trends in Analytical Chemistry*, **2019**, *110*, 150-9.

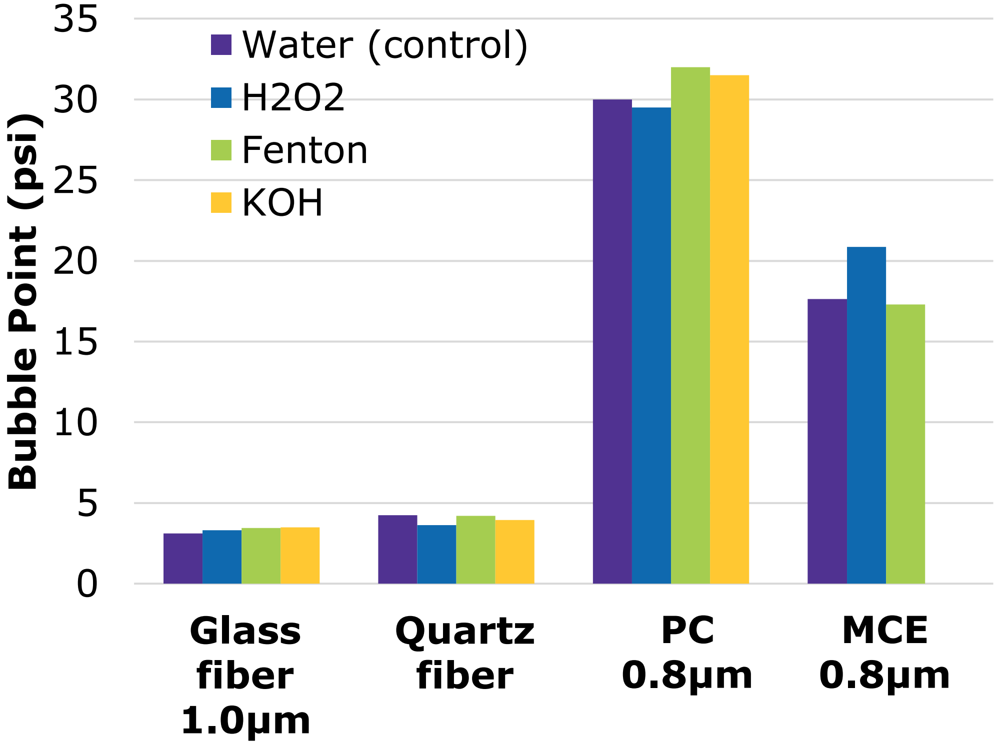
Chemical Digestion

A variety of membranes are compatible filtering digestion reagents

Method: A variety of membranes were used to filter three common the digestion fluids using vacuum filtration [(1) 30% v/v H_2O_2, (2), Fenton's reagent – 1:1 30% (v/v) H_2O_2 + 0.05 mM $FeSO_4$ in Milli-Q® water, and (3) alkaline – 0.05M KOH]. Filtration was observed. Then, filters were dried in an oven for 1 h at 50°C and observed. To evaluate changes in membrane functionality, throughput of water and bubble point were determined (if possible). A water-only "digestion fluid" control was used for comparison.



Flow rates indicate few change in function after exposure to digestion chemicals. *Note: Al2O3 omitted from study.*



Bubble Point indicates slight increase for PC vs. Fenton/KOH and increase for MCE with H_2O_2. *Note: Al2O3 omitted.*

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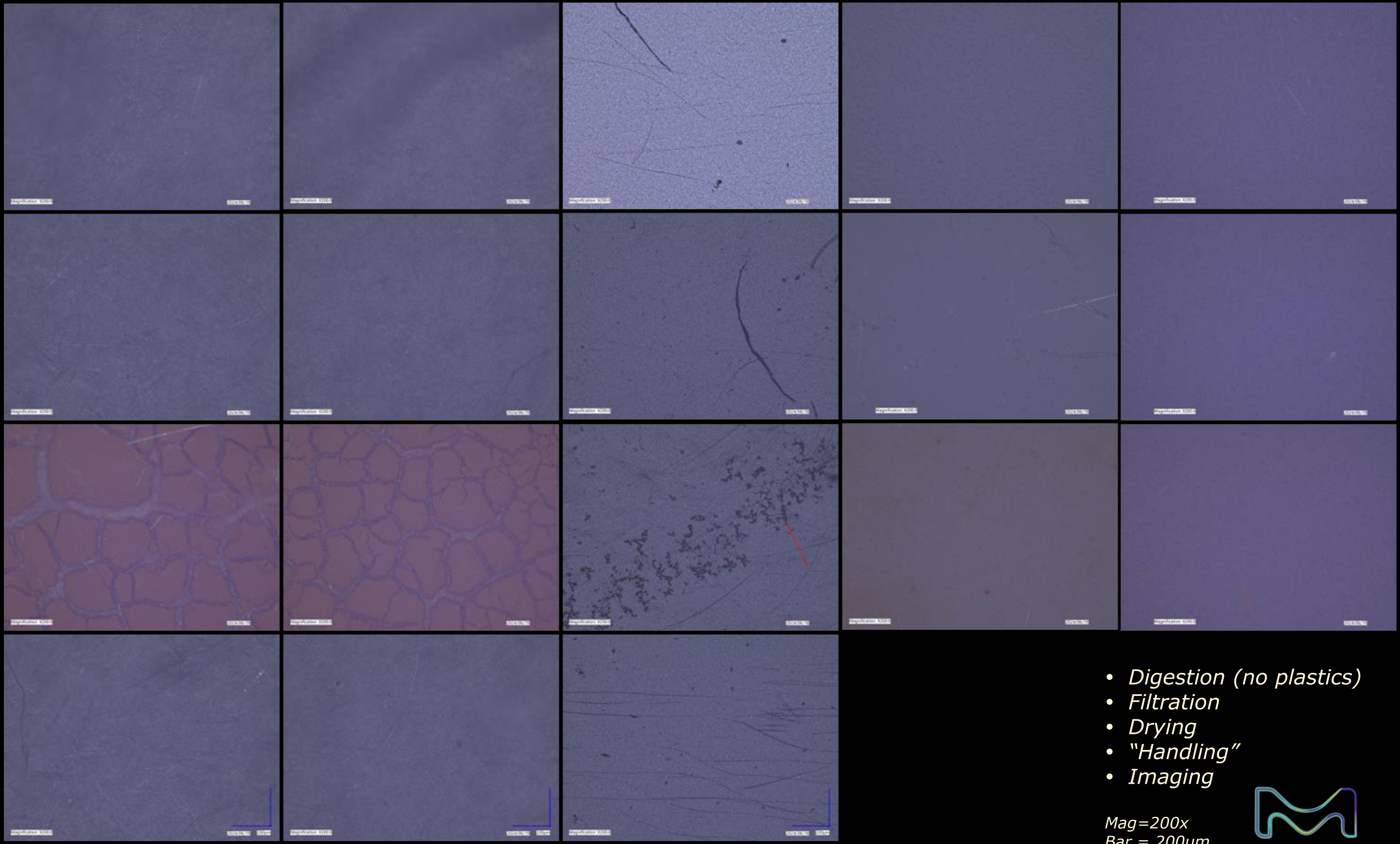
	Glass fiber, 1.0μm	Quartz fiber	Polycarb, 0.8μm	MCE (grids), 0.8μm	MCE (black), 0.8μm
Water (control)					
30% v/v H_2O_2					
Fenton's Reaction					
10% (v/v) KOH					



- *Digestion (no plastics)*
- *Filtration*
- *Drying*
- *"Handling"*
- *Imaging*

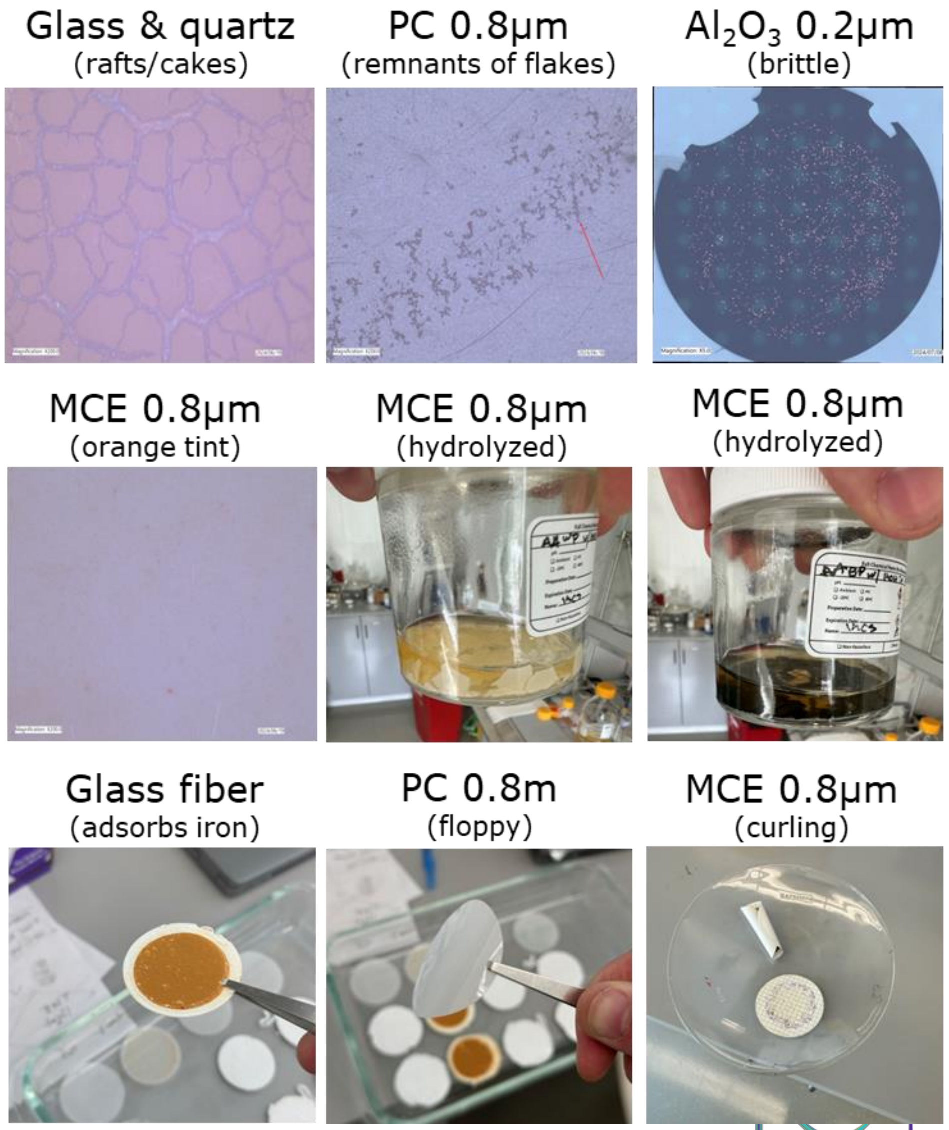
Mag=200x
Bar = 200μm

K. Sydlowski (**2024**)

Chemical Digestion
Compatibility after filtering digests

Method: After filtration, membranes were dried in an oven for 1 h at 50°C and observed, tested for handling by walking through the lab using forceps (~60 ft). Filters were imaged. A water-only "digestion fluid" control was used for comparison.

Filter	Response to drying			Handleability		
	H_2O_2	Fenton	KOH	H_2O_2	Fenton	KOH
GFF, 1.0 µm	Good	Caking	Good	Good	Good	Good
Quartz fiber	Good	Caking	Good	Good	Good	Good
PC, 0.8 µm	Good	Flaking	Good	OK	OK	OK
MCE, 0.8 µm *White/grids*	Good	Flaking	Bad	Good	Good	Bad
MCE, 0.8 µm *black*	Good	Flaking/ sorption	Bad	Good	Good	Bad
Al_2O_3, 0.2µm	OK	OK	OK	OK	OK	OK



K. Sydlowski (**2024**)

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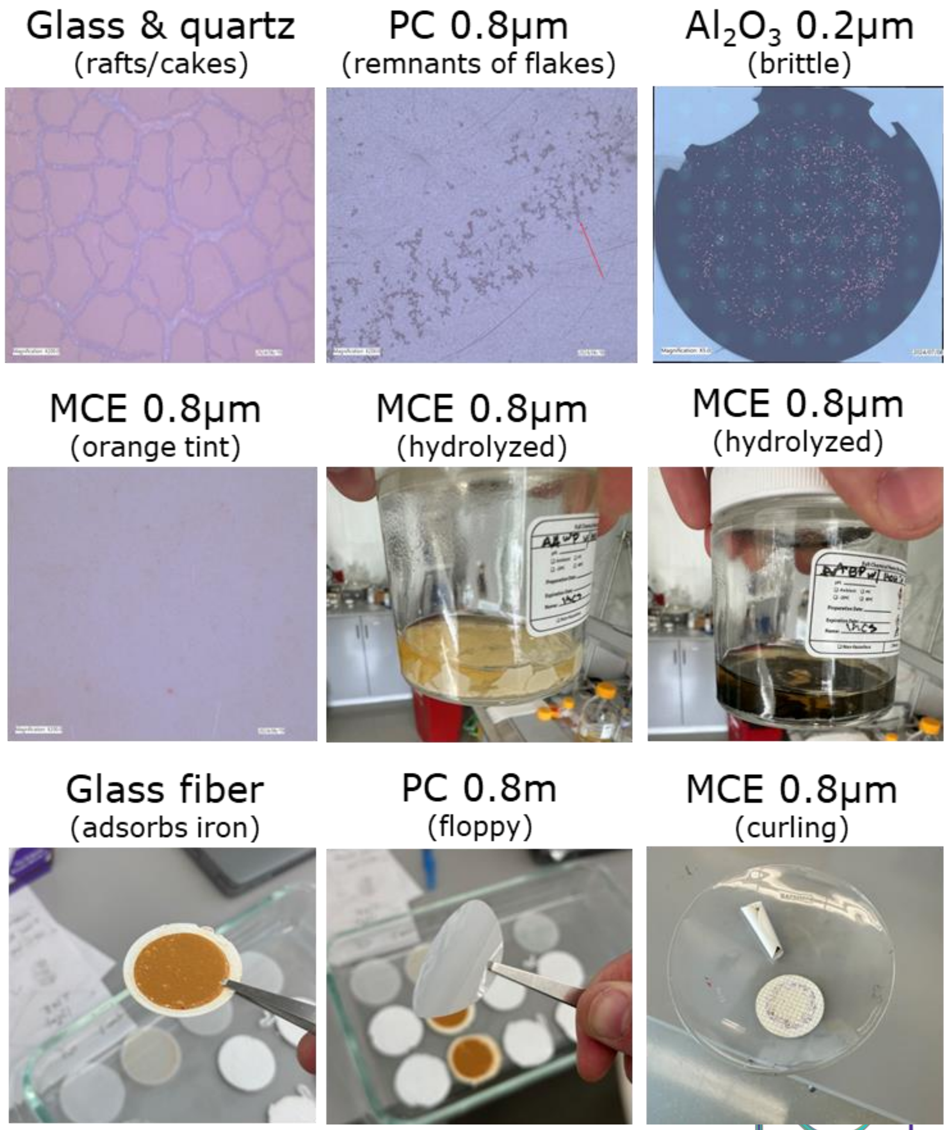
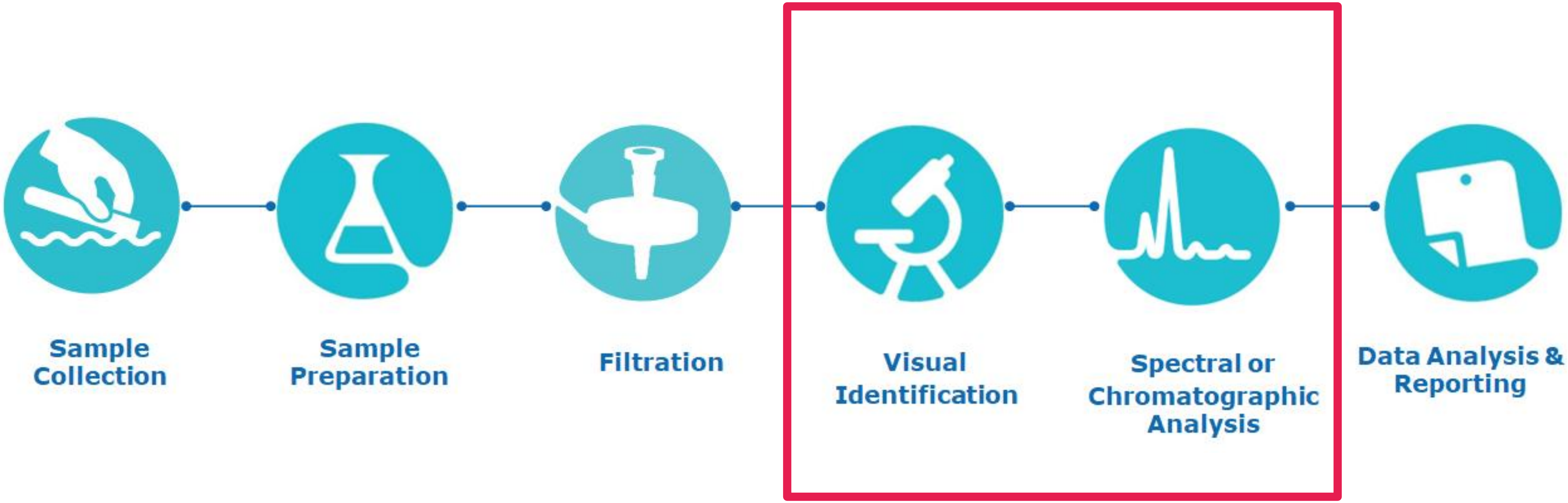
Al_2O_3, 0.2µm	OK	OK	OK	OK	OK	OK



What does it look like with plastics present?

K. Sydlowski (**2024**)

The Microplastics Workflow is Diverse
Filtration Involved in Majority of Workflows



Analysis

Fluorescence, Light Microscopy, FTIR & Other

MilliporeSigma, **2024**. *Membrane filters for microplastics analysis*.

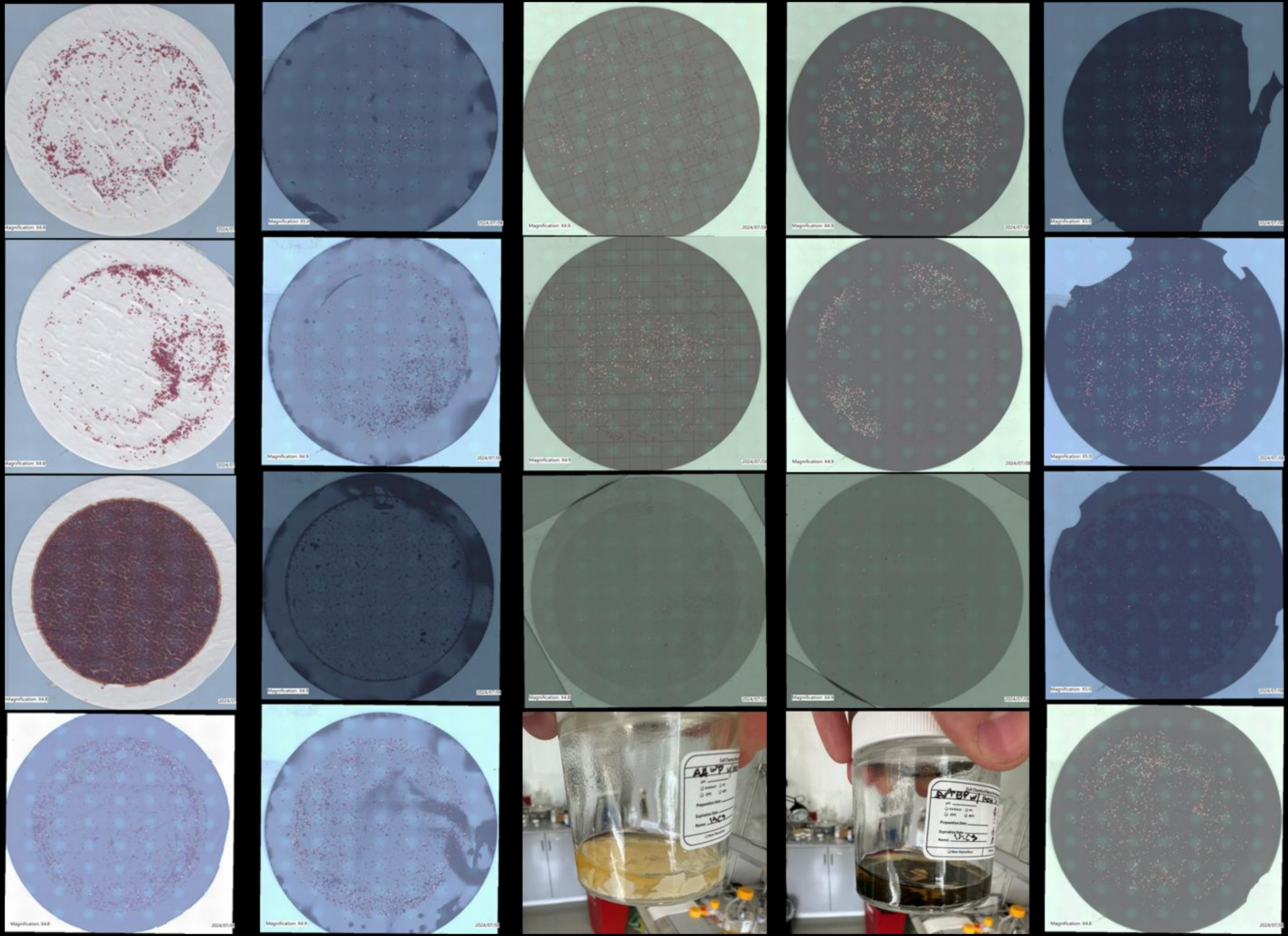
Glass fiber, 1.0µm | Polycarb, 0.8µm | MCE (grids), 0.8µm | MCE (black), 0.8µm | Al_2O_3, 0.2µm

Water (control)

30% v/v **H_2O_2**

Fenton's Reaction

10% (v/v) **KOH**



- *Spike ~5 mg glitter*
- *NaI salt separation*
- *Digestion*
- *Filtration*
- *Drying*
- *"Handling"*
- *Imaging*

Mag=4.9x
Disc size=47mm

K. Sydlowski (**2024**)

Glass fiber, 1.0µm | Polycarb, 0.8µm | MCE (grids), 0.8µm | MCE (black), 0.8µm | Al_2O_3, 0.2µm

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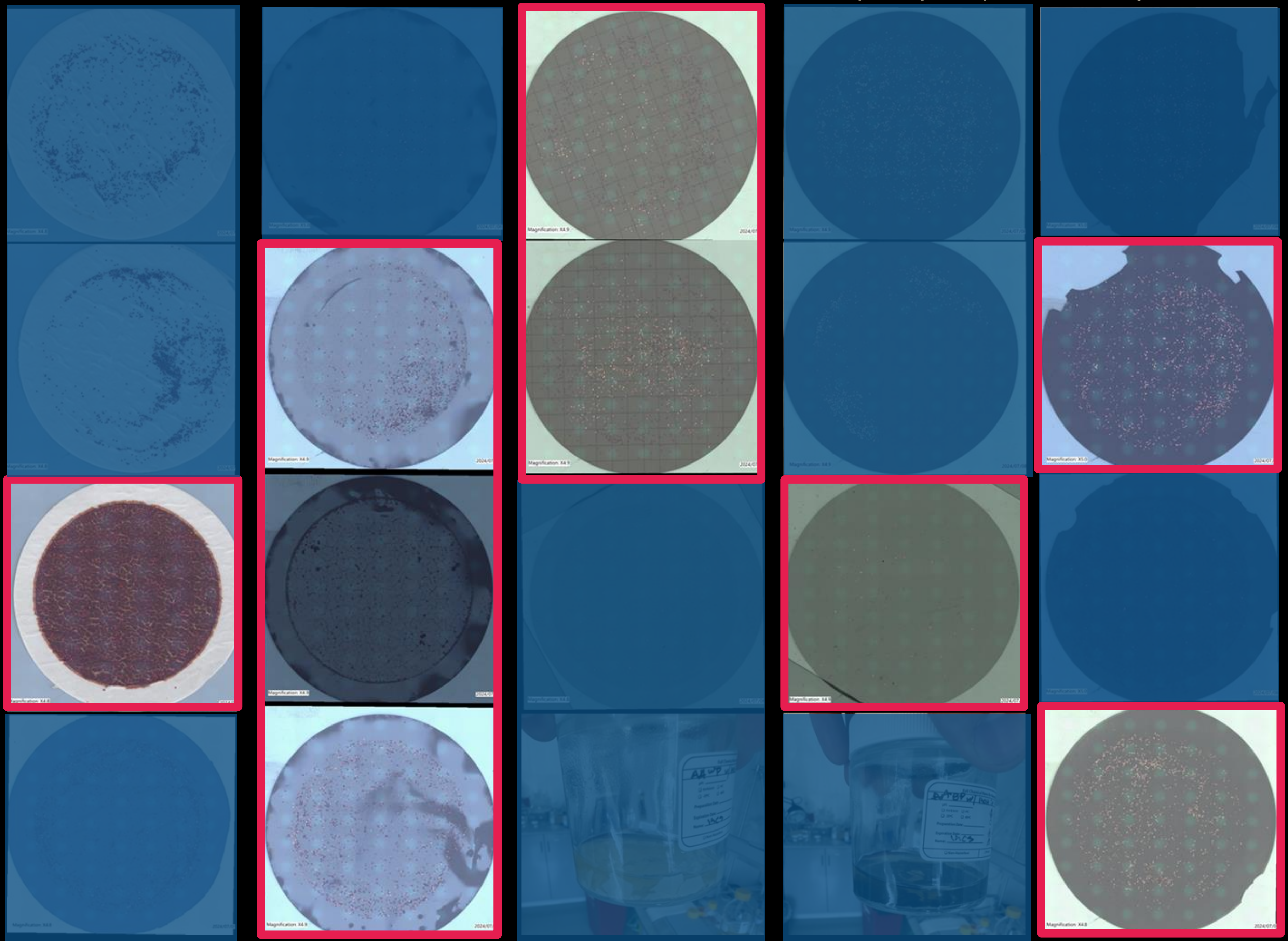

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- *"Handling"*
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Mag=4.9x
Disc size=47mm

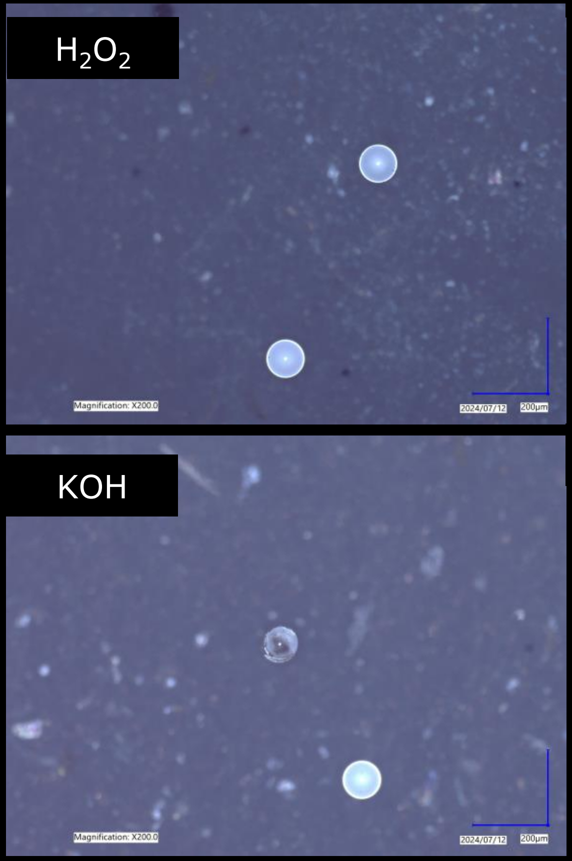
K. Sydlowski (**2024**)

"Recommended" membranes spiked with Polystyrene beads

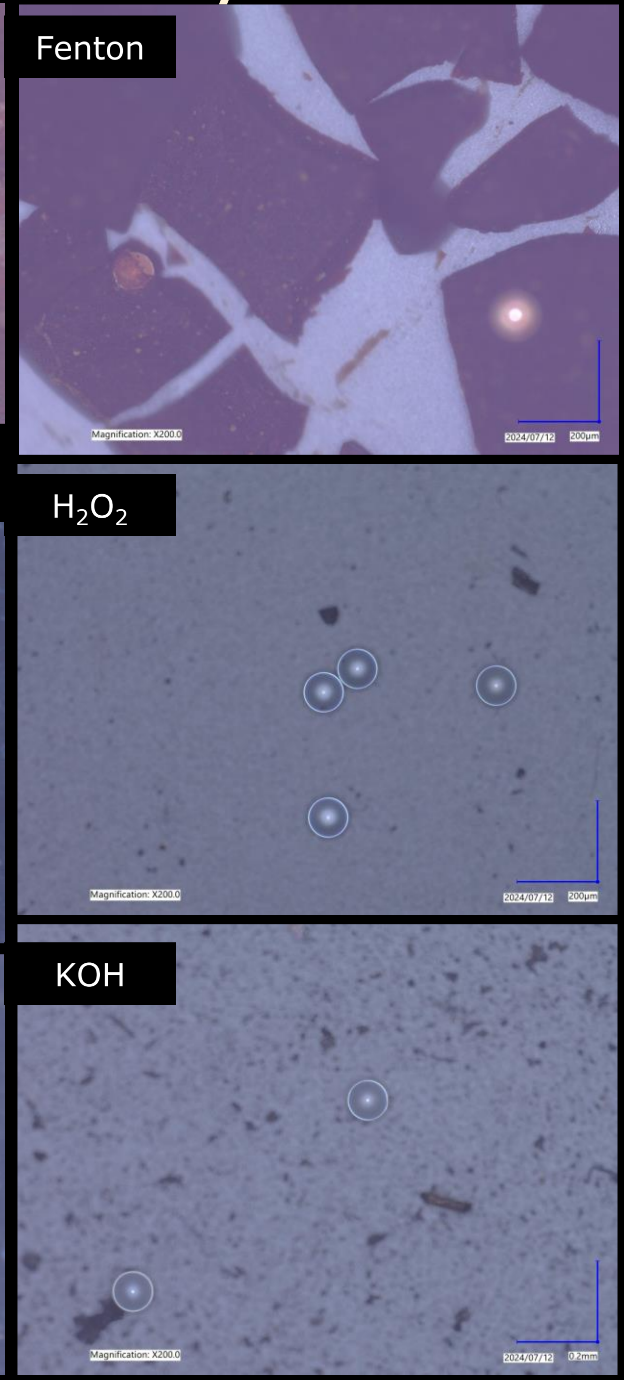
- No damage observed for any of the digestion fluids
- Clearest contrast seen for MCE and Al_2O_3
- Beads "stuck" in cakes on top of glass and quartz (seen with SEM)
- Low retention of PC
- Varied dispersal of beads seen with NaI & different digests

Glass fiber:

Aluminum oxide:



Polycarbonate:



Mixed cellulose ester:

- *Spike with 200µm PS beads*
- *NaI salt separation*
- *Digestion*
- *Filtration*
- *Drying*
- *"Handling"*
- *Imaging*

Mag=200x
Bar = 200µm

K. Sydlowski (**2024**)

Chemical Digestion & Image Analysis

Membrane Compatibility – Image Quality, Function & Handleability

Filter Type	**Water** (control) + NaI separation	**Hydrogen peroxide** (30%v/v) + NaI separation	**Fenton's Reaction** + NaI Separation	**Potassium Hydroxide, alkaline** (10%v/v) + NaI Separation
Glass Fiber	**Recommended**	**Recommended** • *Possible polymer aggregation*	**Recommended**	**Recommended**
Quartz Fiber	**Recommended**	**Recommended** • *Possible polymer aggregation*	**Recommended**	**Recommended**
Polycarbonate	**Okay** • *Scratches*	**Not recommended** • *Flaking, low retention*	**Okay** • *Scratches*	**Okay** • *Scratches*
Mixed Cellulose Ester (MCE) white	**Recommended**	**Recommended**	**Not recommended** • *Curling/deformation*	**Not recommended** • *Complete hydrolysis*
Mixed Cellulose Ester (MCE) White/grids	**Recommended**	**Recommended**	**Not recommended** • *Curling/deformation*	**Not recommended** • *Complete hydrolysis*
Mixed Cellulose Ester (MCE) black	**Recommended**	**Okay** • *Possible NaI interaction*	**Not recommended** • *Curling/deformation*	**Not recommended** • *Complete hydrolysis*
Aluminum Oxide	**Okay** *(brittle)*	**Okay** *(brittle)*	**Okay** *(brittle)*	**Okay** *(brittle)*

K. Sydlowski (**2024**)

Chemical Digestion & Image Analysis

Membrane Compatibility – Image Quality, Function & Handleability

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Glass Fiber	**Recommended**	**Recommended** • *Possible polymer aggregation*	**Recommended**	**Recommended**
Quartz Fiber	**Recommended**	**Recommended** • *Possible polymer aggregation*	**Recommended**	**Recommended**
Polycarbonate	**Okay** • *Scratches*	**Not recommended** • *Flaking, low retention*	**Okay** • *Scratches*	**Okay** • *Scratches*
Mixed Cellulose Ester (MCE) white	**Recommended**	**Recommended**	**Not re** • *Curli*	**nded** *lysis*
Mixed Cellulose Ester (MCE) White/grids	**Recommended**	**Recommended**	**Not re** • *Curli*	**nded** *rolysis*
Mixed Cellulose Ester (MCE) black	**Recommended**	**Okay** • *Possible NaI interaction*	**Not recommended** • *Curling/deformation*	**Not recommended** • *Complete hydrolysis*
Aluminum Oxide	**Okay** *(brittle)*	**Okay** *(brittle)*	**Okay** *(brittle)*	**Okay** *(brittle)*

Next steps: Use best performing filters to analyze New England beach sand samples *(coming soon!)*.

K. Sydlowski (**2024**)

Image Analysis
Fluorescence Microscopy Using Nile Red[1]

What: Detection of microplastic particles in drinking water using Nile Red fluorescent dye

- Why:
  - *Common, accurate stain*
  - *Selective adsorption and fluorescence for polyethylene, polypropylene, polystyrene, nylon, etc.*
  - *Can be semi-automated for particle counting*
- Sample matrix: drinking water (other matrices limited)
- Detection limits: 6.5 µm to 5 mm
- Dye: 10 µg/mL and exposure to sample for 30 min

Workflow:

750 mL

\+

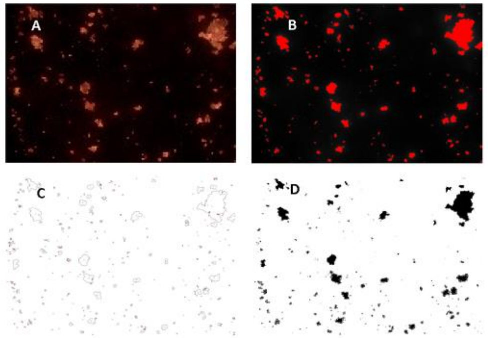
10 µg/L (acetone)

30 min

1 µm glass fiber, 47 mm (method) + DI water control

Leica EZ4HD

Image filter (0.67x-4.5x mag), blue fluorescent light

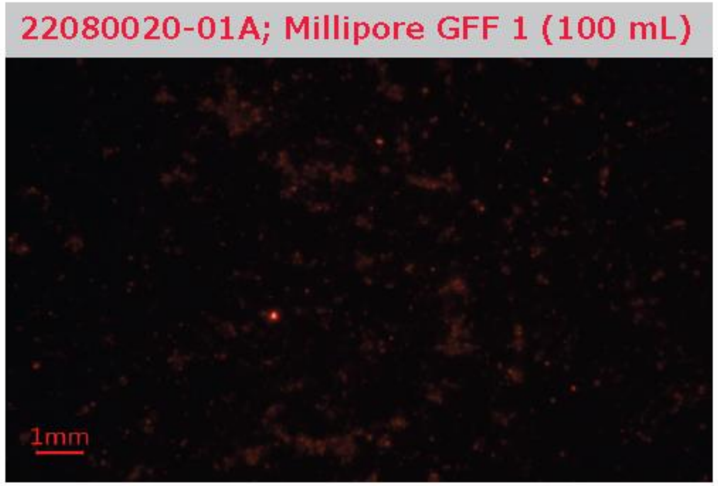


Analysis: 4 quadrants, Binarize, threshold, count/size (microplastics/L)

Mason, S.A, Welch, V.G, Neratko, J. *Front. Chem.*, **2018**, *6*, 1-10.

Image Analysis
Fluorescence Microscopy Using Nile Red[1]

A



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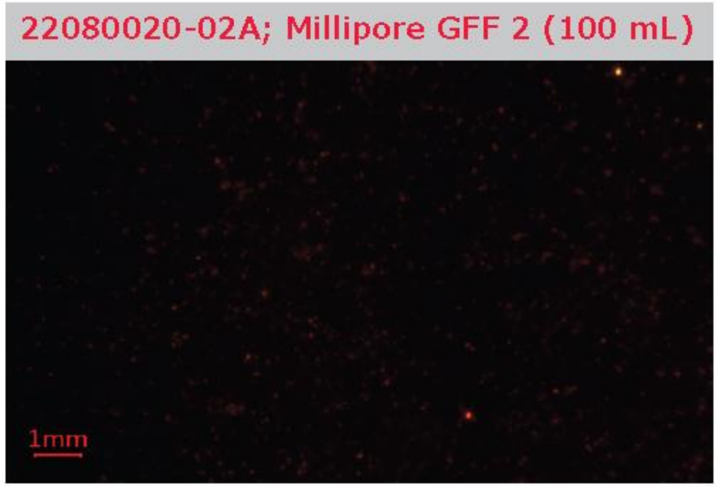

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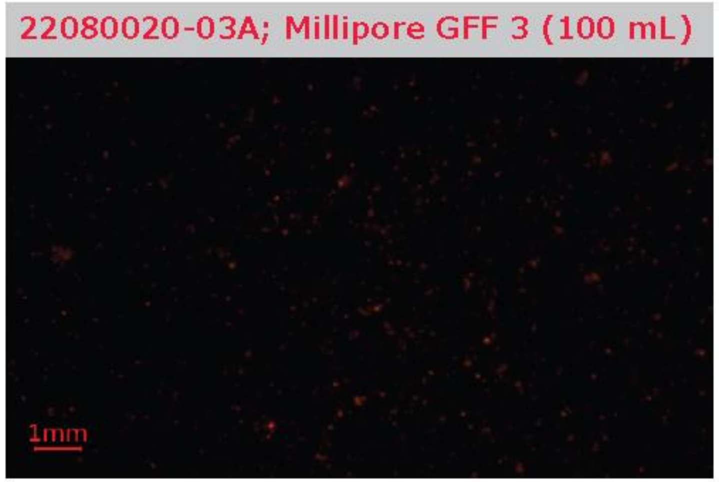

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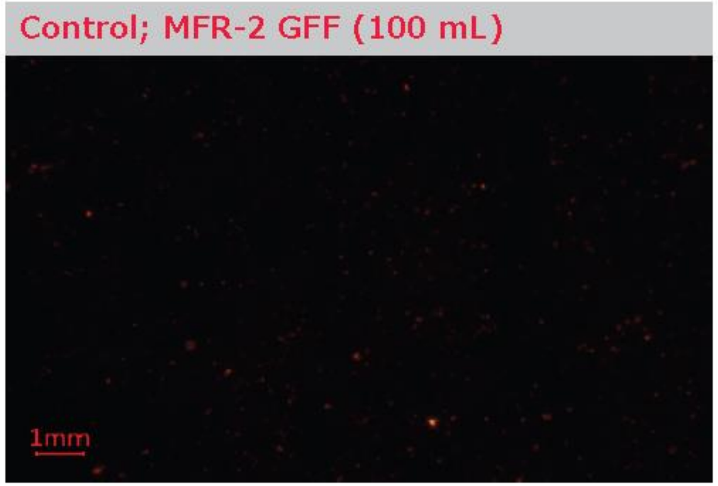
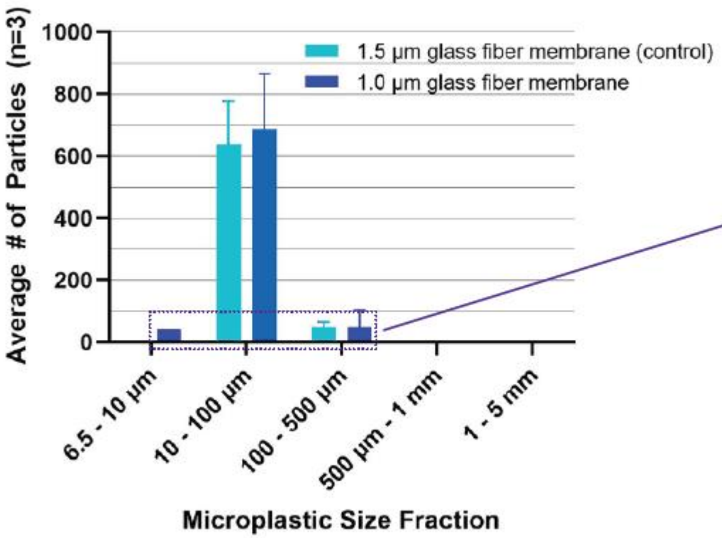
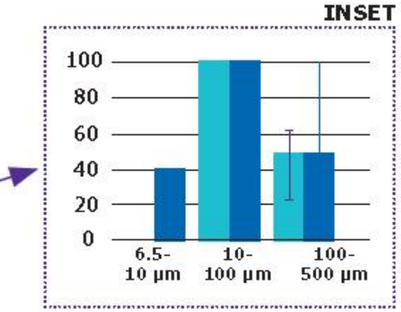


E

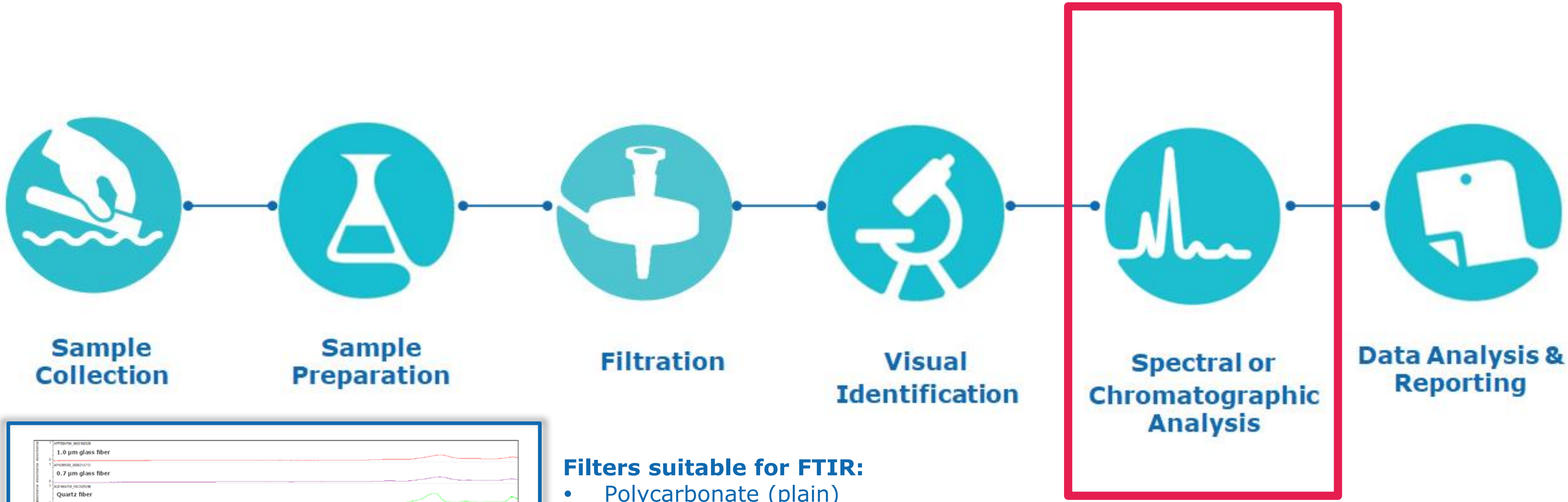




*recommend using flat side (vs. fibrous A side) of glass fiber

Mason, S.A, Welch, V.G, Neratko, J. *Front. Chem.*, **2018**, *6*, 1-10.

The Microplastics Workflow is Diverse
Filtration Involved in Majority of Workflows



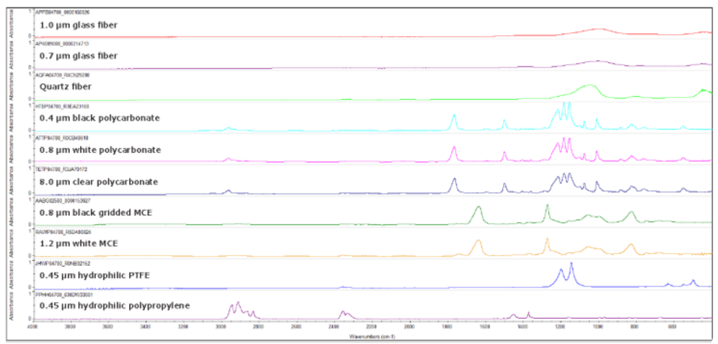


Figure 6. Background IR spectra [absorbance (0-1.0)] vs. wavenumber (cm^{-1}) for various filter membranes discussed through this article. (Nicolet iS5 with iD7 BASE ATR accessory (Thermo Fisher Scientific), system mode: reflection, ATR, resolution 4 cm^{-1}; 16 scans).

Filters suitable for FTIR:
- Polycarbonate (plain)
- Black polycarbonate
- Silver
- Aluminum oxide
- PTFE (in certain cases)
- Stainless steel
- Silicon
- Gold-coated membranes

- FTIR spectroscopy
- Raman spectroscopy
- Pyrolysis-GC-MS

MilliporeSigma, **2024**. *Membrane filters for microplastics analysis*.

Spectral and Chromatographic Characterization
Membrane Substrate Considerations

Filter Considerations for Spectroscopy (IR and Raman)

Spectral interference
- – Signal masking
- – Thickness
- Reflectivity
- Signal enhancement
- Laser compatibility
  - – Handleability & fragility
- Disc size & instrument compatibility
- Filter dryness
- Particle size as it relates to particle retention
- Filter availability & cost
- If coated, coating reactivity

- IR transparency vs. subtractable background?
- Possible loss of particles
- Curling & deformation can lead to artefacts and difficulties fitting in sample holders/clamps
- IR transparent generally cost more

Filter Considerations for GC/MS

- Filter diameter
  - – Ability to fit in pyrolysis cup
- Subsampling and/or punching out filter sections
- Sturdiness vs. pyrolysis method
- Low or highly distinguished background from polymers

MilliporeSigma, **2024**. *Membrane filters for microplastics analysis*.

Spectral and Chromatographic Characterization
Membrane Substrate Considerations

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Filter Considerations for GC/MS

- Filter diameter
  - Ability to fit in pyrolysis cup
- Subsampling and/or punching out filter sections
- Sturdiness vs. pyrolysis method
- Low or highly distinguished background from polymers

- Small enough diameters rare = need for subsampling or folding/crumpling membrane
- Membrane should be easy to deform without losing particles

- Inorganic membranes

MilliporeSigma, **2024**. *Membrane filters for microplastics analysis*.

Microplastics in the Environment – Summary & Conclusion
Optimizing Membrane Filter Selection

MilliporeSigma, **2024**. *Membrane filters for microplastics analysis*.

Technique/Application	Recommended Millipore® Membrane Filter(s)					
	Glass fiber	Quartz fiber	Polycarbonate	Mixed cellulose ester	Polypropylene	Aluminum oxide
Production of MAG water	●	●	●	●	N.T.	N.T.
Visual analysis	●	●	●	●	●	N.T.
Nile Red Fluorescence	●	●				N.T.
Drying & Handling	●	●	●	●	●	
Chem. digestion/30% H_2O_2	●	●		●	N.T.	●
Chem. digestion/Fenton Rxn	●	●	●		N.T.	●
Chem. digestion/KOH	●	●	●		N.T.	●
Salt Separation			●			●
Oil flotation	●	●			●	N.T.
Spectroscopy			●			●
Pyrolysis-GC/MS	●	●				

Microplastics in the Environment – Summary & Conclusion
Optimizing Membrane Filter Selection

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Technique/Application	Recommended Millipore® Membrane Filter(s)					
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Production of MAG water	●	●	●	●	N.T.	N.T.
Visual analysis	●	●	●	●	●	N.T.
Nile Red Fluorescence	●	●				N.T.
Drying & Handling	●	●	●	●	●	
Chem. digestion/30% H_2O_2	●	●		●	N.T.	●
Chem. digestion/Fenton Rxn	●	●	●		N.T.	●
Chem. digestion/KOH	●	●	●		N.T.	●
Salt Separation			●			●
Oil flotation	●	●			●	N.T.
Spectroscopy ***Next step***			●			●
Pyrolysis-GC/MS ***Next step***	●	●				

- Microplastics methods are being developed, with focus on certain matrices and portions of workflow (sampling, etc.)
- **Many technical hurdles** in collecting and analyzing microplastics
  - **While cut disc filter membranes are always involved** in sample prep, there may not be one membrane that applies to all chosen methods
- Through this study our team **expanded the "recommended membranes by method" table** to include chemical digestion methods, handling, and basic salt and oil separation techniques

Thank you

Maricar Dube

Amy Laws

Ryan Amara

Mayra Jimenez

Kevin Sydlowski

Ranjani Muralidharan

Vivek Joshi

Taylor Reynolds

Dave Brewster

Janet Smith

Curious2024 Future Insight™--Microplastics Hackathon, Mainz Germany (10-11 JUL 2024)

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