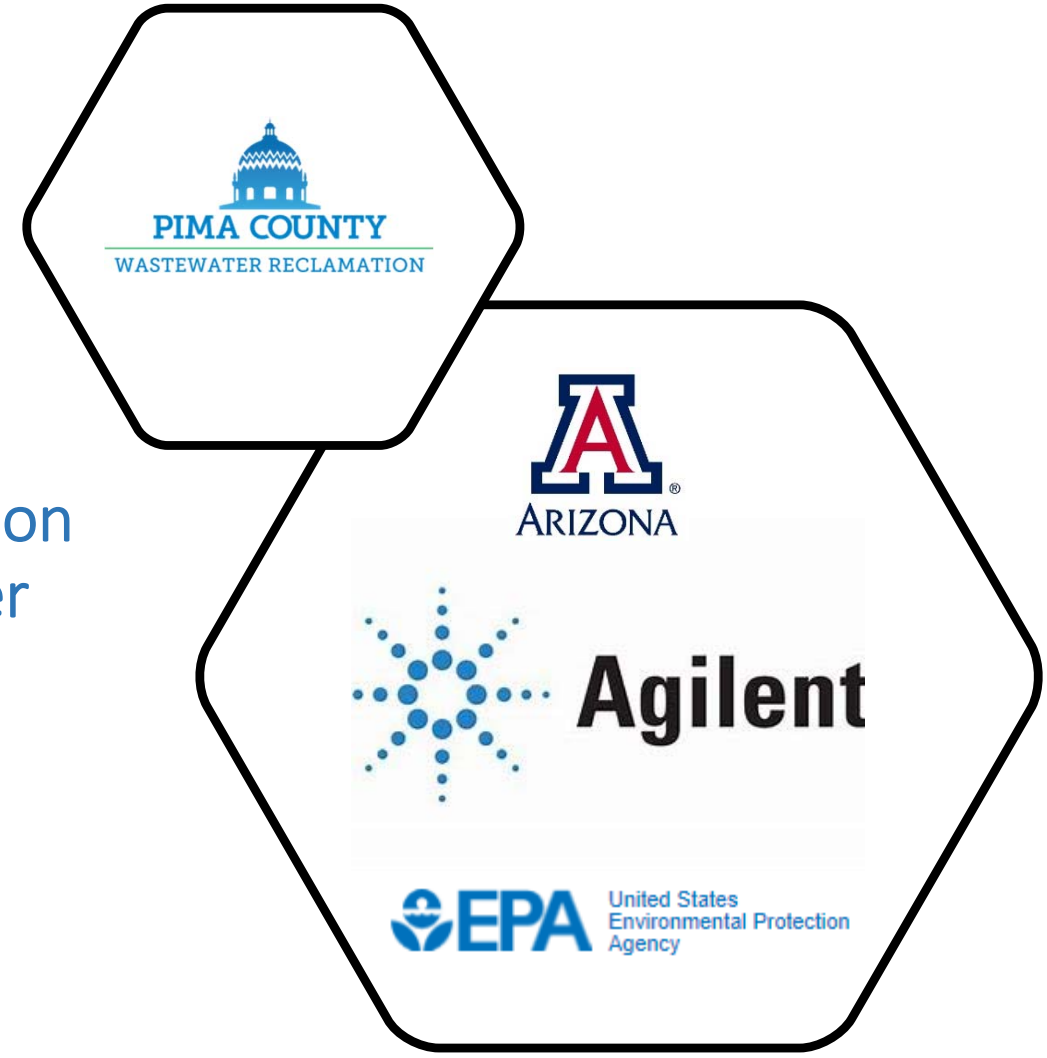
Standardized Method for the Identification and Quantification of Microplastics in Wastewater Using LDIR Microscopy

By

Jeff Prevatt



Recent Headlines

PLASTICS

Powered by RebelMouse

Microplastics Detected in Human Stool Samples for First Time

By Lorraine Chow | Oct. 23, 2018 09:07AM EST

HEALTH + WELLNESS

Microplastics. *MPCA Photos / Flickr / CC BY-NC 2.0*

OCTOBER 31, 2018

Is there a risk to human health from microplastics?

by BfR Federal Institute for Risk Assessment

According to EFSA, there is a possibility of oral absorption of microplastic particles of a certain size, although the fate and possible degradation in the gastrointestinal tract have not been sufficiently investigated so far due to a lack of analytical methods and valid studies.

Recent Headlines

Oh, yuck! You're eating about a credit card's worth of plastic every week

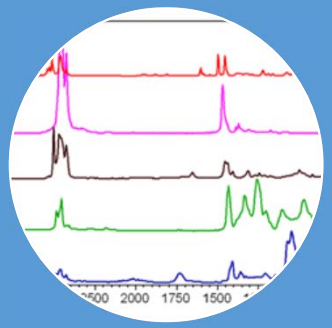
Doyle Rice, USA TODAY Published 6:24 p.m. ET June 12, 2019 | Updated 1:11 a.m. ET June 13, 2019

The analysis *No Plastic in Nature: Assessing Plastic Ingestion from Nature to People* prepared by Dalberg, based on a study commissioned by WWF and carried out by University of Newcastle, Australia, suggests people are consuming about 2000 tiny pieces of plastic every week. That's approximately 21 grams a month, just over 250 grams a year.

Standardization is Needed

Sample Collection

Sample Preparation



Sample Analysis

Reproducibility

Sample Collection

Desired Features

- Representative of source
- Account for diurnal patterns
- Volume proportional

Wastewater Sampling Apparatus

1) Pump capable of providing 20' of head and up to 3 gpm flow
2) Stainless steel transfer tubing
3) Adjustable valve for flow control
4) Programmable timer
5) Sieve Stack (1,000 µm – 20 µm)
6) Container for sieves with drainage

Sampling Options

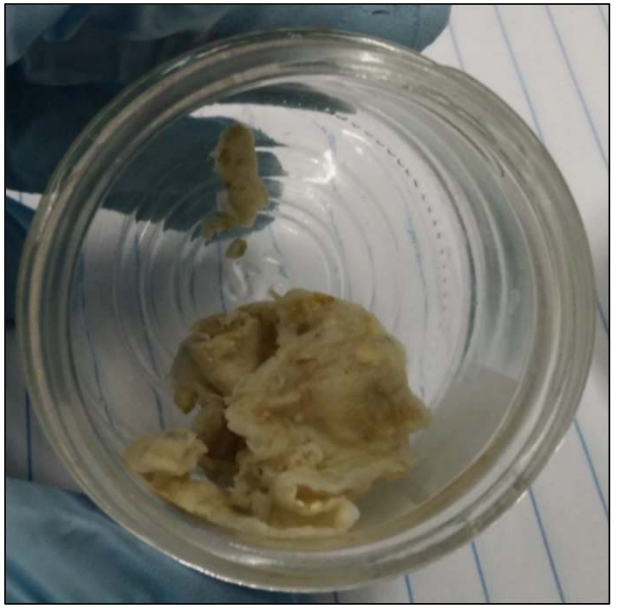
Hose Bib Valve

Flow Meter

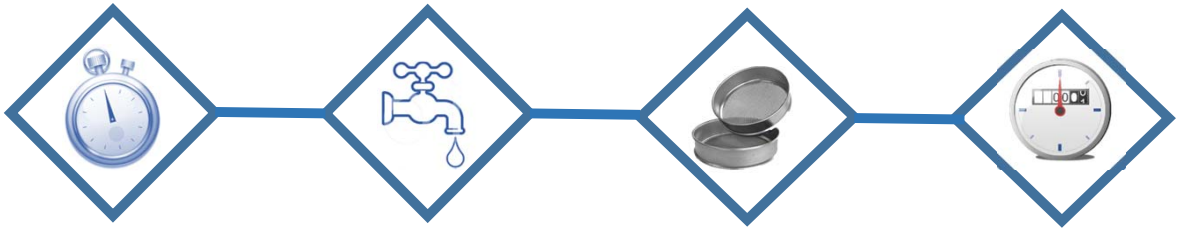
- Easy Setup & Calibration
- Improved Flow Control
- Recorded Total Volume
- Submersible or Peristaltic Pump

Sample Retrieval

Sieved material rinsed and collected in sample container



Sample Collection



- Straightforward
- Simple to follow
- Reproducible

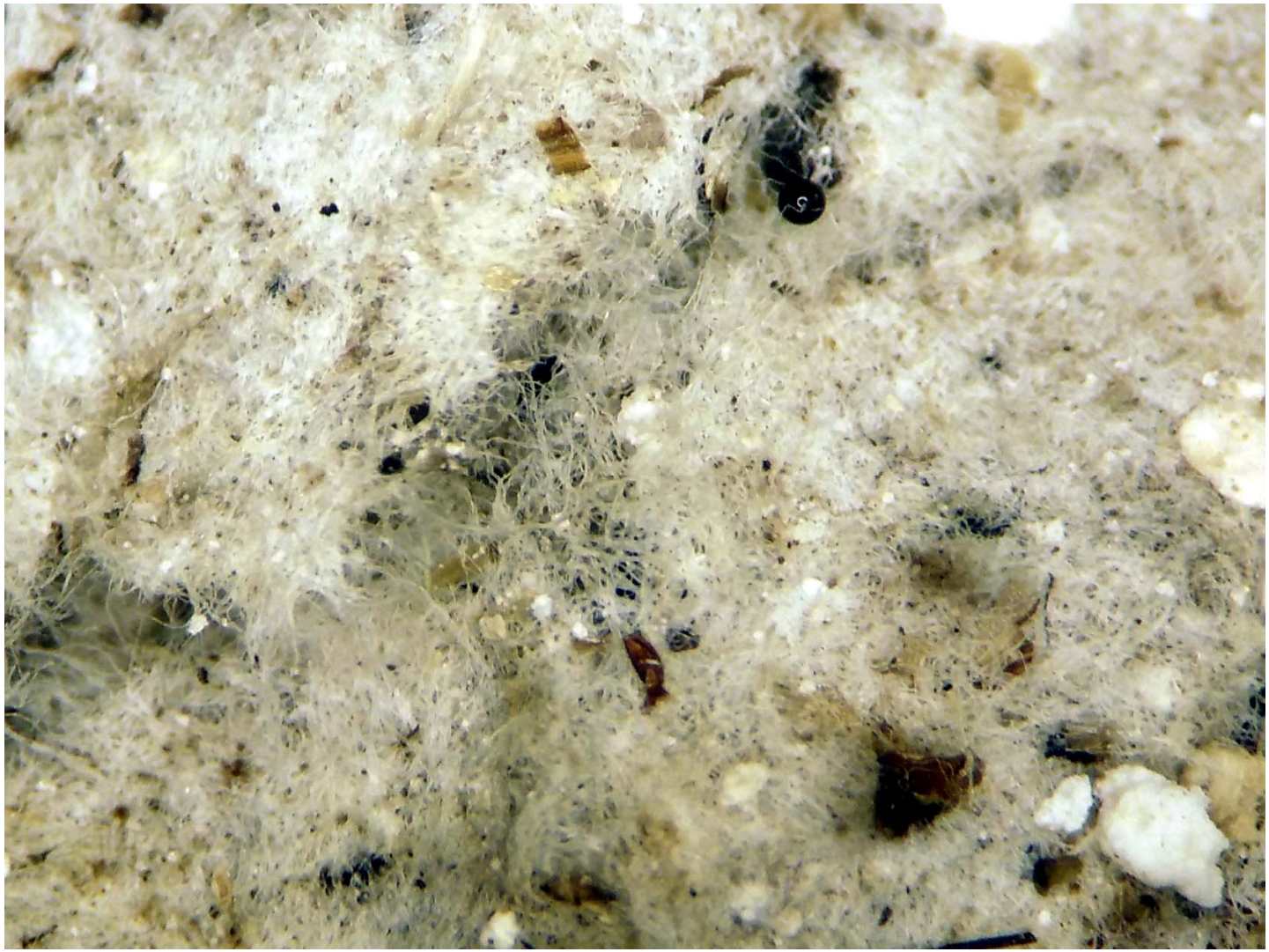
Sample Preparation

Desired Outcome

Procedure MUST:

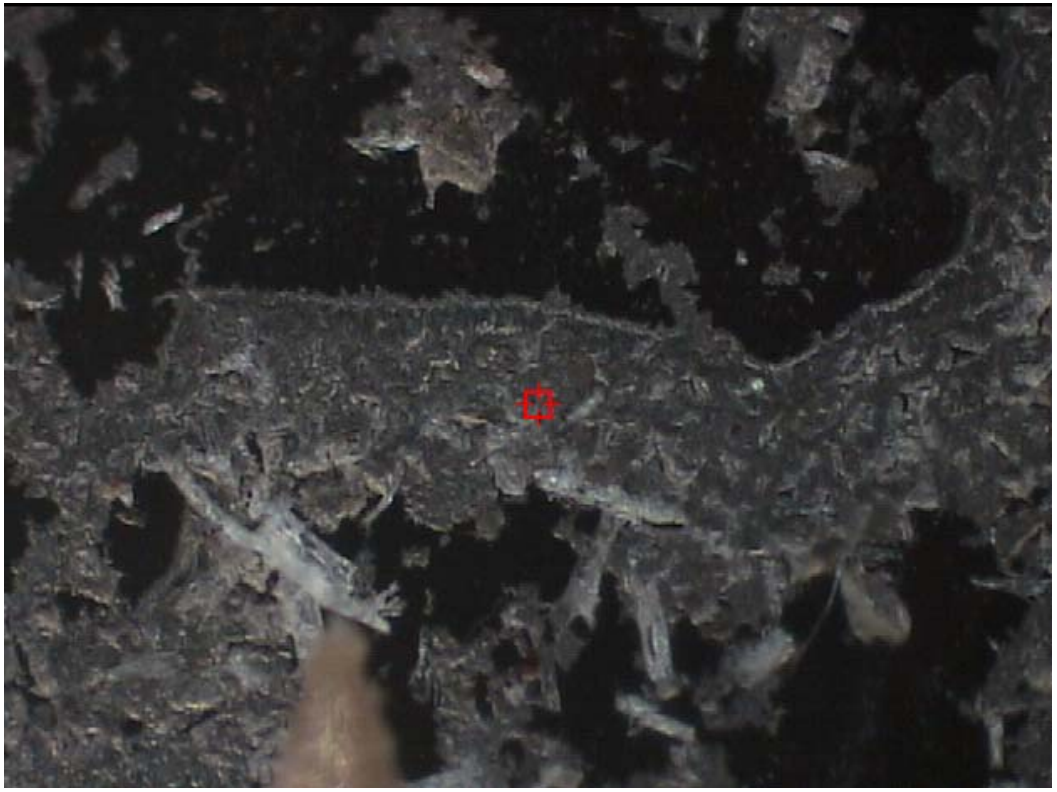
- Account for interferences
- Easy to follow & replicate
- Produce accurate results
- Minimize loss or damage of particles

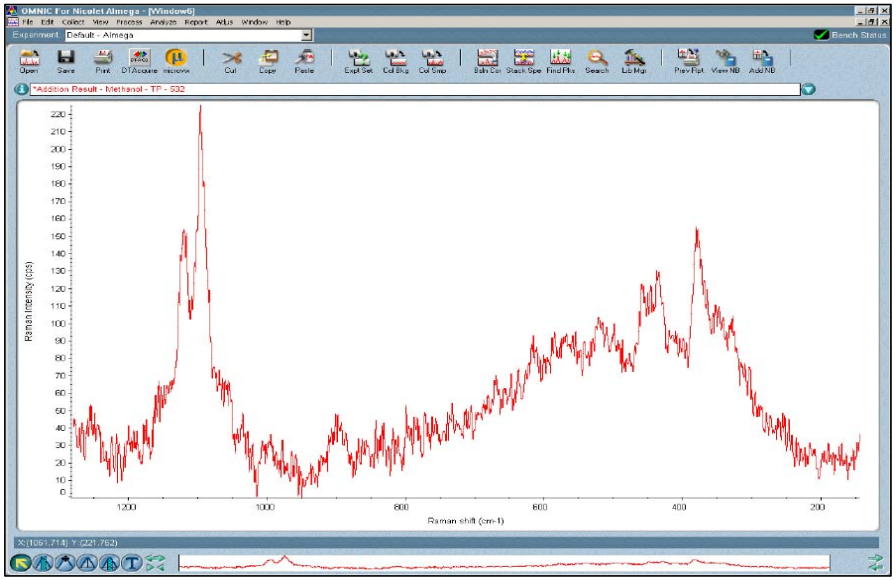
~20x magnification

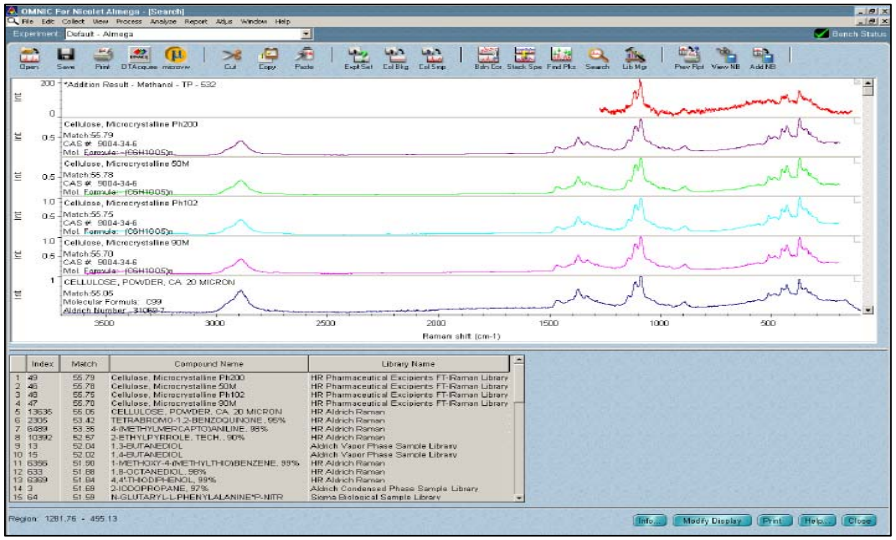


Interferences

Cellulose is abundant
(toilet paper)

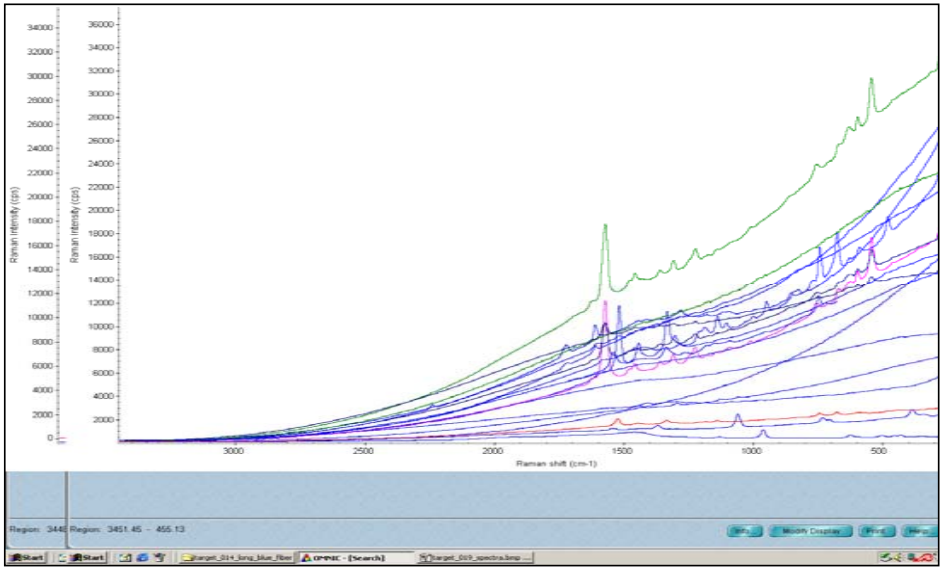


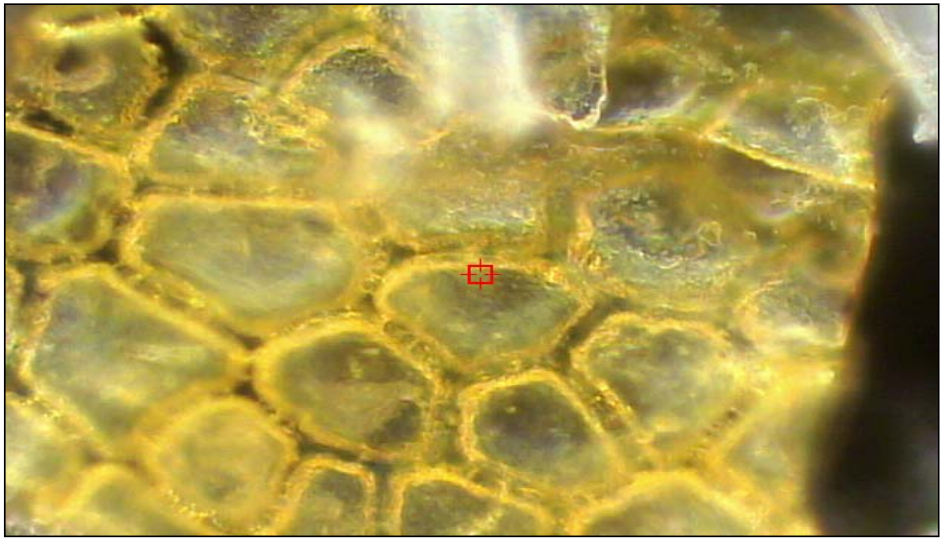




Interferences

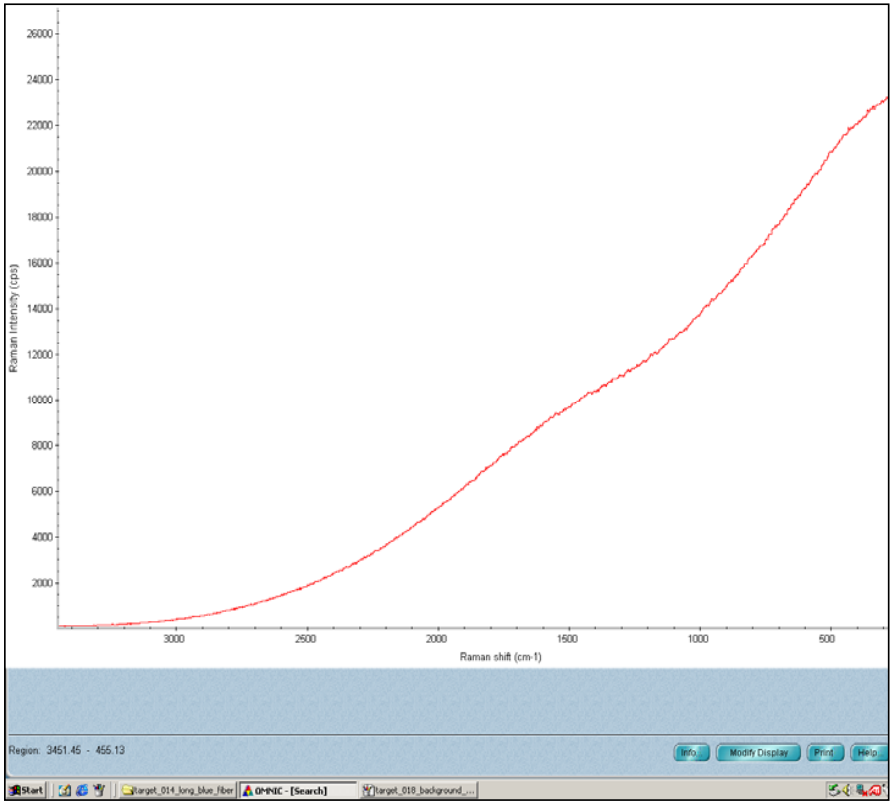
Abundant chitin background material



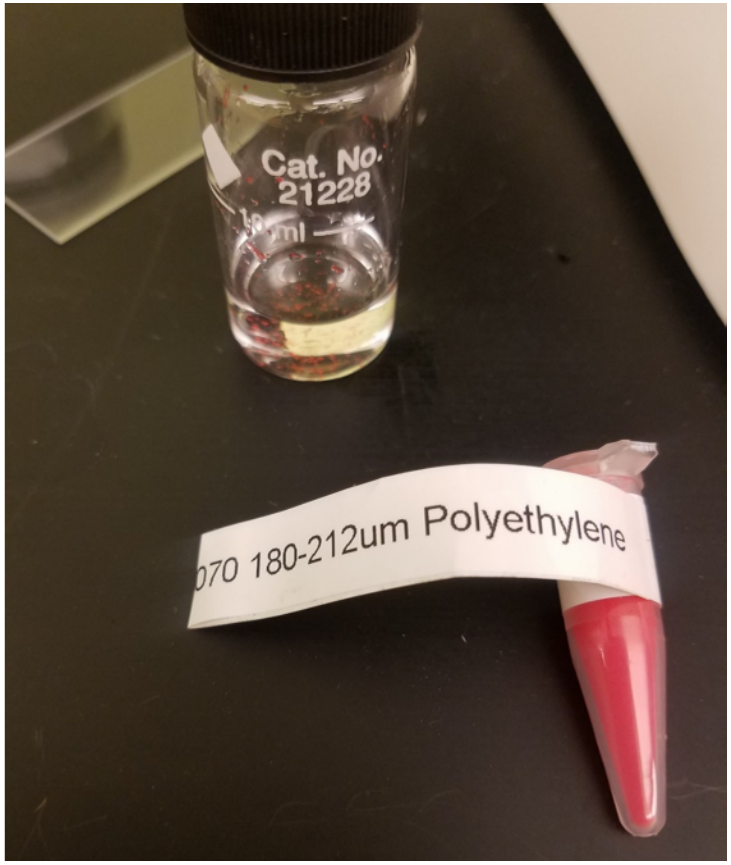


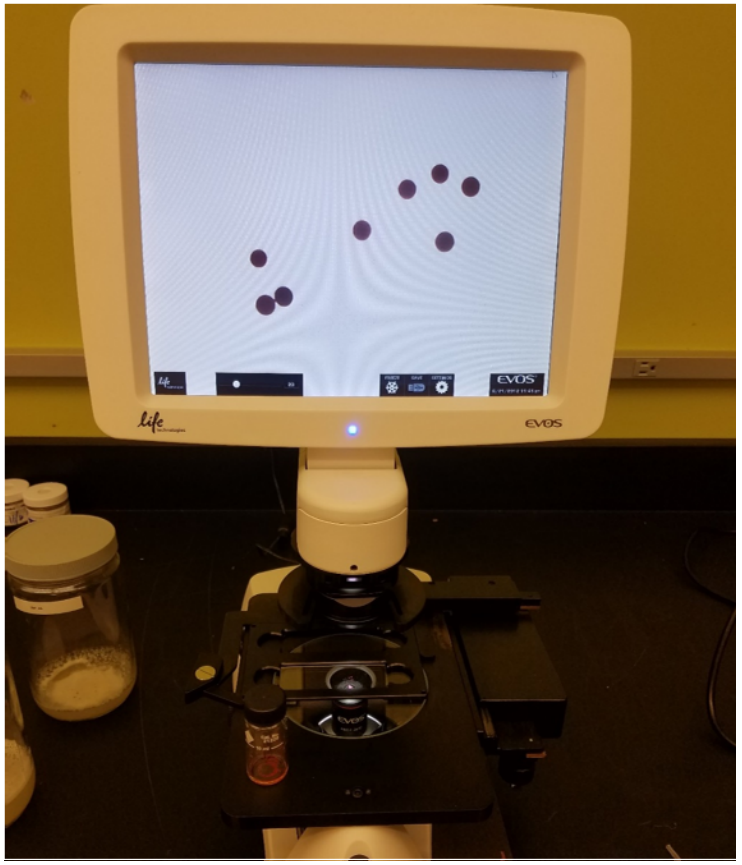
Interferences

Skin and lipid material is abundant



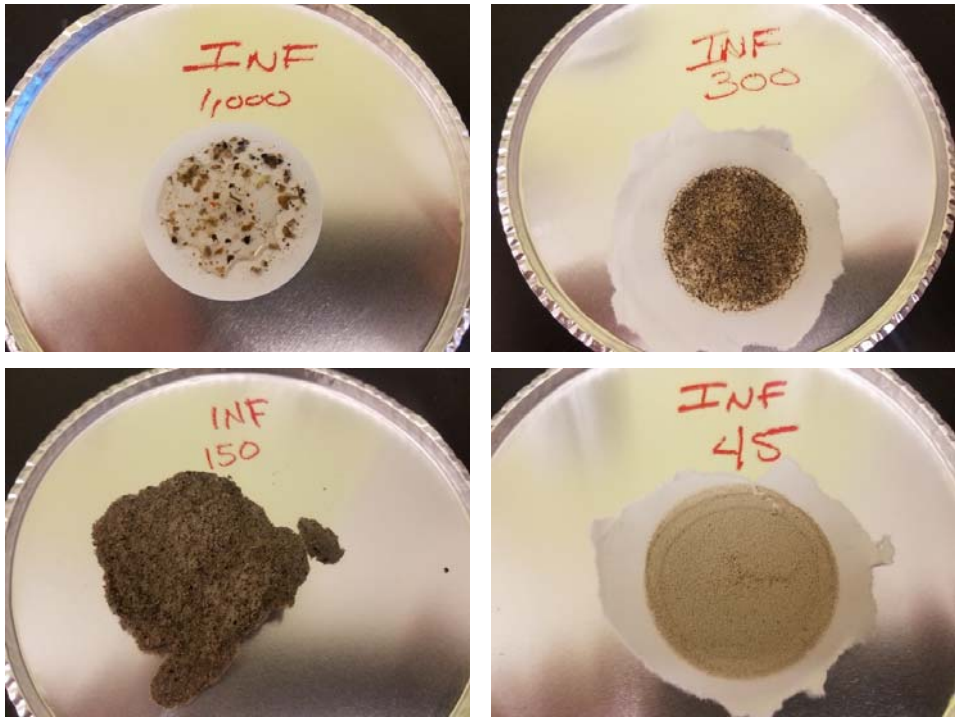
Extraction Procedure Reference Spikes



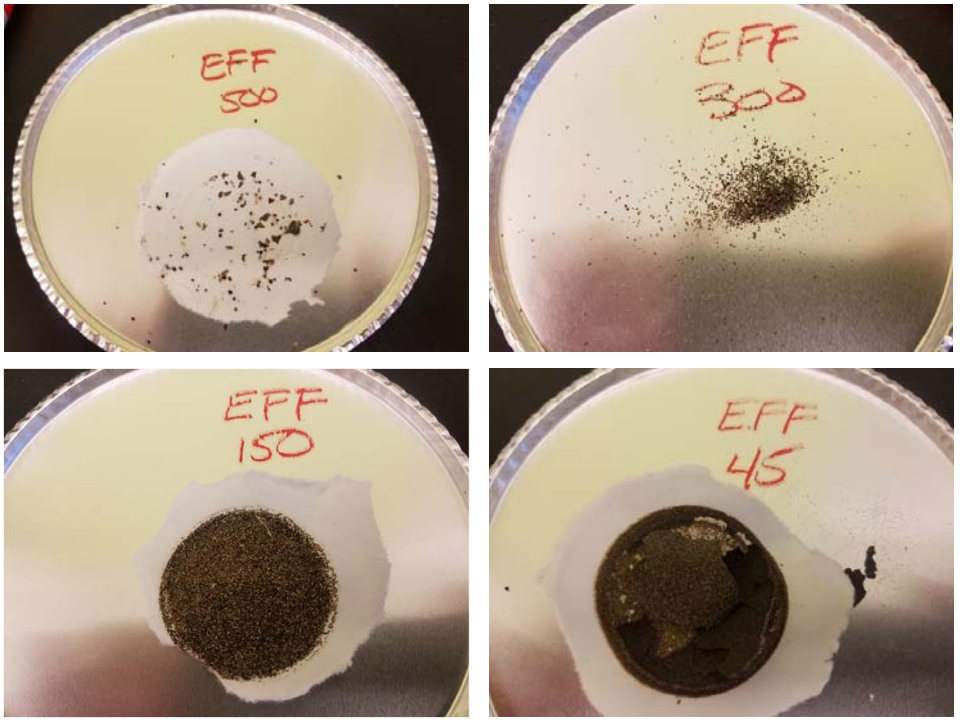


Sieve Fractions

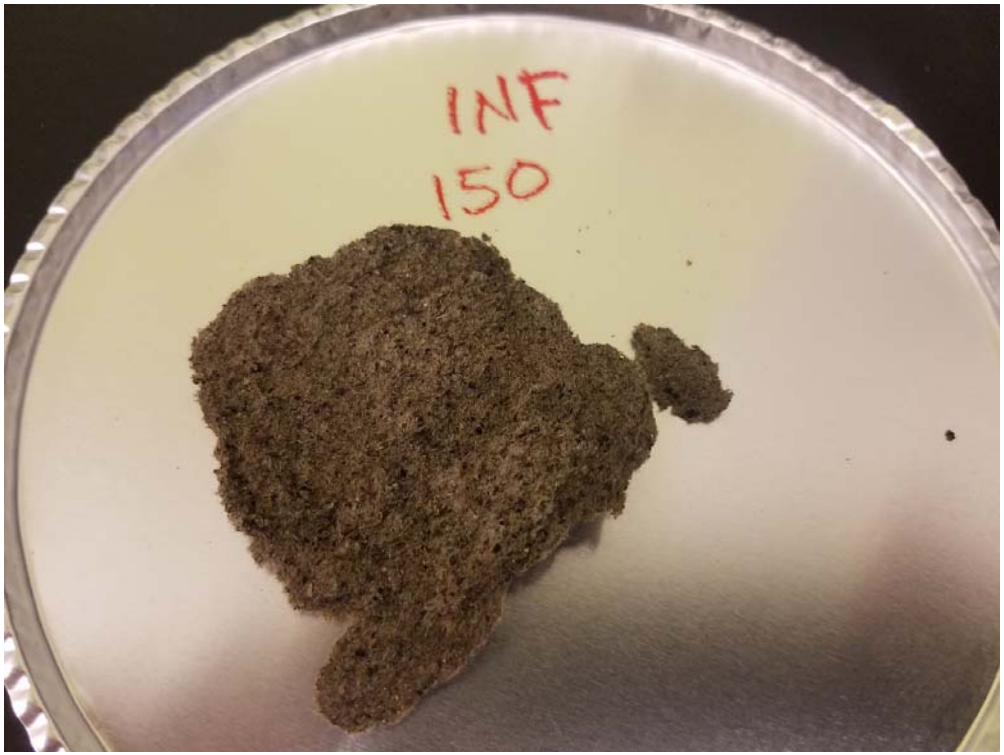
Influent



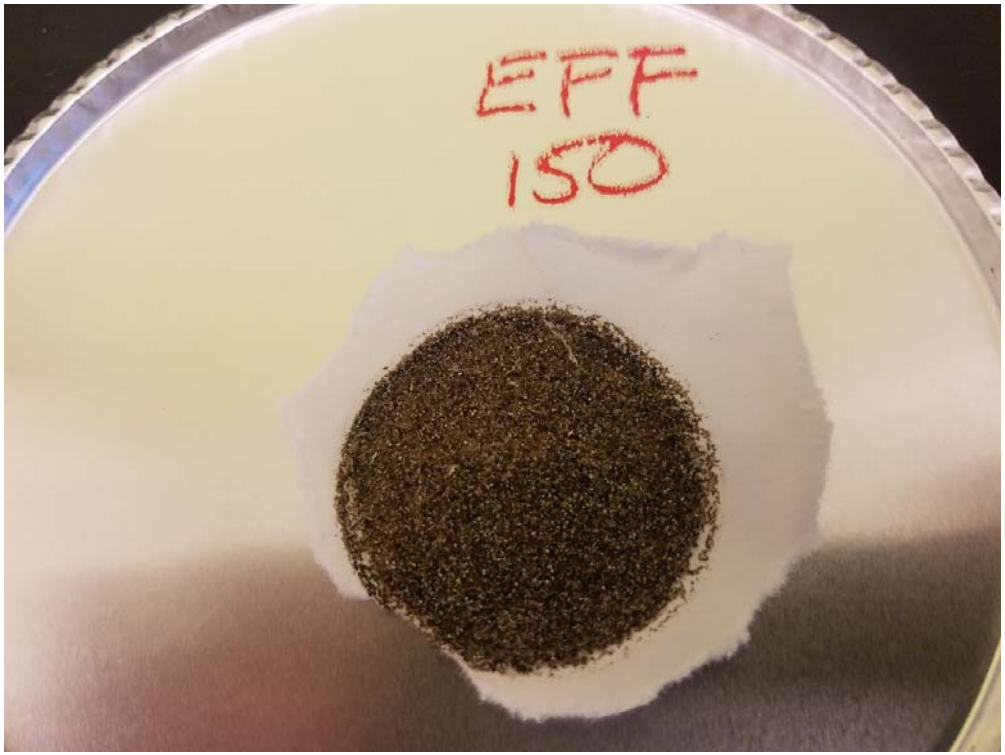
Effluent



Influent 150 micron

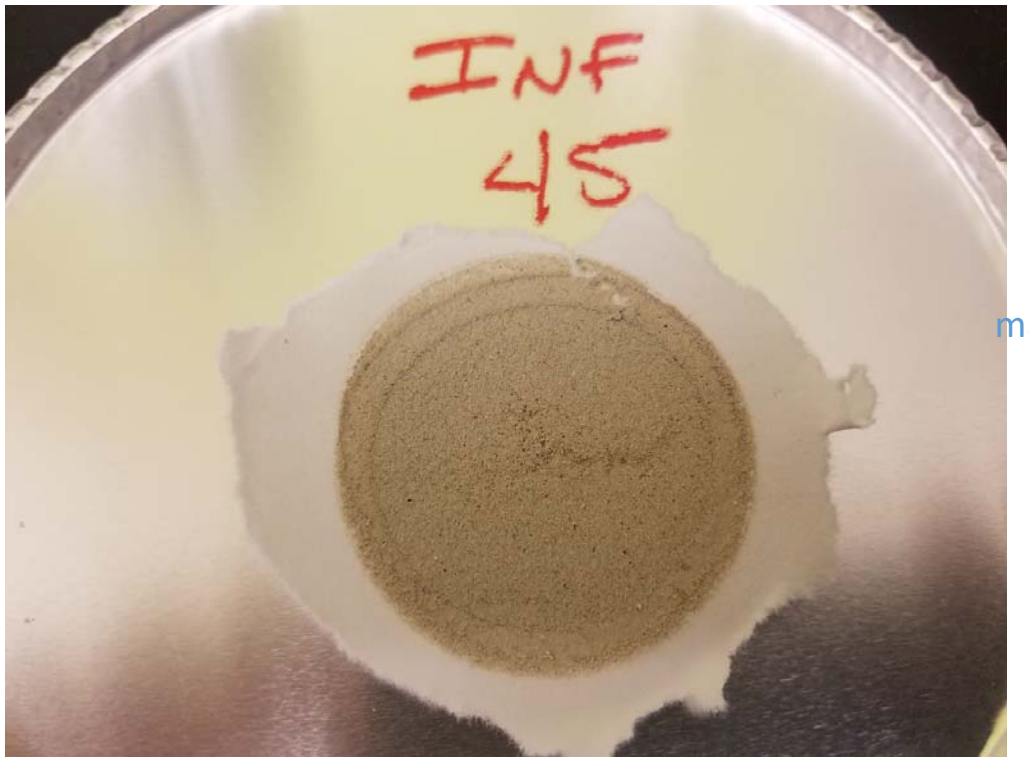


Effluent 150 micron

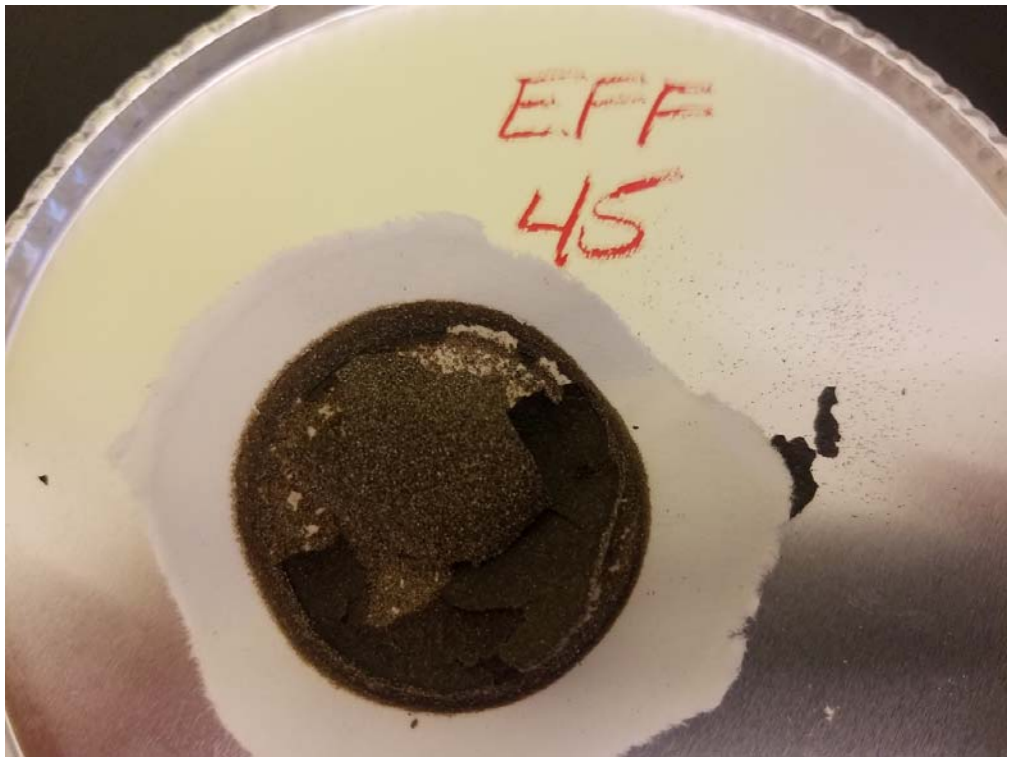


Influent 45 micron

Effluent 45 micron

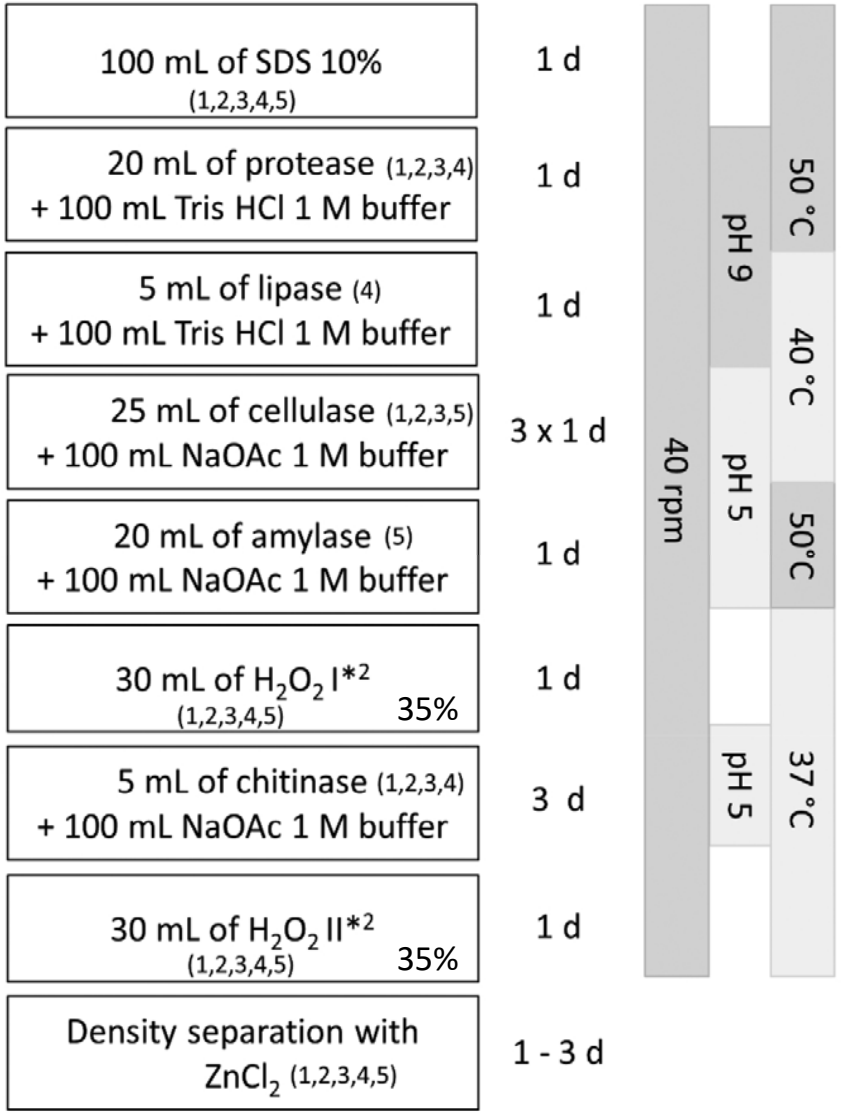


mat



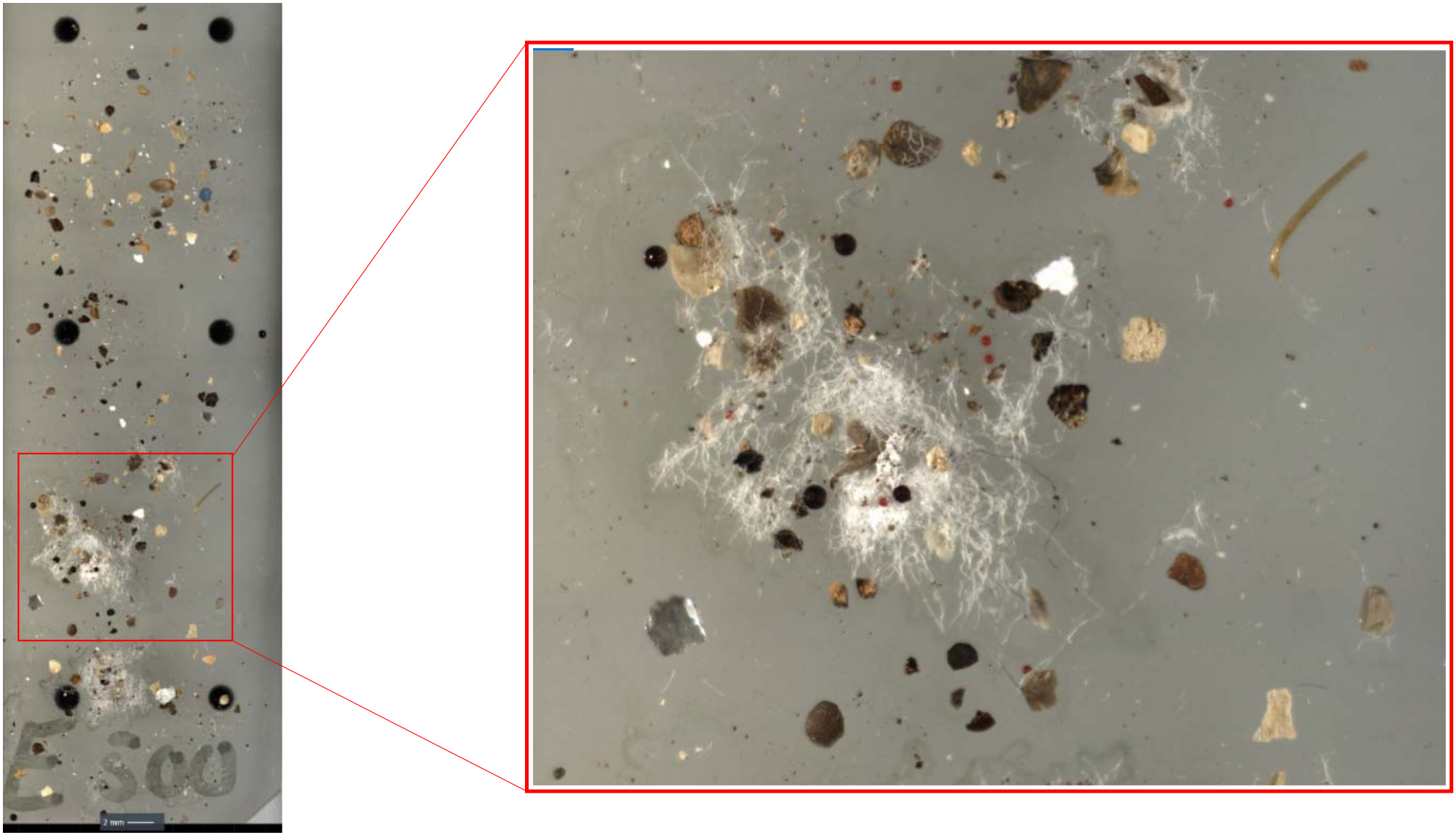
Universal Enzymatic Purification Protocol

Löder 2017

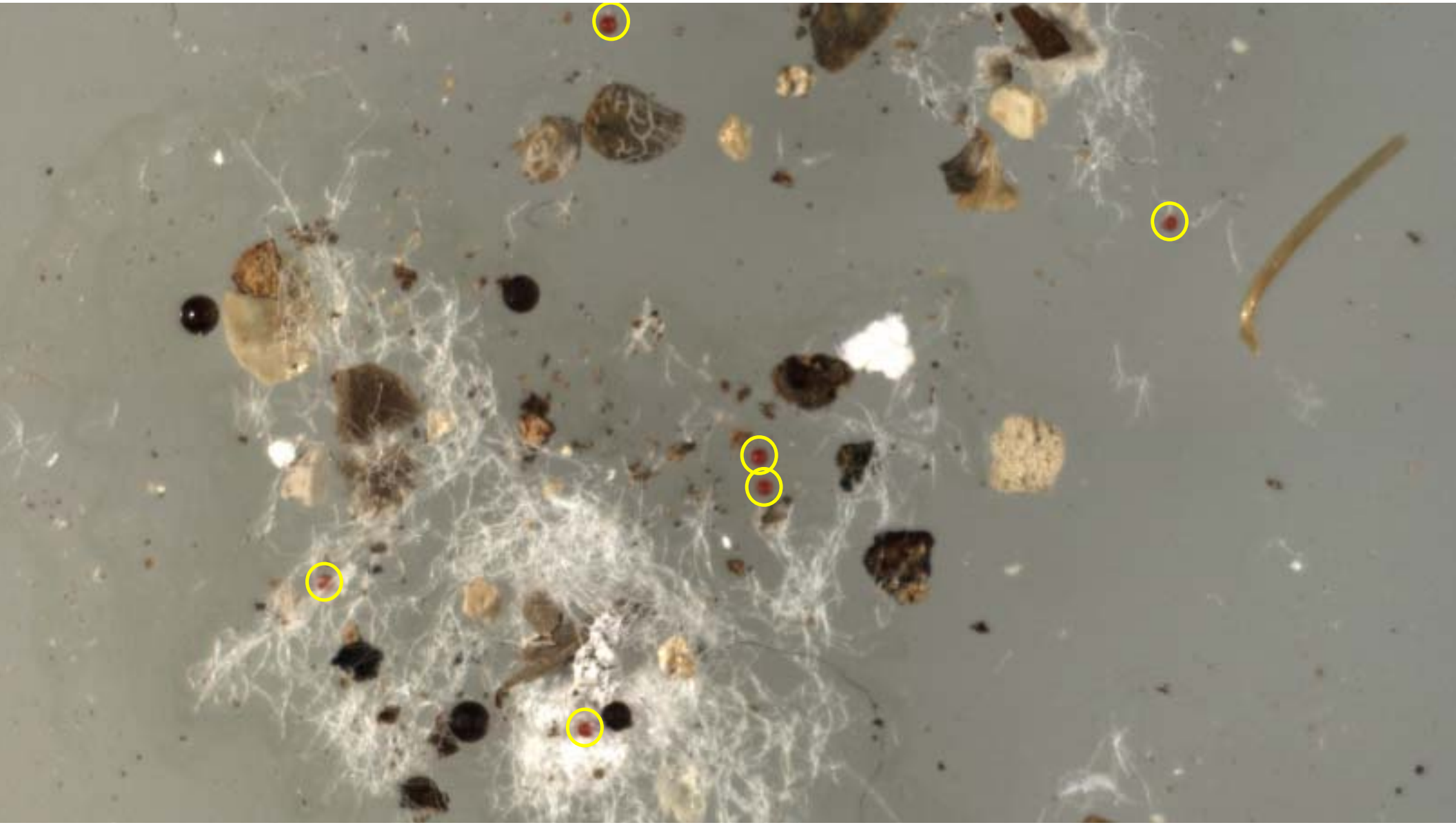


Extraction Procedure

1. Filter, dry & weigh sample (24 hrs)
2. Wet peroxide digestion (24 hrs)
3. Filter sample, protease digestion (24 hrs)
4. Filter sample, lipase digestion (24 hrs)
5. Filter sample, cellulase digestion (24 hrs x 3)
6. Filter sample, final wet peroxide, filter sample (3 hrs)

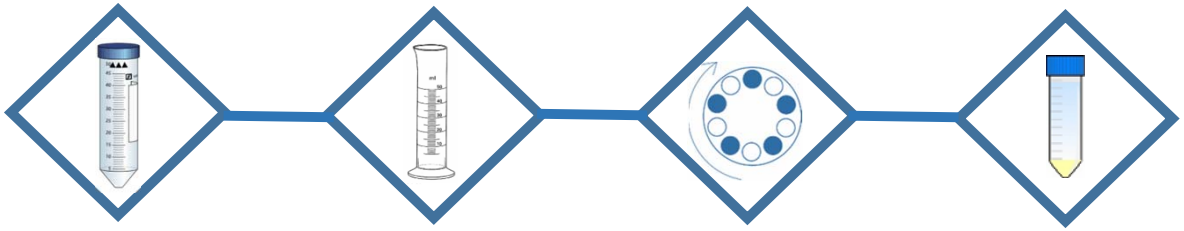

2 mm


2 mm



Sample Preparation for Microplastics

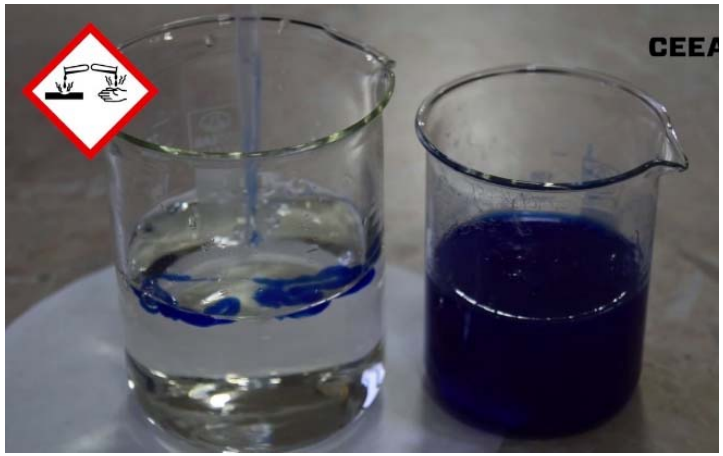
Sample Preparation



- Easy to replicate
- Addresses Interferences
- Minimizes damage and loss of particles

Standardized Preparation

Modified
Schweizer's Reagent

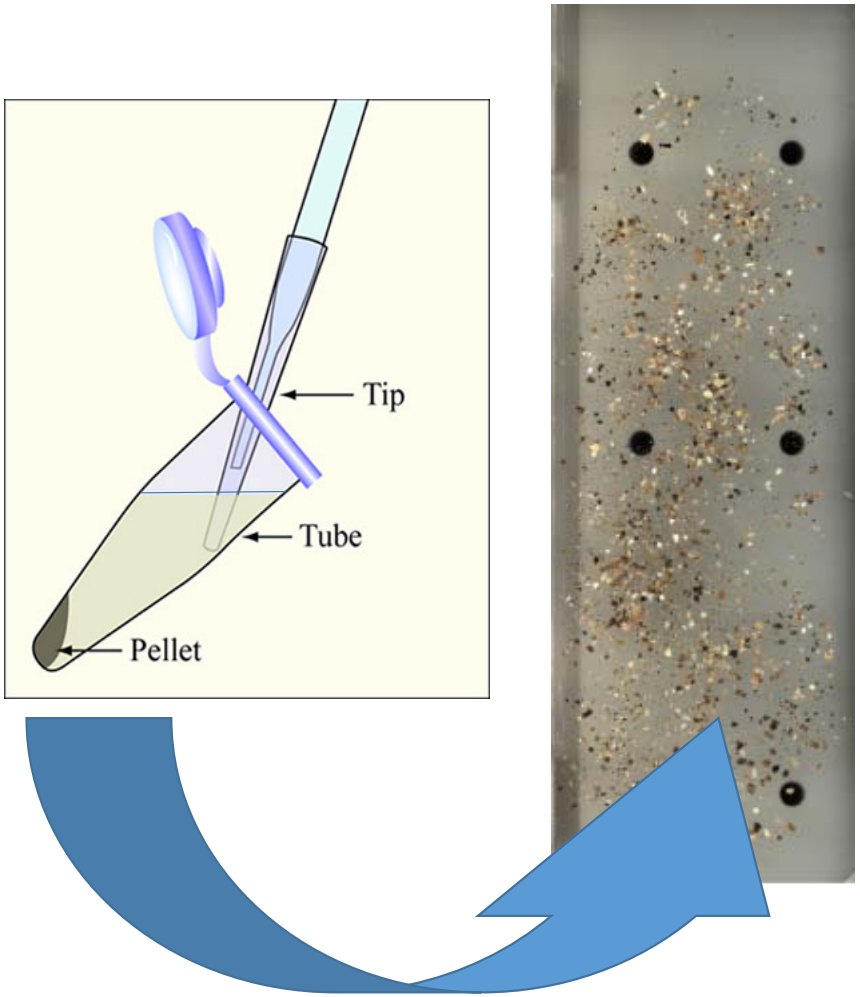


Test Tube Shaker

Centrifuge

Extraction Procedure

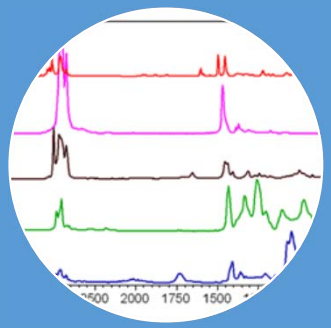
1. Wet peroxide digestion (1 hr)
2. Centrifuge & decant excess liquid (15 min)
3. Cellulose digestion with modified Schweizer's Reagent (5 min)
4. Centrifuge & decant excess liquid (5 min)
5. Schweizer's Reagent quench with 30% NH_4OH (5 min)
6. Buffer sample to pH 8 with Tris-HCl (5 min)
7. Simultaneous protease & lipase digestion (20 hrs)
8. Centrifuge & decant excess liquid (5min)
9. Water wash (5 min)
10. Centrifuge, & decant excess liquid, add 10 mL of CH_4OH



Observed Improvements

- Eliminates filtration and associated sample loss
- Cellulose is completely removed during the first step
- Preparation time is ideal
- Entire sieved contents are plated onto a single slide
- Reference spike recovery near 100%

Desired Characteristics



Sample Analysis

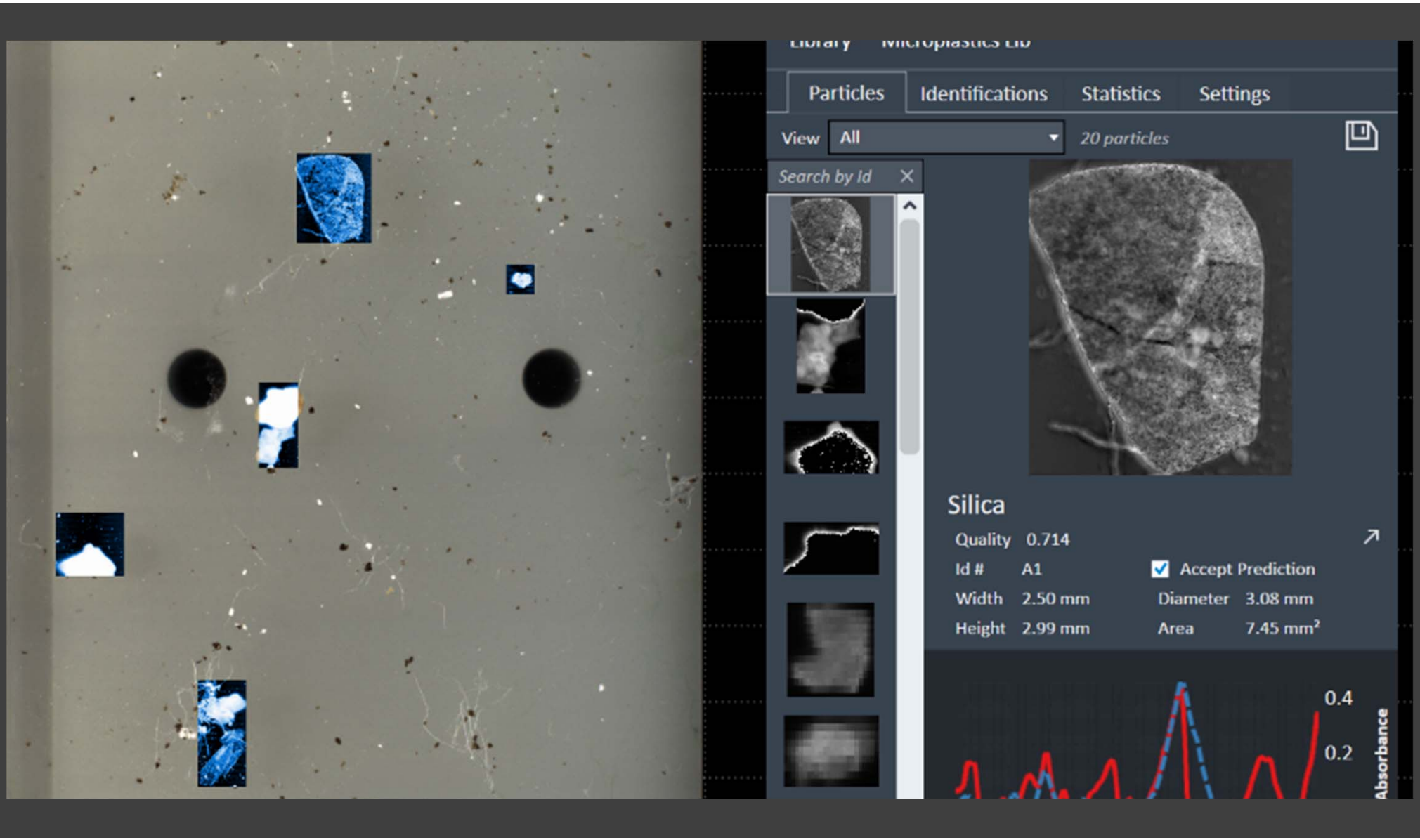
1. Spectroscopic analysis
2. Identify targets
3. Isolate individual particles
4. Positive identification
5. Quantification of particle count and size

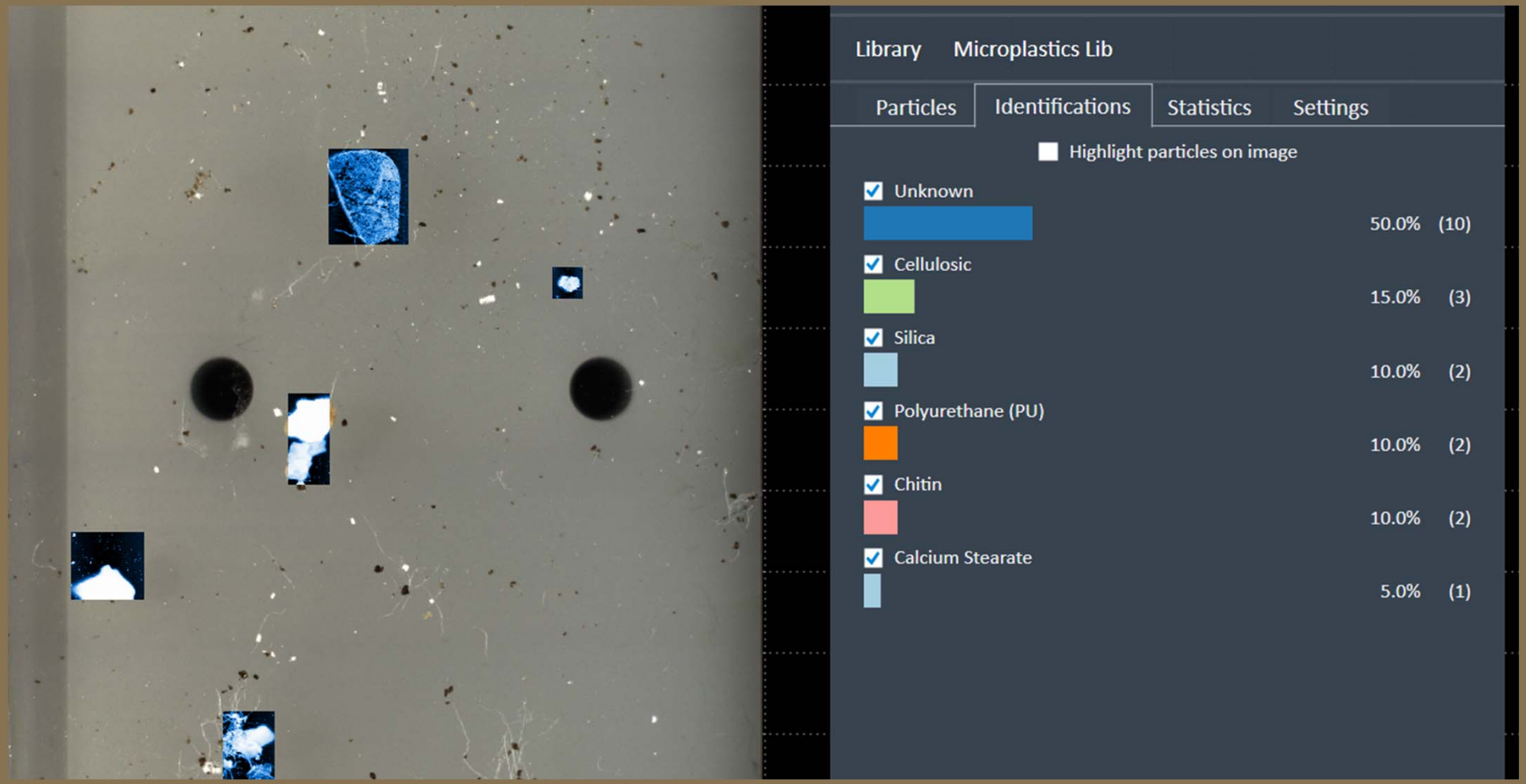
Agilent LDIR 8700

Chemical Imaging System

Agilent LDIR 8700

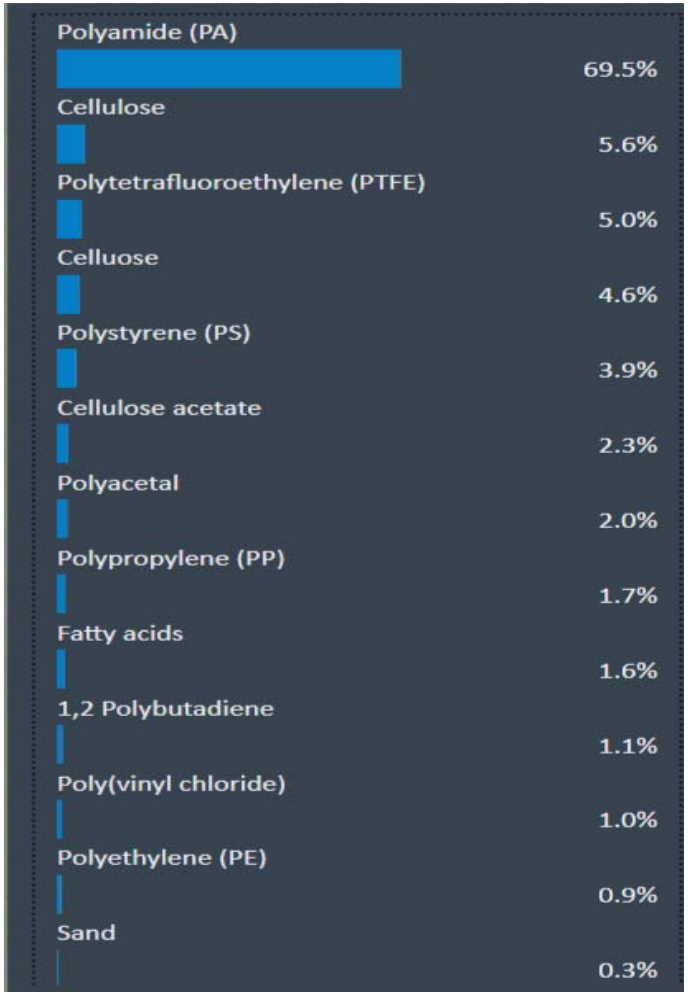
Specification	Description
Optical Resolution	1 micron
Spectral Resolution	20 micron
Wavelength Coverage	1,800 – 1,000 wavenumbers (cm^{-1})
ATR Resolution	
Light Source	Quantum Cascade Laser
Detector	TE cooled MCT
Time Required for Analysis	Minutes
Reference Library	IR and buildable


Library
Microplastics Lib
Particles
Identifications
Statistics
Settings
View
All
20 particles
Search by Id
Silica
Quality 0.714
Id # A1
Accept Prediction
Width 2.50 mm
Diameter 3.08 mm
Height 2.99 mm
Area 7.45 mm²
0.4
0.2
Absorbance

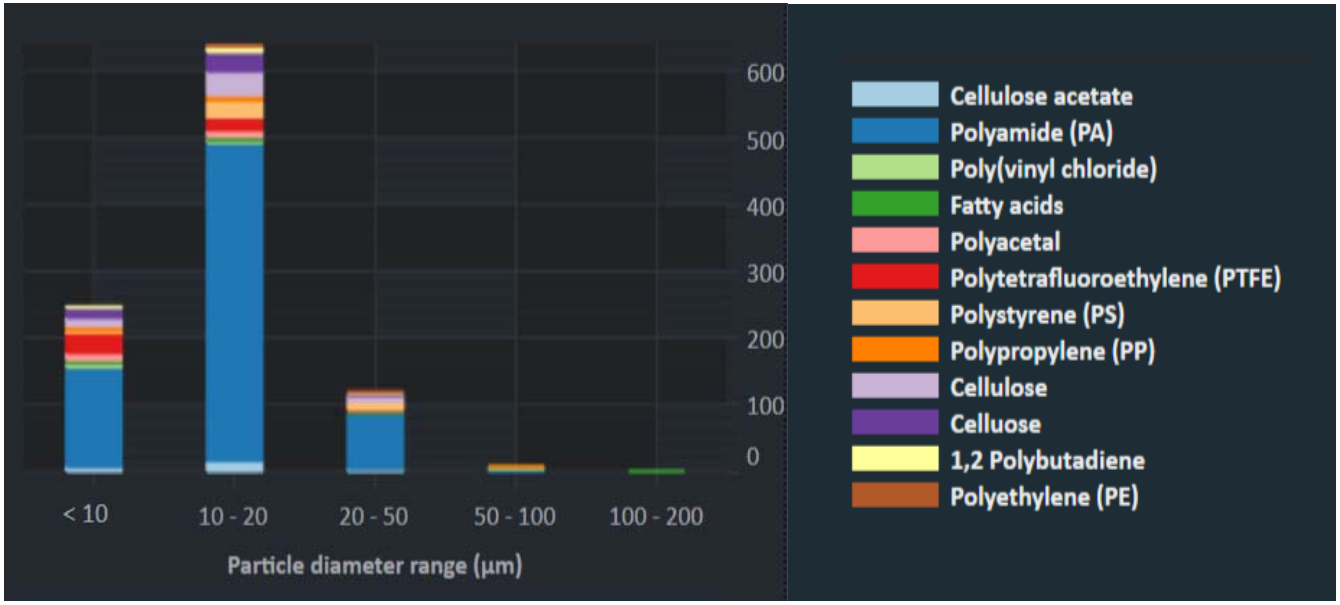

Library
Microplastics Lib
Particles
Identifications
Statistics
Settings
Highlight particles on image
Unknown
50.0% (10)
Cellulosic
15.0% (3)
Silica
10.0% (2)
Polyurethane (PU)
10.0% (2)
Chitin
10.0% (2)
Calcium Stearate
5.0% (1)

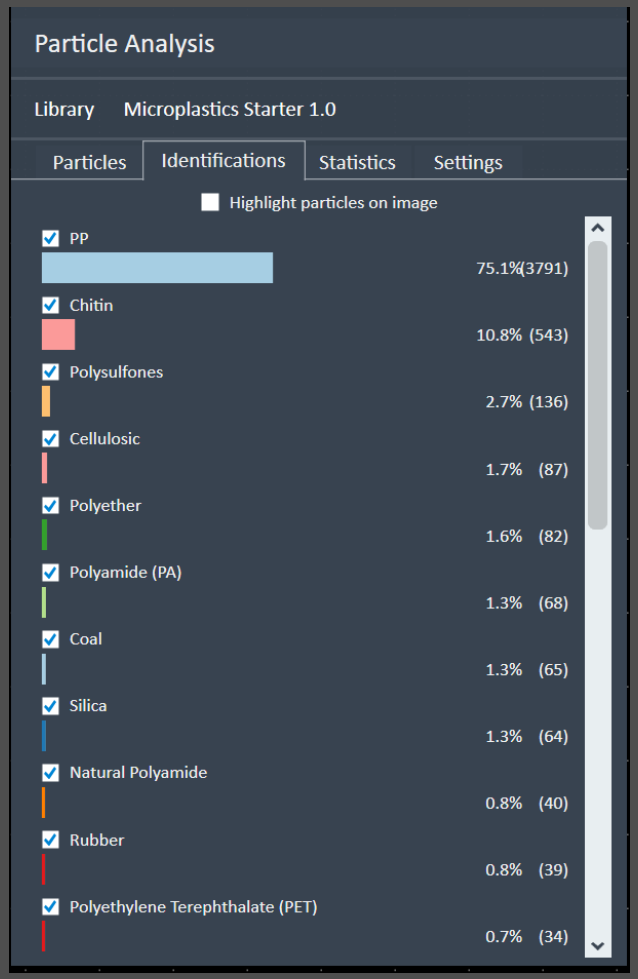

PE REference Sphere
Particle Analysis
Spectral Matching
Select All
Filter
9 spectra , 1 selected
Particle Analysis A4
1:53 PM
Particle Analysis A1
1:53 PM
Particle Analysis A5
1:54 PM
Particle Analysis A2
1:54 PM
Particle Analysis A3
1:54 PM
Particle Analysis A6
1:54 PM
Polyethylene (PE)
11:28 AM
Particle Analysis B1
1:59 PM
Particle Analysis A1
2:01 PM
1.000
0.800
0.600
0.400
0.200
0.000
Absorbance
1800 1700 1600 1500 1400 1300 1200 1100 1000
Wavenumber (cm⁻¹)
Particle Analysis
Library Microplastics Starter 1.0
Particles Identifications Statistics Settings
View All
1 particle
Search by Id
Particle Analysis A1
Quality 0.967
Id # A1
Accept Prediction
Width 215.00 µm
Diameter 214.13 µm
Height 214.00 µm
Area 36011.93 µm²
1.2
0.8
0.4
0
Absorbance
1800 1600 1400 1200 1000
Wavenumber (cm⁻¹)
1 mm

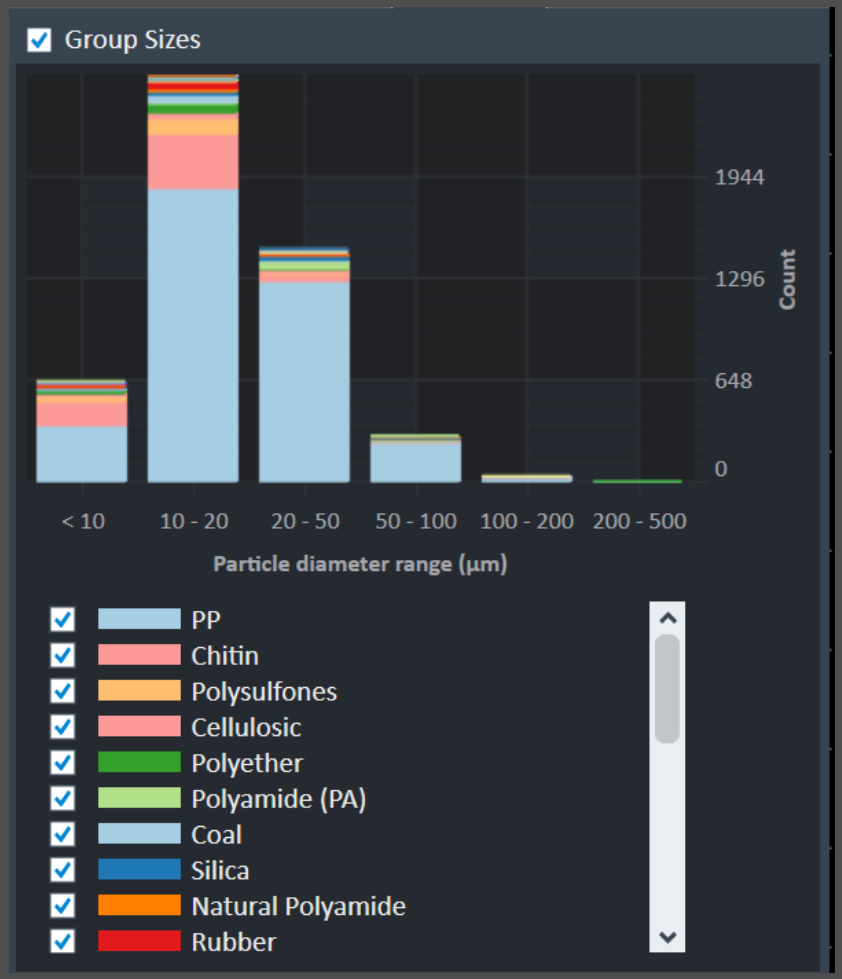
Influent 300 micron



- 1028 particles detected
- Polyamides were most prevalent
- Wool, silk, nylon from textiles
- Undigested protein-rich debris
- Followed by cellulosic particles, PTFE (Teflon), polystyrene




Particle Analysis
Library Microplastics Starter 1.0
Particles
Identifications
Statistics
Settings
Highlight particles on image
PP 75.1% (3791)
Chitin 10.8% (543)
Polysulfones 2.7% (136)
Cellulosic 1.7% (87)
Polyether 1.6% (82)
Polyamide (PA) 1.3% (68)
Coal 1.3% (65)
Silica 1.3% (64)
Natural Polyamide 0.8% (40)
Rubber 0.8% (39)
Polyethylene Terephthalate (PET) 0.7% (34)


Group Sizes
1944
1296
648
0
Count
< 10
10 - 20
20 - 50
50 - 100
100 - 200
200 - 500
Particle diameter range (µm)
PP
Chitin
Polysulfones
Cellulosic
Polyether
Polyamide (PA)
Coal
Silica
Natural Polyamide
Rubber

Instrument Validation

	Sample Analyzed	LDIR Particle Count	Usable Partiles Identified	Acrylonitrile Butadiene	Alkyd Varnish	Calcium Stearate	Cellulosic	Chitin	Coal	Natural Polyamide	Polyamide (PA)	Polyethylene (PE)	Polyethylene Terephthalate (PET)	Polyimide	Polypropylene (PP)	Polytetrafluoroethylene (PTFE)	Polyurethane (PU)	Rubber	Silica
	Run 1	136	132	7	5	4	12	1	0	60	9	2	2	0	9	1	19	1	0
	Run 2	138	131	3	1	5	12	1	1	63	7	2	1	0	9	1	25	0	0
	Run 3	136	131	7	3	5	12	0	0	65	10	2	1	0	8	0	16	2	1
	Run 4	140	135	7	6	5	12	0	1	63	11	2	1	1	8	1	17	0	0
	Run 5	136	127	8	4	6	7	2	2	60	10	2	1	1	8	0	12	3	1
	Run 6	137	130	1	1	5	12	1	2	60	11	2	2	0	8	0	24	1	0
	Run 7	137	127	0	2	6	13	1	1	58	9	2	1	0	8	0	26	0	0
	Run 8	135	135	0	3	6	11	1	3	58	9	1	0	1	9	1	25	1	0
True Number:															10				
Mean Detected:		137	131	4.13	3.13	5.25	11.4	0.88	1.25	60.9	9.5	1.88	1.13	0.38	8.38	0.5	20.5	1	0.25
Percent Recovery:															83.8				
Std Dev:		1.55	3.07	3.48	1.81	0.71	1.85	0.64	1.04	2.53	1.31	0.35	0.64	0.52	0.52	0.53	5.21	1.07	0.46
Relative Std Dev:		1.13	2.34	84.4	57.8	13.5	16.2	73.2	82.8	4.16	13.8	18.9	57	138	6.18	107	25.4	107	185

Particle Analysis

Library Microplastics Lib

Particles | Identifications | Statistics | Settings

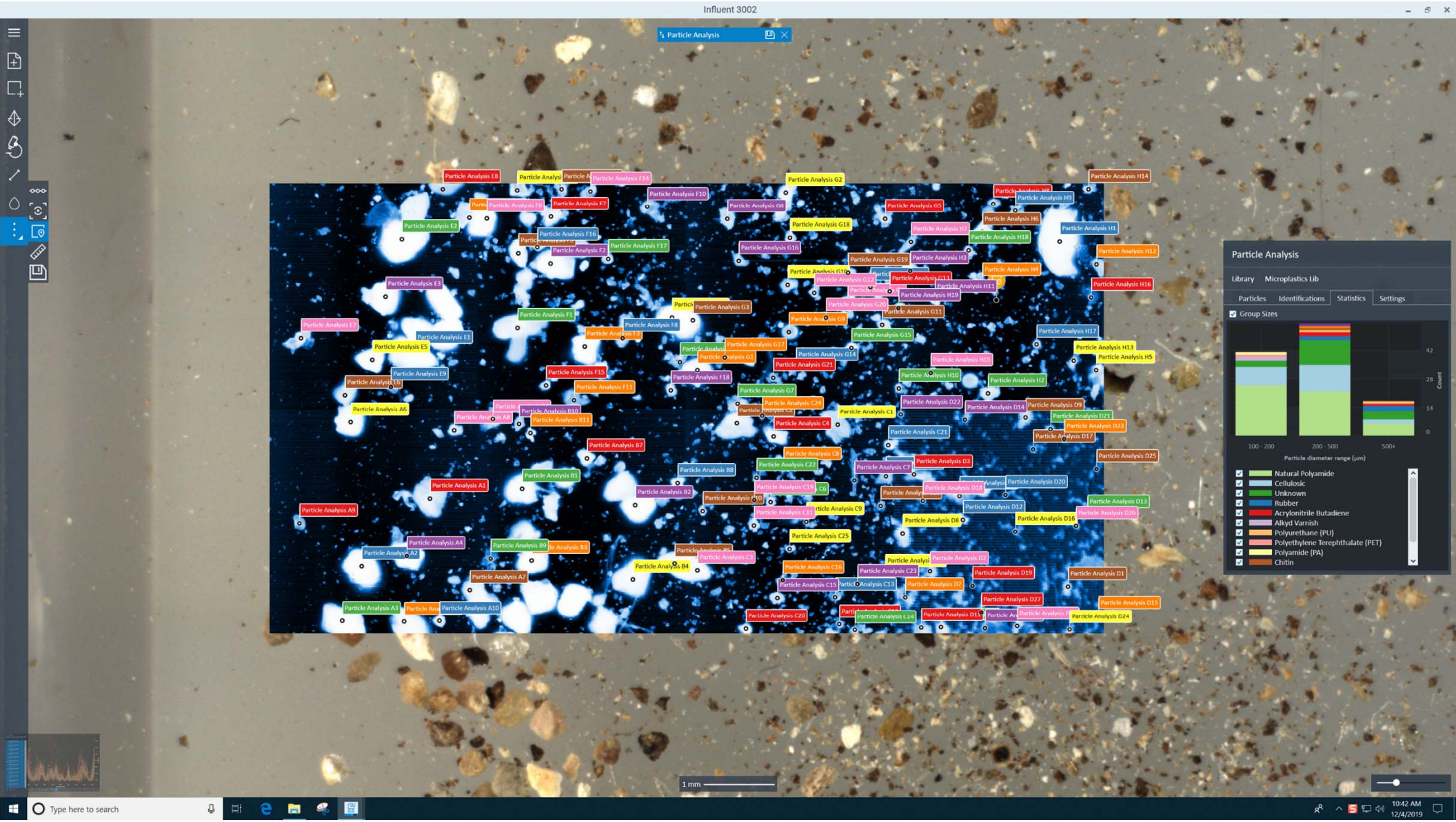
☑ Auto Scan

Particle Sensitivity

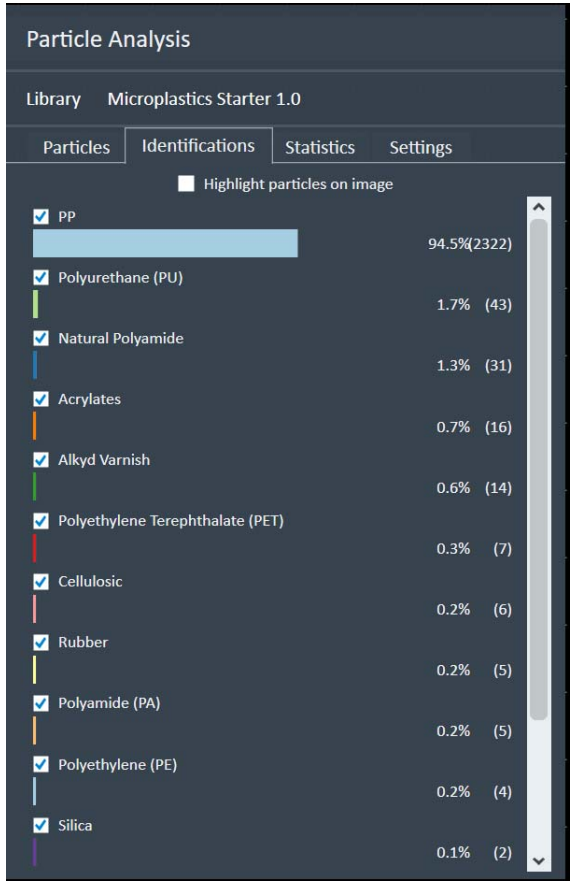
Particle Diameter (µm)

Minimum 100 ☐ Auto

Maximum 5000 ☑ Auto


Influent 3002
Particle Analysis
Particle Analysis
Library
Microplastics Lib
Particles
Identifications
Statistics
Settings
Group Sizes
42
28
14
0
Count
100 - 200
200 - 500
500+
Particle diameter range (µm)
Natural Polyamide
Cellulosic
Unknown
Rubber
Acrylonitrile Butadiene
Alkyd Varnish
Polyurethane (PU)
Polyethylene Terephthalate (PET)
Polyamide (PA)
Chitin
1 mm
Type here to search
10:42 AM
12/4/2019


Particle Analysis
Library Microplastics Starter 1.0
Particles Identifications Statistics Settings
Auto Scan
Collect Visible Image
Particle Sensitivity
Classification Ranges
Particle Diameter (µm)
Minimum 20 Auto
Maximum 2000 Auto


Particle Analysis
Library Microplastics Starter 1.0
Particles Identifications Statistics Settings
Highlight particles on image
PP 94.5% (2322)
Polyurethane (PU) 1.7% (43)
Natural Polyamide 1.3% (31)
Acrylates 0.7% (16)
Alkyd Varnish 0.6% (14)
Polyethylene Terephthalate (PET) 0.3% (7)
Cellulosic 0.2% (6)
Rubber 0.2% (5)
Polyamide (PA) 0.2% (5)
Polyethylene (PE) 0.2% (4)
Silica 0.1% (2)

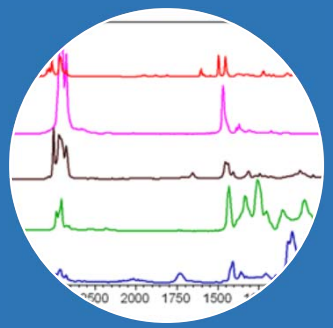

Particle Analysis
Library Microplastics Starter 1.0
Particles Identifications Statistics Settings
Group Sizes
Particle diameter range (µm)
PP
Polyurethane (PU)
Natural Polyamide
Acrylates
Alkyd Varnish
Polyethylene Terephthalate (PET)
Cellulosic
Rubber
Polyamide (PA)
Polyethylene (PE)


Particle Analysis
Library Microplastics Starter 1.0
Particles Identifications Statistics Settings
PP
Wavenumber (cm⁻¹)

Excel Data Export

#	Id	Width	Height	Diameter	Aspect Ratio	Area	Perimeter	Eccentrici	Circularity	Solidity	Identification	Quality	Is Valid
1	A1	721	529	540.5484	1.363601028	229487.5	2990.279552	0.794483	0.322512	0.780205	Cellulosic	0.70778	true
2	A2	589	494	535.8316	1.191709808	225500	1874.091612	0.664369	0.806817	0.969267	Rubber	0.686543	true
3	A3	625	430	524.1045	1.453488372	215737.5	1919.619399	0.617248	0.735708	0.970479	Natural Polyamide	0.699224	true
4	A4	657	415	473.4825	1.58367215	176075	1940.954526	0.581468	0.587323	0.896284	Unknown	0.626958	true
5	A5	550	375	443.7235	1.466666667	154637.5	1651.040754	0.570457	0.712868	0.974171	Unknown	0.644938	true
6	A6	430	395	425.4491	1.088607595	142162.5	1483.761536	0.696801	0.811459	0.967915	Cellulosic	0.669999	true
7	A7	230	805	393.5601	0.285714286	121650	2004.680365	0.863211	0.380392	0.880883	Unknown	0.630628	true
8	A8	210	167	186.5242	1.256410171	27325	648.7005711	0.671909	0.815984	0.96427	Natural Polyamide	0.69458	true
9	A9	216	154	177.8764	1.404301234	24850	622.8427076	0.544338	0.80497	0.965986	Polyethylene Terephthalate (	0.653054	true
10	A10	182	169	174.7167	1.073770495	23975	599.4112474	0.720755	0.83853	0.974099	Natural Polyamide	0.699323	true
11	B1	897	816	750.3297	1.099287452	442175	4734.751766	0.725563	0.247862	0.750706	Natural Polyamide	0.682812	true
12	B2	906	629	683.9621	1.439613179	367412.5	3268.269461	0.526536	0.432243	0.756129	Unknown	0.647627	true
13	B3	770	492	591.9153	1.565899468	275175	2582.79218	0.545498	0.51837	0.850585	Acrylonitrile Butadiene	0.651467	true
14	B4	596	578	551.8541	1.031010139	239187.5	2116.015492	0.806203	0.671291	0.924262	Rubber	0.692184	true
15	B5	405	470	405.4714	0.861702128	129125	1608.11182	0.652729	0.627462	0.919285	Natural Polyamide	0.653315	true
16	B6	277	391	309.3025	0.708571501	75137.5	1132.045807	0.751547	0.736781	0.94468	Cellulosic	0.660203	true
17	B7	417	241	287.183	1.730526346	64775	1331.959578	0.584122	0.458812	0.857805	Natural Polyamide	0.670752	true
18	B8	262	220	211.3641	1.193002971	35087.5	967.9036713	0.790561	0.47065	0.755789	Natural Polyamide	0.666217	true
19	B9	305	167	177.1142	1.822222374	24637.5	1106.898619	0.729224	0.252692	0.689161	Natural Polyamide	0.74989	true
20	B10	159	208	170.7548	0.762520215	22900	642.8427058	0.792272	0.696363	0.906931	Natural Polyamide	0.708936	true
21	B11	155	172	155.8941	0.901204853	19087.5	612.3401785	0.756879	0.639696	0.879608	Natural Polyamide	0.663842	true
22	C1	840	500	554.4006	1.679999951	241400	3014.507912	0.664947	0.333821	0.813137	Natural Polyamide	0.718071	true
23	C2	825	435	527.8415	1.896551706	218825	2834.507915	0.865484	0.342256	0.777664	Natural Polyamide	0.656713	true
24	C3	600	446	447.0112	1.34631437	156937.5	2155.304814	0.713552	0.424541	0.759804	Natural Polyamide	0.683267	true
25	C4	363	336	310.0477	1.079202306	75500	1450.538229	0.700405	0.450919	0.80138	Natural Polyamide	0.684362	true
26	C5	255	403	293.2701	0.633367615	67550	1540.538228	0.810585	0.357676	0.798345	Cellulosic	0.771951	true
27	C6	269	588	283.4737	0.456880747	63112.5	1849.741321	0.811755	0.231795	0.605396	Natural Polyamide	0.725266	true
28	C7	149	667	253.8226	0.223171385	50600	1688.111818	0.958237	0.22313	0.647266	Natural Polyamide	0.708144	true
29	C8	422	173	237.2617	2.443395967	44212.5	1169.325024	0.564417	0.406335	0.833805	Polyurethane (PU)	0.712773	true

LDIR Analysis of Microplastics

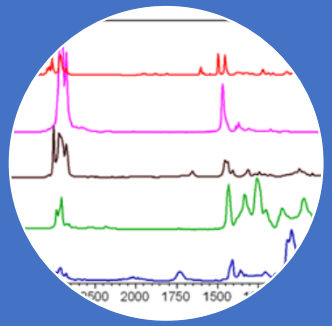


Sample Analysis

- Identification of MP materials
- Quantification of MP particles
- Particle size distribution
- Reproducible

Sample Collection

Sample Preparation



Sample Analysis

Reproducibility Achieved

Thank You!

Stay Positive…….Test Negative