Bilgewater Emulsion Formation and Breaking by In Situ Multiplexed Chemical Imaging and Microfluidics

WP18-1660

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PI's Organization: Pacific Northwest National Laboratory
DOD Co-performer: Danielle Paynter
Co-Performer Organization: Naval Surface Warfare Center, Carderock Division

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SERDP
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SON WPSON-18-C1, Advancing emulsion science for application in armed forces vessels

Project Team

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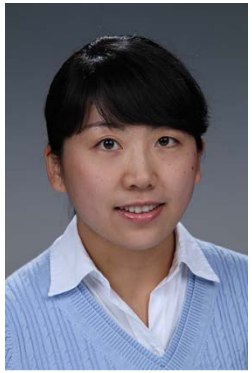
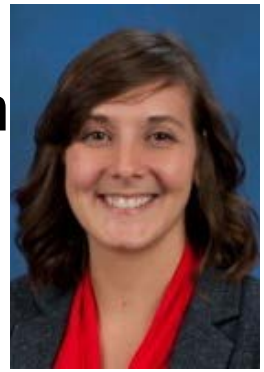
- Team members: Dr. Jiyoung Son (postdoc), Yanjie Shen (graduate student), Dr. George Bonheyo, Jenn Yao (staff scientist), Rachel Komorek (research associate), and EMSL supporting staff



- Co-performer: Danielle Paynter, bilge emulsion, Naval Surface Warfare Center, Carderock Division



Background

- Limited scope project initiated in 2018
- Objectives:
  - ♦ Build a fundamental knowledge base concerning the occurrence and behaviors of shipboard oil/water emulsions
  - ♦ Obtain a better understanding of how oil/water emulsions are created, stabilized, or worsened in this environment
  - ♦ ***Assist the development of methodologies to treat bilge wastewater***
  - ♦ ***Identify the factors that drive the formation and breaking of bilge***, e.g., size distribution, hydrophobicity/hydrophilicity, affinity to potential coagulants, and feasibility of microbial biodegradation

Technical Objectives

- Investigate the fundamental physiochemical processes in the formation, stabilization, and breaking of shipboard emulsions using a vacuum compatible microreactor to image the chemical processes

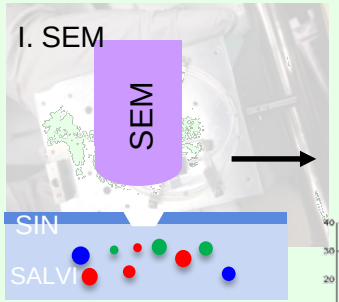
- Hypothesis: **Surface chemical changes at the l-l interface are critical for mass and charge transfer** leading to emulsion formation, stabilization, and breaking

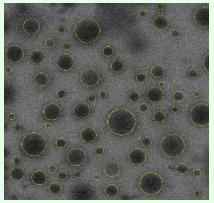
- Questions to answer:
  - How do the NAVY model emulsifiers affect emulsion stabilization in ship bilgewater conditions?
  - How does microbial biochemistry and biodegradation affect emulsion formation, stabilization, and worsening?

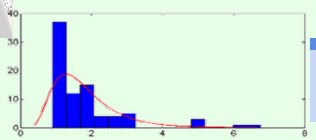
Technical Approach

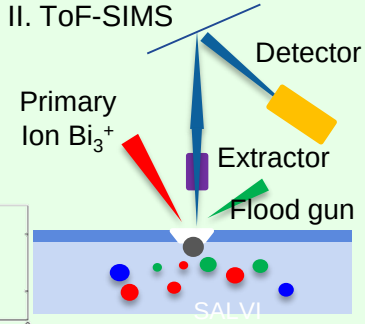
Limited Scope

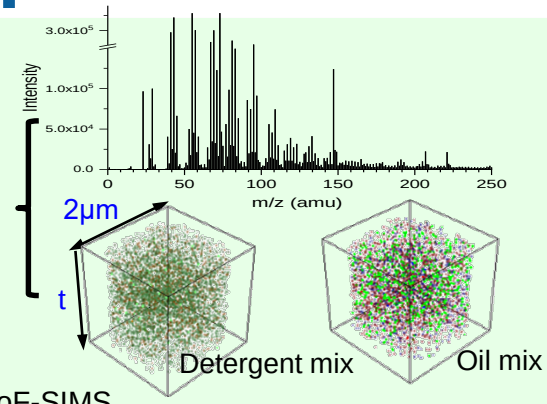
Task 1
In Situ Imaging of Bilgewater



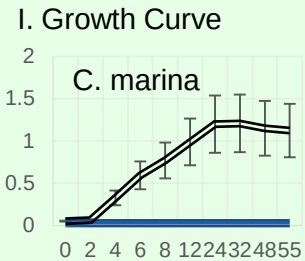


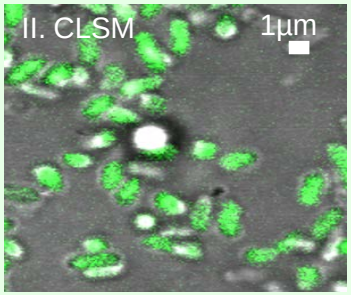


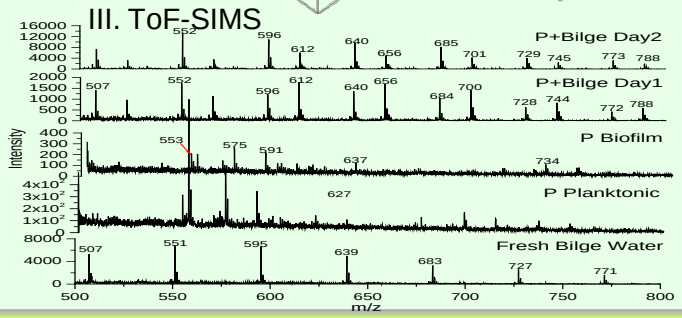




Task 2
Investigate Microbial Effects



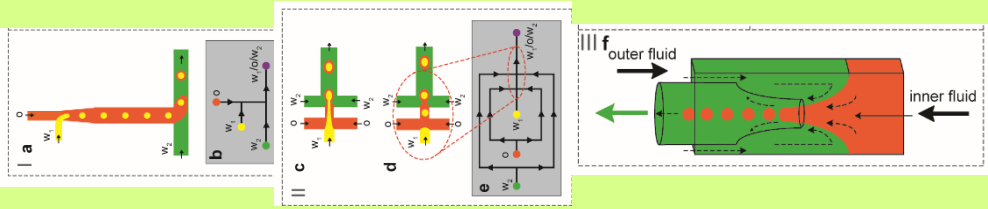




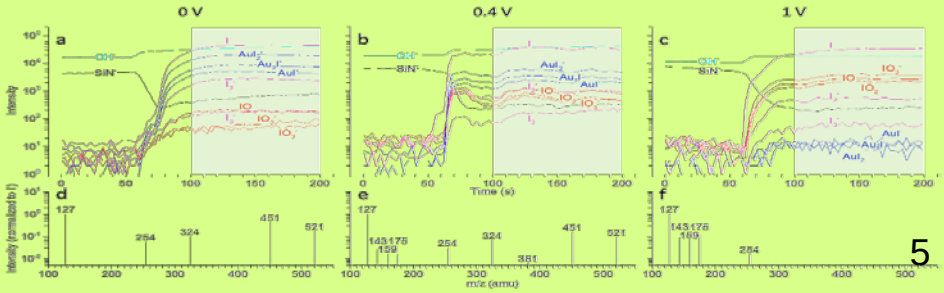
Future Work

Task 5
Comsol Modeling

Task 3
Droplet Fluidics Design & Fabrication



Task 4
In Situ, In Vivo, and In Operando Study of Emulsion Stabilization & Breaking



Task 1. Bilgewater Multimodal Imaging

Hypothesis:

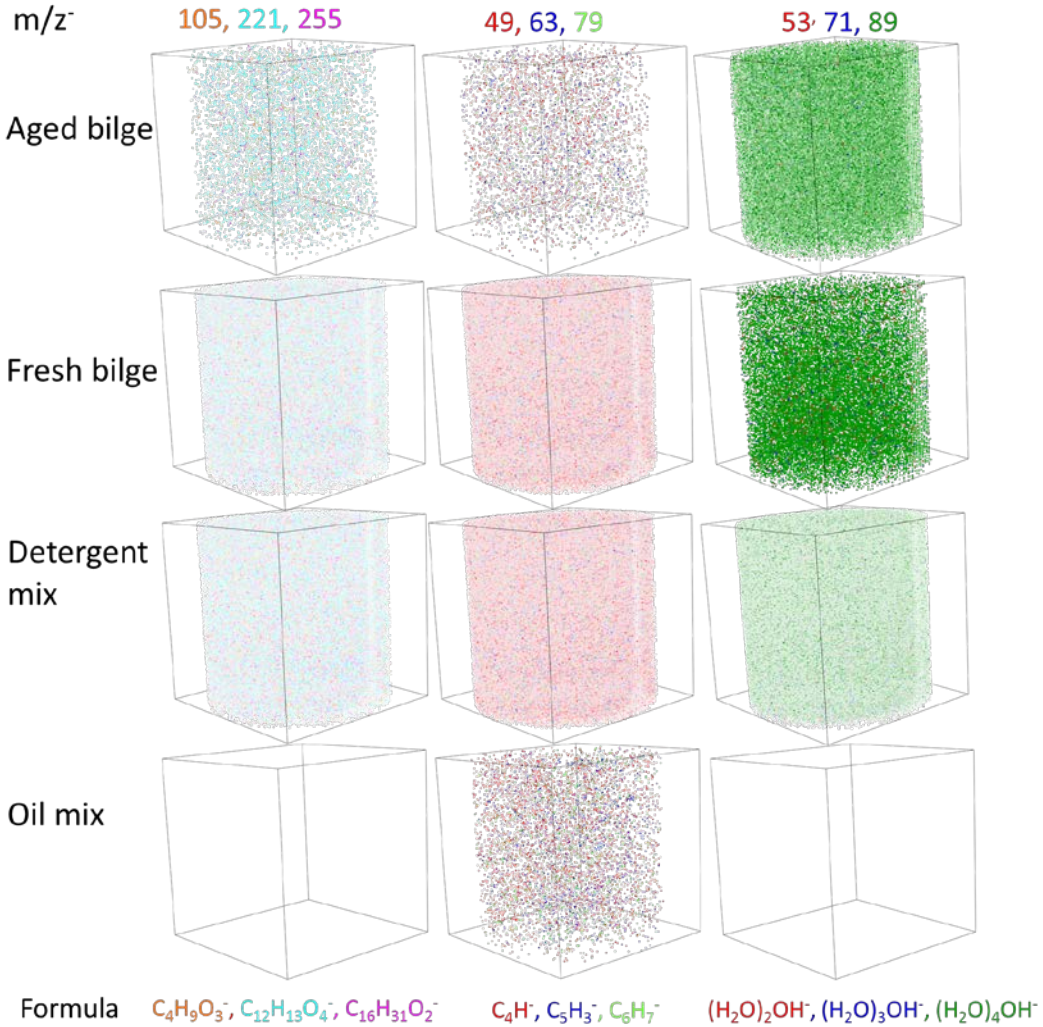
Bilgewater emulsion is more likely to form with favorable oil and water mixing ratios under high mechanical perturbation

Outcome:

- In situ SIMS molecular imaging of l-l interfaces
- In situ SEM of droplet size distribution (DSD)
- Other in situ chemical mapping of emulsion characteristics

Approach:

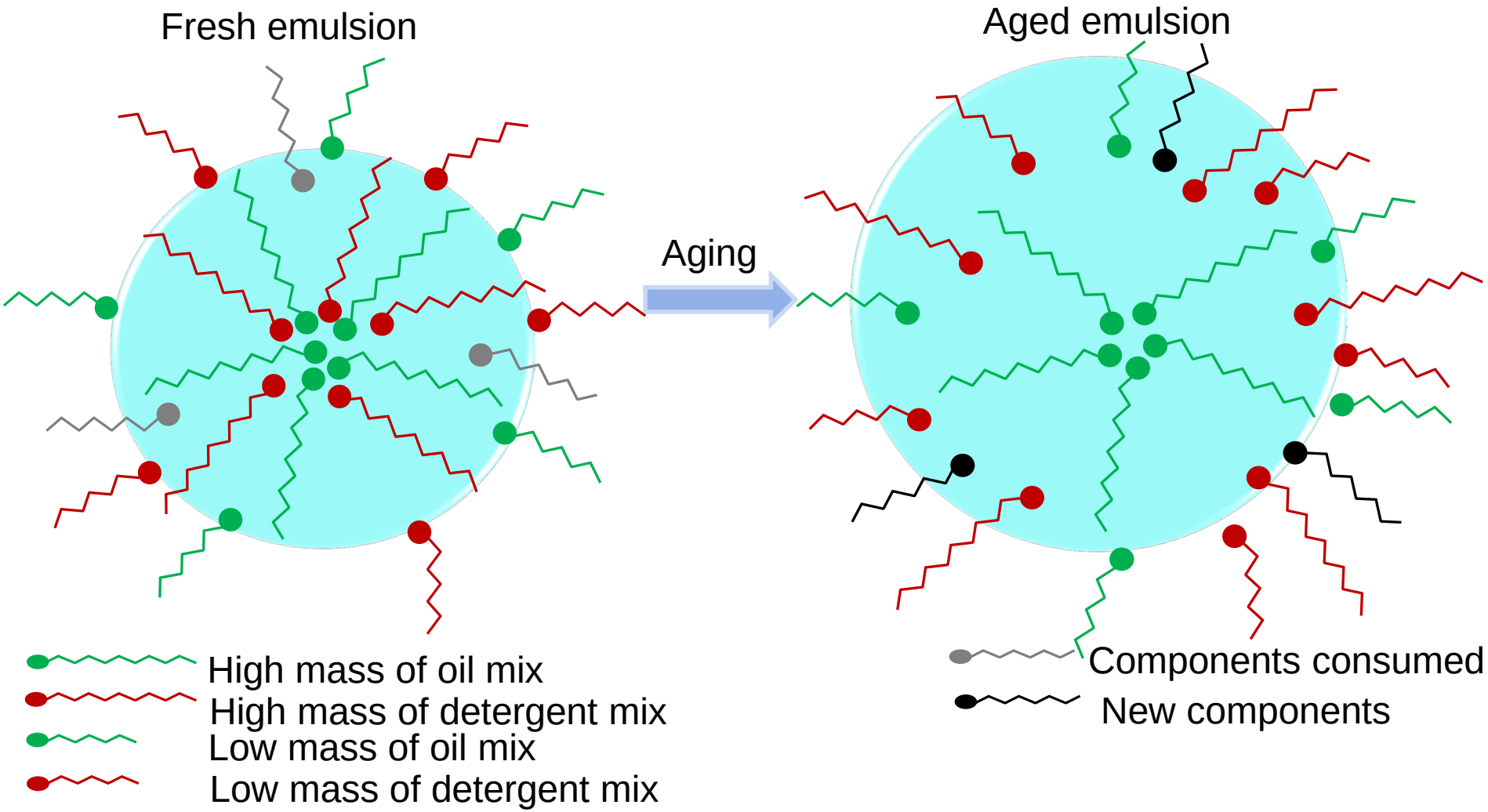
- Direct chemical mapping of the l-l interface by in situ liquid SIMS, SEM, and other chemical imaging tools



The evolution of the l-l interface of oil-in-water bilge imaged by in situ liquid SIMS

Shen et al., in preparation, 2019

Findings from static SIMS & optical imaging



- Go/No Go: ToF-SIMS is feasible to characterize bilge surface evolution

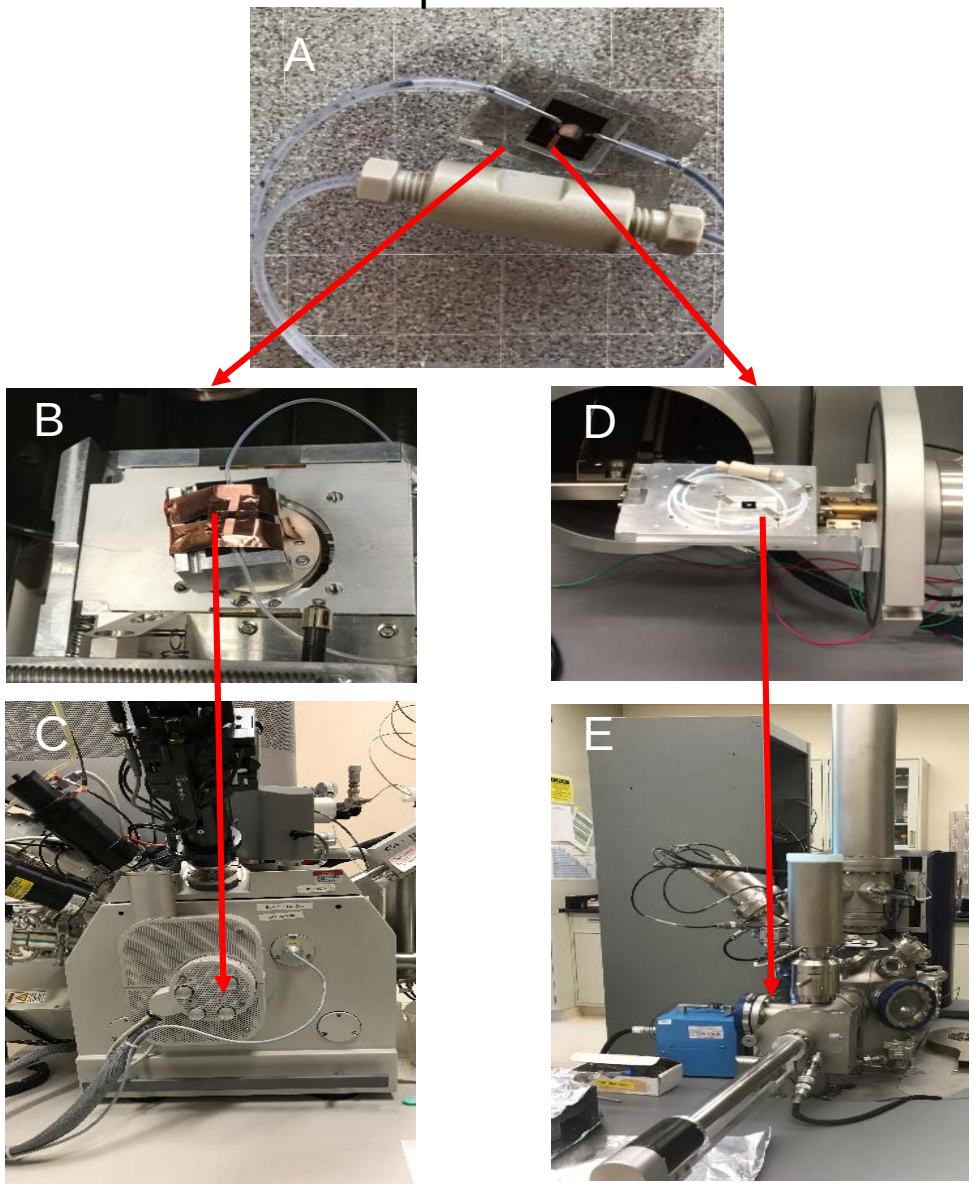
Son et al., Chemosphere, 2019

Task 1: In situ bilge multimodal imaging

- SAVLI is a vacuum compatible microfluidic platform for multimodal chemical imaging
- Easily adaptable to many instruments: NMR, optical microscopy, SEM, SIMS
- Demonstrated in situ SEM and in situ ToF-SIMS to study bilge emulsion
- Complemented surface characterization with optical imaging, i.e., CLSM and optical microscopy, liquid NMR

Shen et al., in preparation, 2019

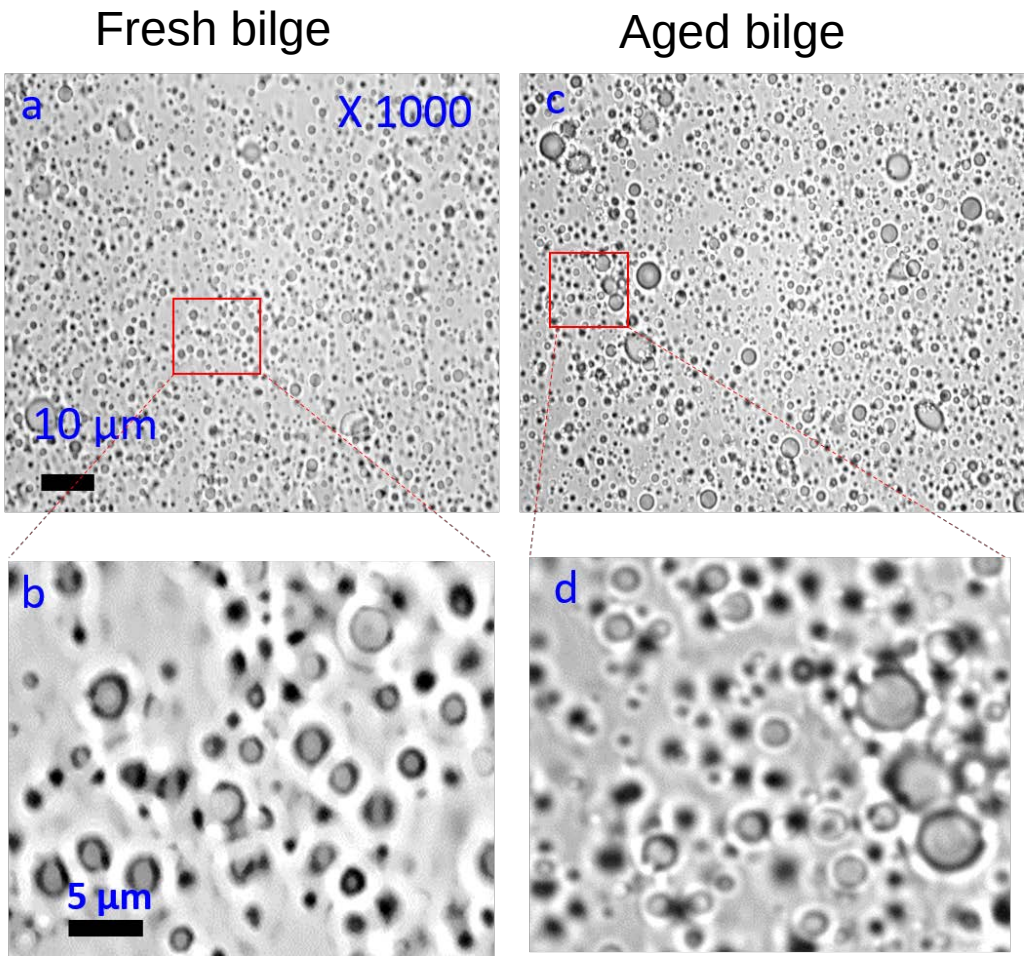
Workflow of in situ liquid SEM and ToF-SIMS

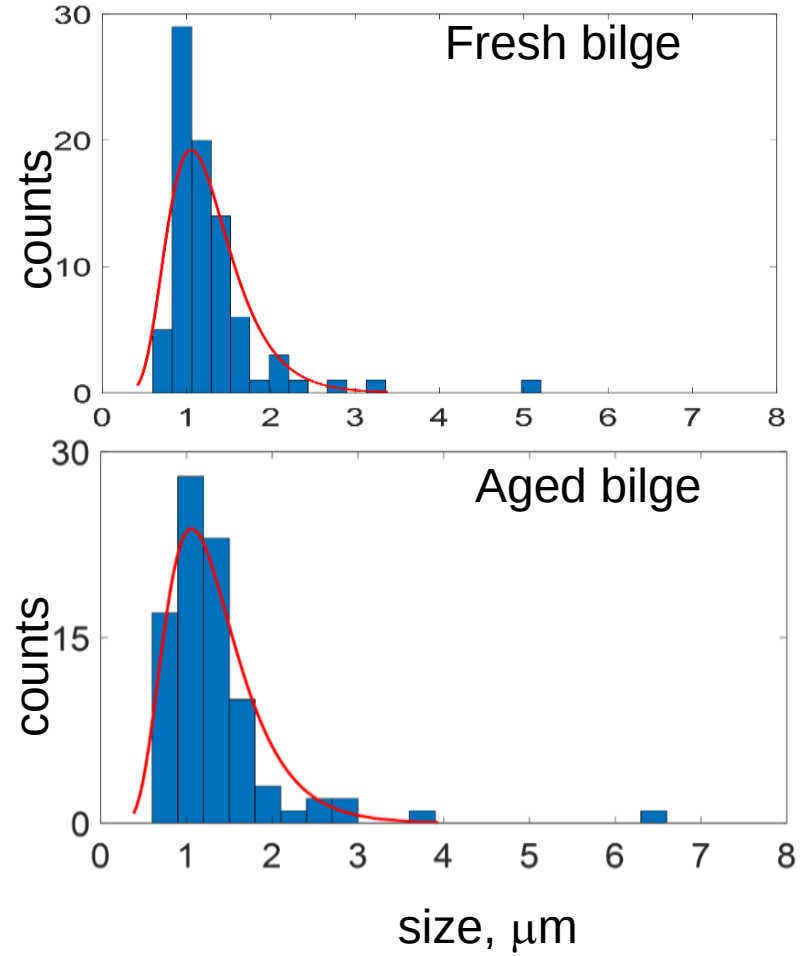


In situ SEM

In situ ToF-SIMS

Task 1: Droplet Size Distribution (DSD)

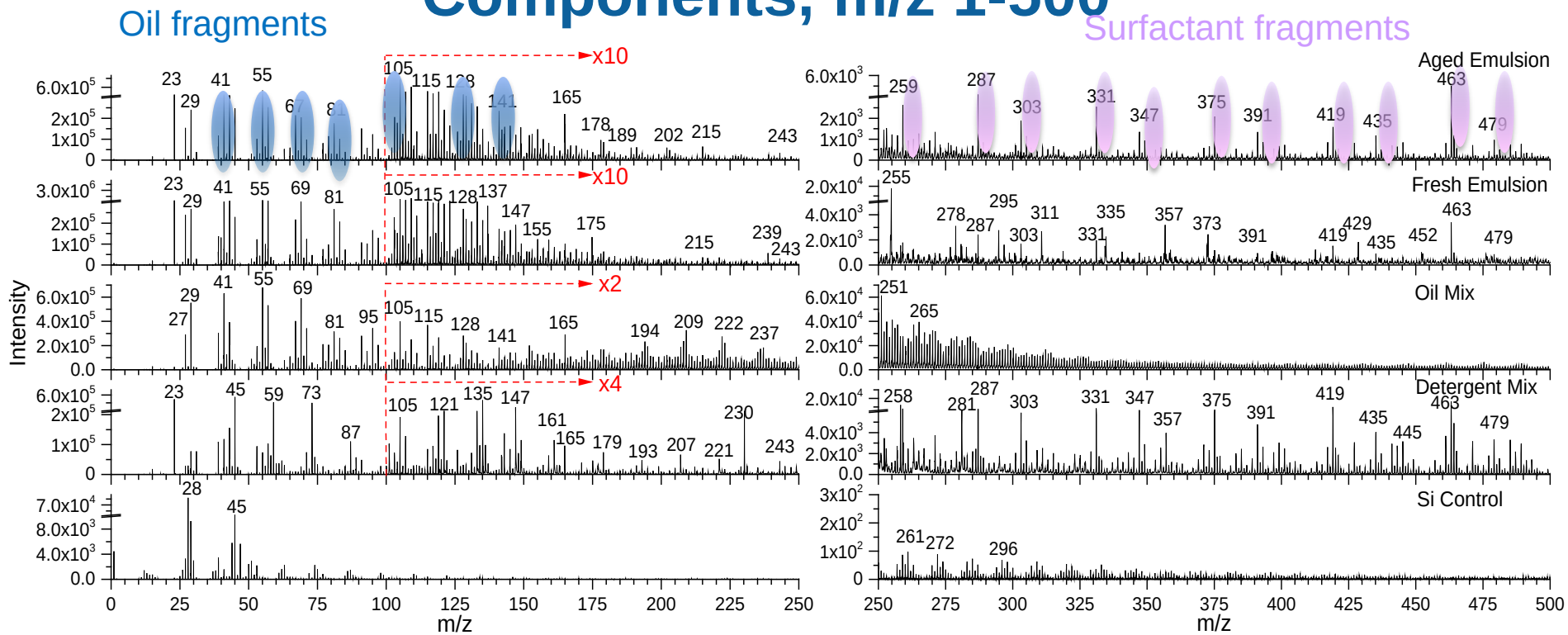




- Fresh bilge is mostly monomodal, with a mean of 1.26±0.04 µm
- Aged bilge is mostly monomodal, with a mean of 1.38±0.04 µm

Son et al., Chemosphere, 2019

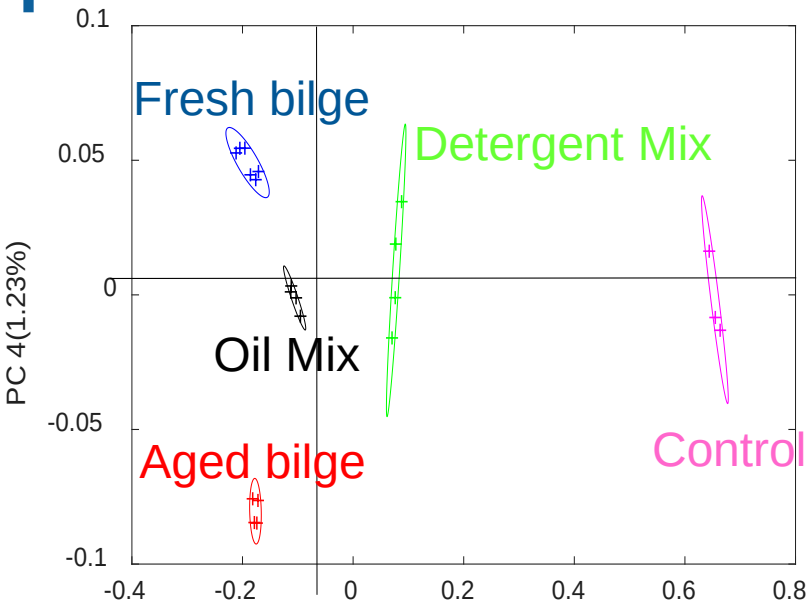
Positive SIMS Spectral Analysis of Bilge and Its Components, m/z 1-500



- Dry SIMS analysis offers high mass resolution and accuracy, necessary for peak identification
- Aged emulsion (dried in N_2 in 7 days) and fresh emulsion(dried by N_2 in several hrs.) show spectral differences and similarities in the positive and negative spectra
- Detergent mix and diesel mix have different signature peaks
- Characteristic peaks of detergent mix and oil mix are observed in both fresh and aged bilge
- Some organic components existed in fresh bilge disappear in the aged bilge; some new organics form in the aged bilge

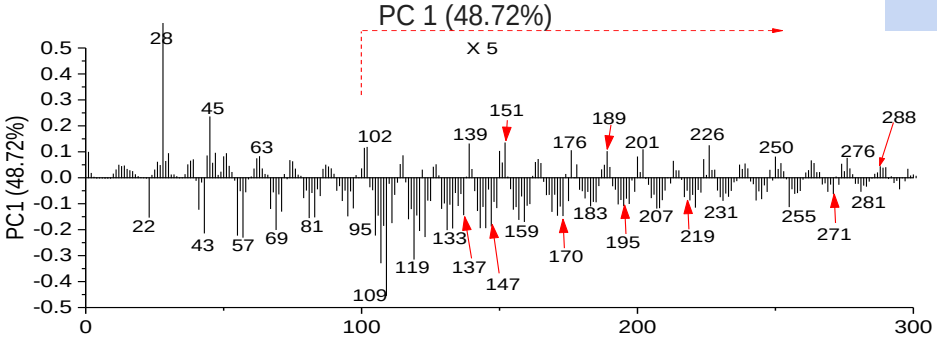
Son et al., Chemosphere, 2019

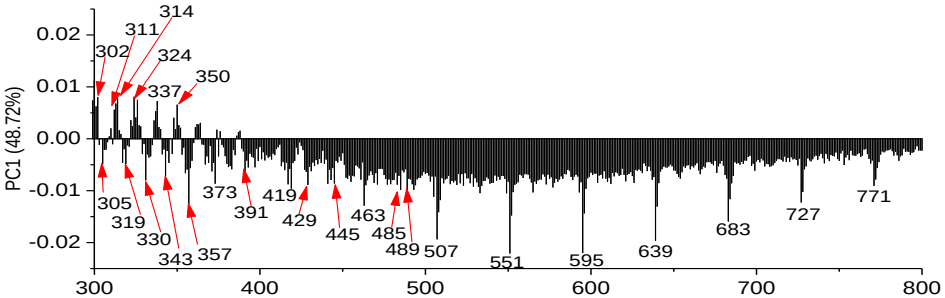
Spectral PCA shows surface composition evolution

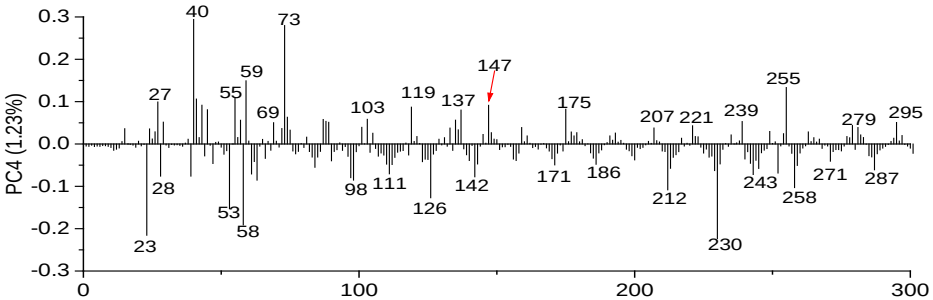


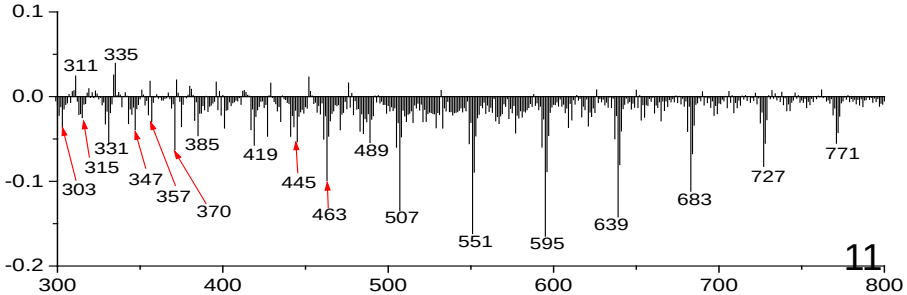
Positive spectral PCA results show

- PC1 vs. PC4 scores plot shows nice separation among all samples
- PC4+ fresh bilge, PC4- aged bilge
- Oil mix share similarities between fresh and aged bilge
- PC1+ detergent, PC- oil mix, fresh and aged bilge









In Situ Liquid SEM Imaging of Synthetic Bilgewater

- We used in situ liquid SEM to characterize bilgewater droplet size distribution (DSD) of fresh and aged emulsion samples
- Representative images of fresh emulsion show mono-modal fit with the mean prominent size of 1.68 μm
- Some coagulation occurs

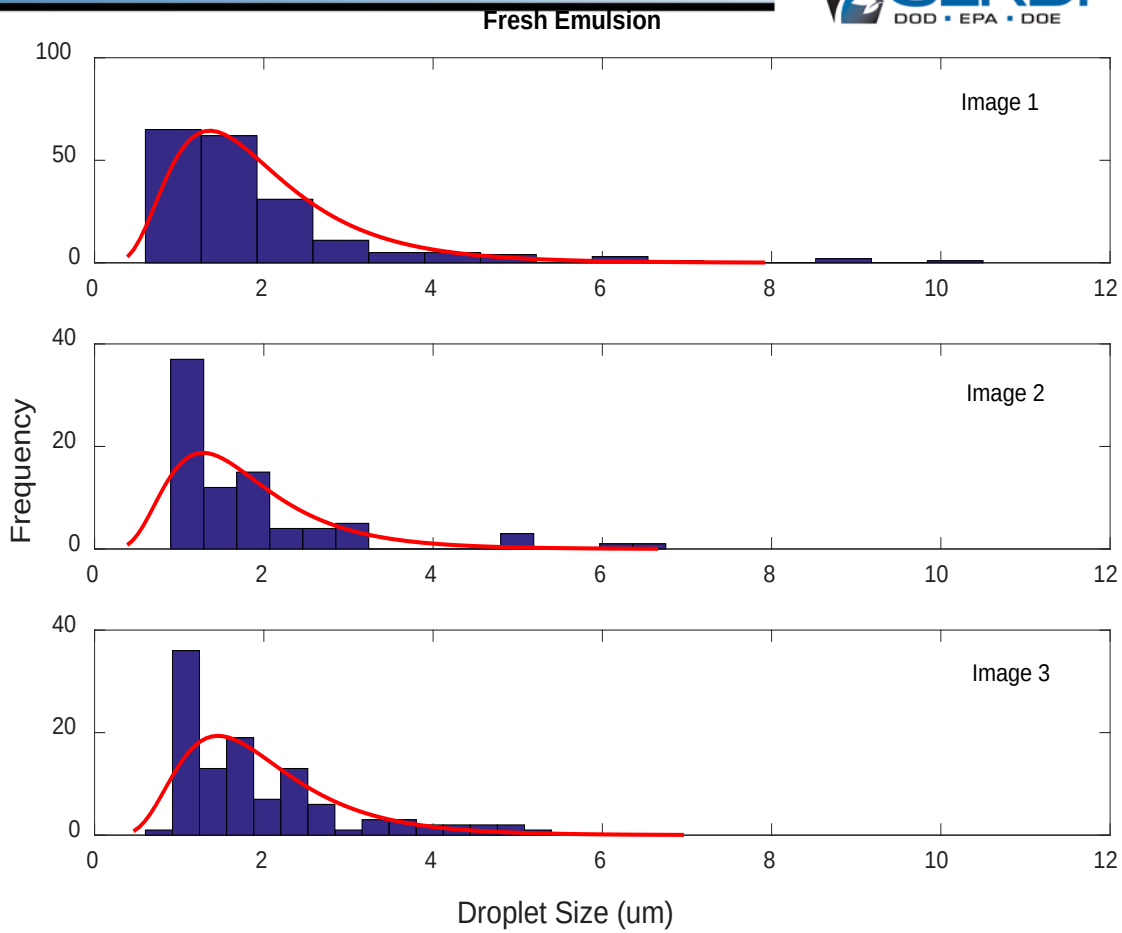
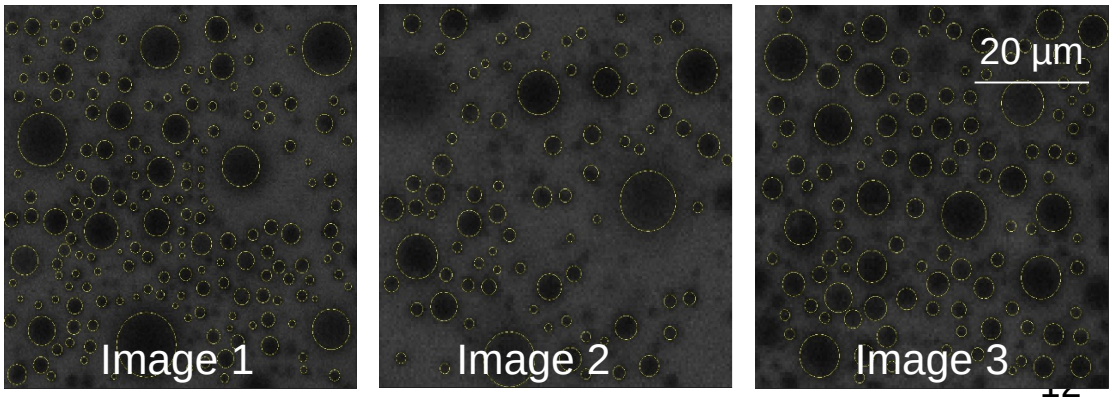


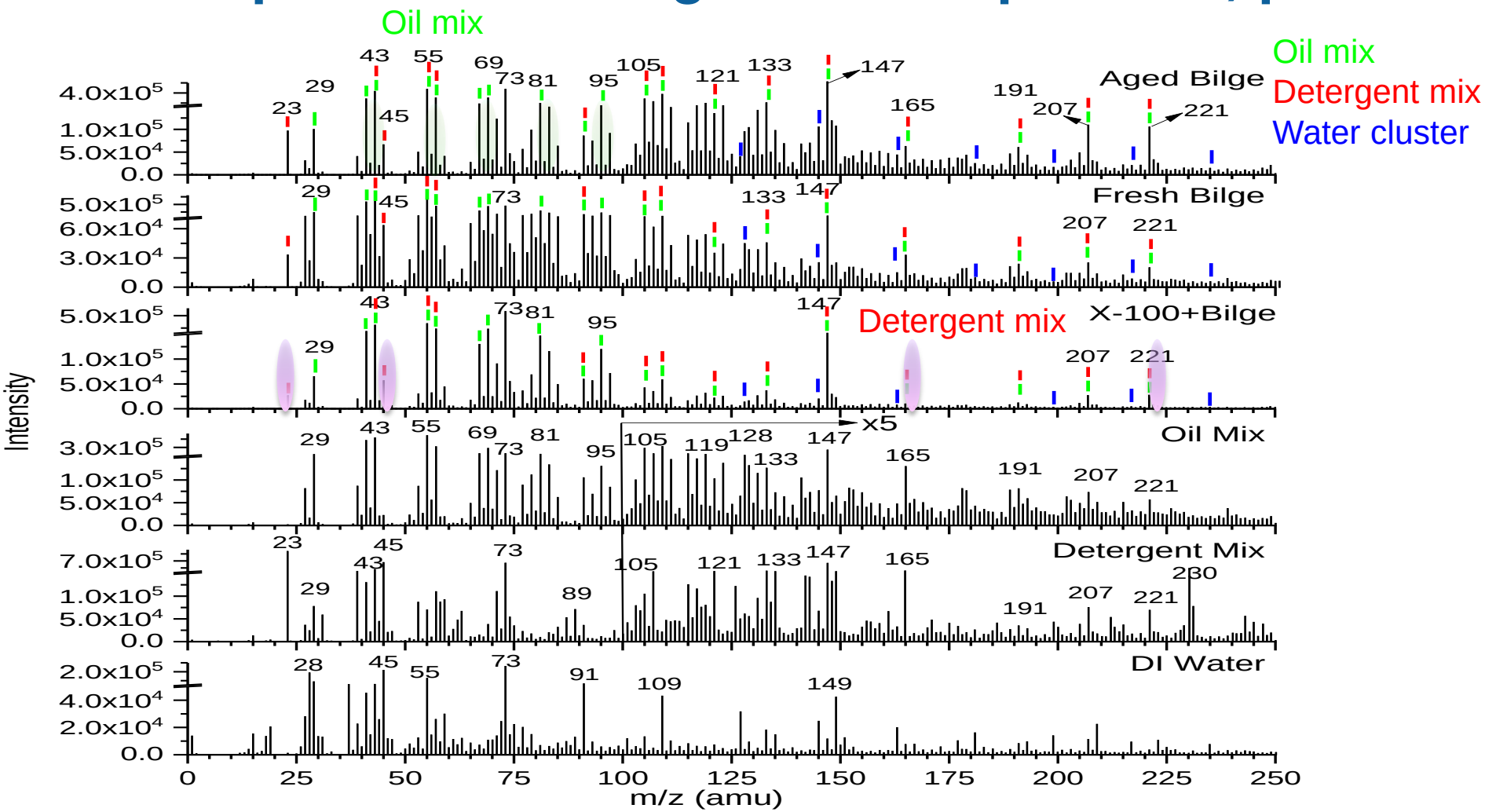
Table 1. DSDs of fresh bilge

No.	Mean, μm	Median, μm	variance, μm
1	2.03	2.88	1.98
2	1.83	1.50	1.32
3	1.20	1.87	1.02



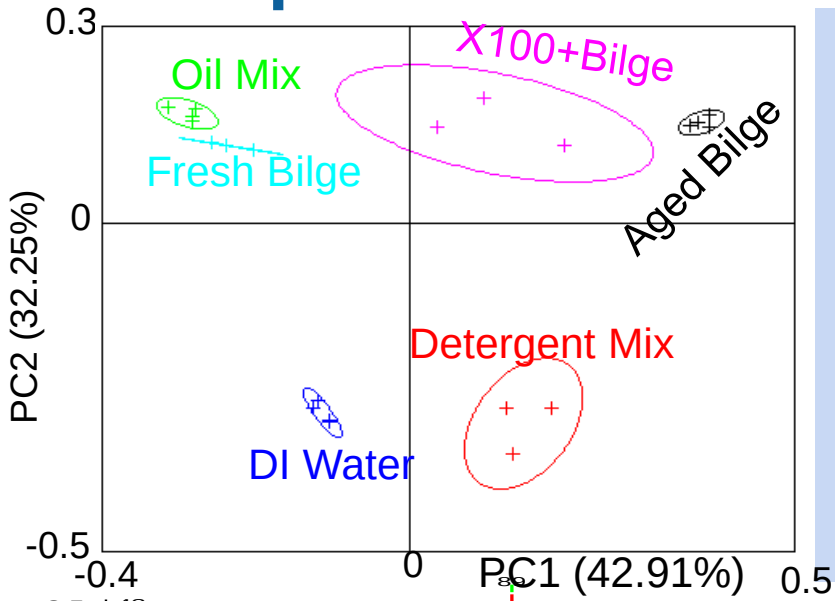
Sheng et al., in preparation, 2019

In situ liquid SIMS of bilge and components, positive

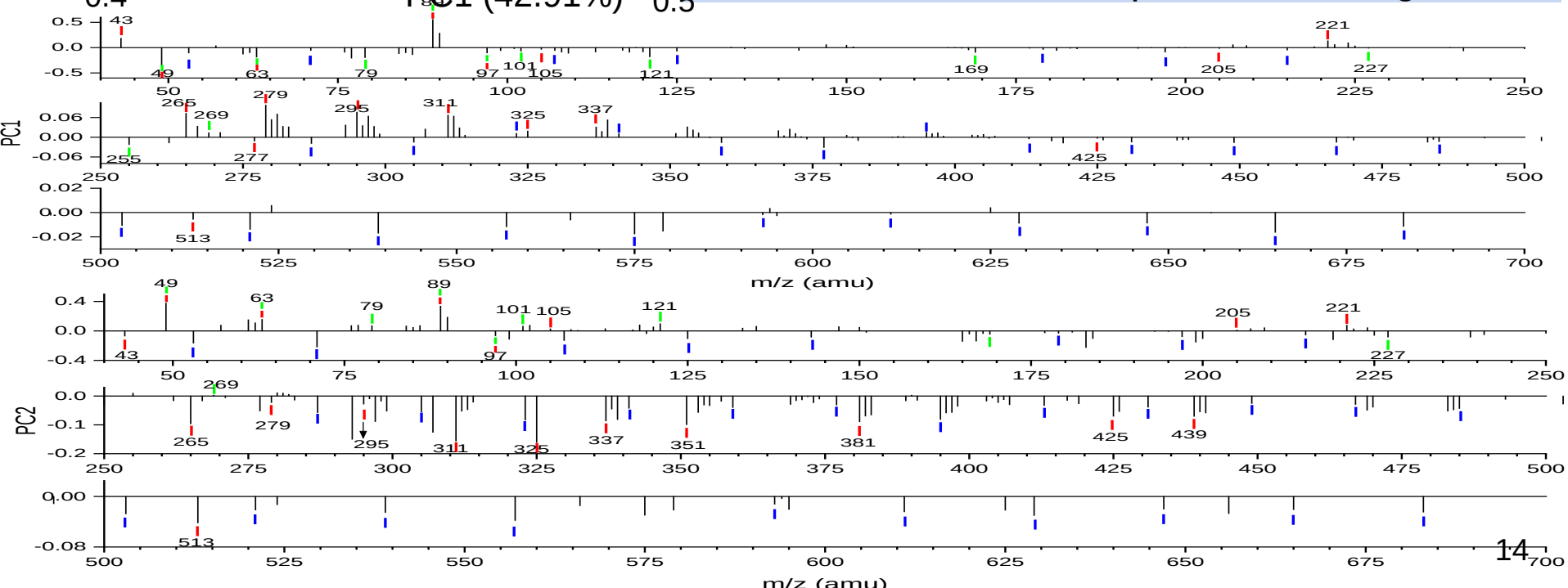


- Characteristic peaks of oil mix and detergent mix are observed in both fresh and aged bilge
- Oil mix and detergent mix both have distinctive peaks in the lower mass range and they show up in fresh emulsion right away and persist in the aged emulsion
- DI water shows distinctive water cluster peaks

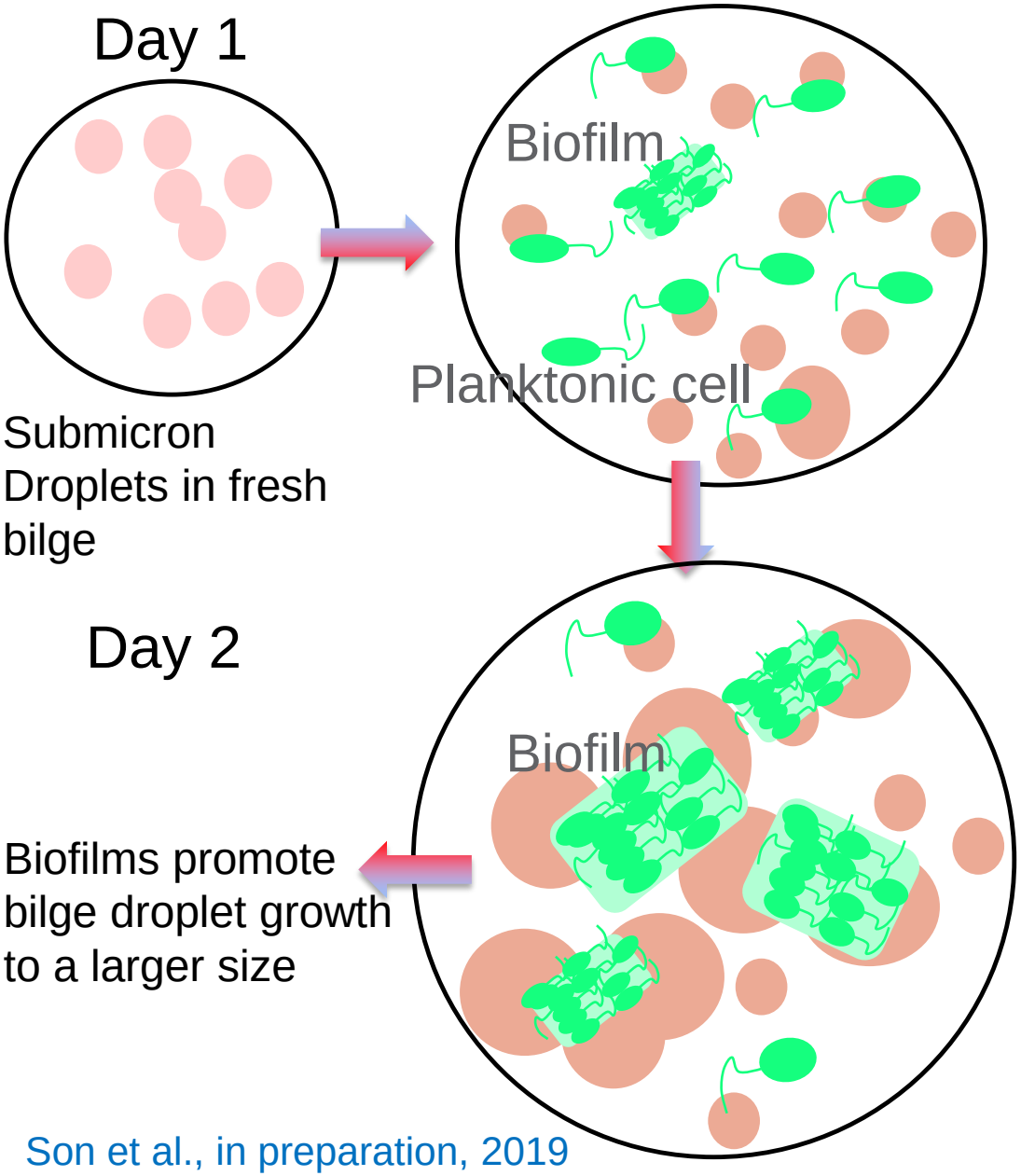
Spectral PCA confirms surface evolution



- PC1 vs. PC2 scores plot shows nice separation among all samples
- PC1+: detergent & DI water; PC-: oil mix, fresh / aged emulsion, X100+EM
- Indicates oil mix more significant to fresh bilge; detergent significant to aged bilge
- PC2+: oil mix, fresh, aged bilge, and X100+bilge;
- PC2-: DI water a component of detergent mix



Task 2. Investigating Microbial Effects on Bilgewater



Son et al., in preparation, 2019

Hypothesis:

Biological processes of microorganisms and cell products can induce emulsion droplet surface changes, resulting in bilge stabilization or breaking

Outcome:

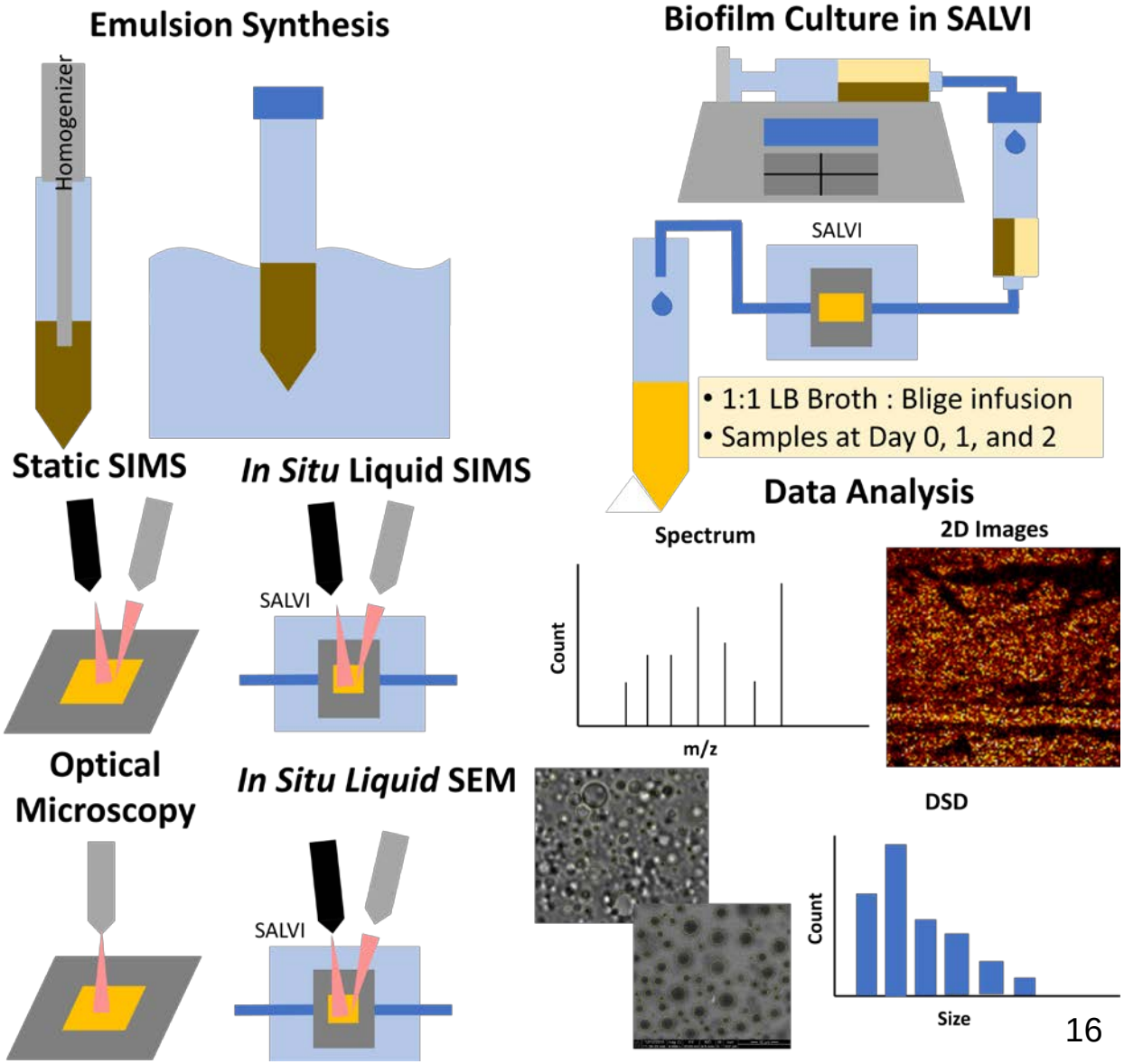
- Understand the effect of representative microorganisms on emulsion formation and stabilization
- Establish whether microbes play a role on emulsion under typical environmental conditions in bilgewater

Approach:

- Use *Pseudomonas* fluorescens, *Arthrobacter*, or *Cobetia marina* and introduce bilgewater as OM
- Investigate how biofilms affect bilgewater formation
- Establish understanding in using biofilms to treat emulsion in the future

Results: Task II
Investigating Microbial Effects on Bilgewater

- Navy bilge model was used as a source of carbon for biofilm growth
- Specifically, 1:1 LB broth to bilge, was used growth medium for *P.* biofilms
- The bilge DSDs with and without *P.* biofilms were determined optically
- Bilge surface changes w/ and w/o *P.* biofilms analyzed by ToF-SIMS



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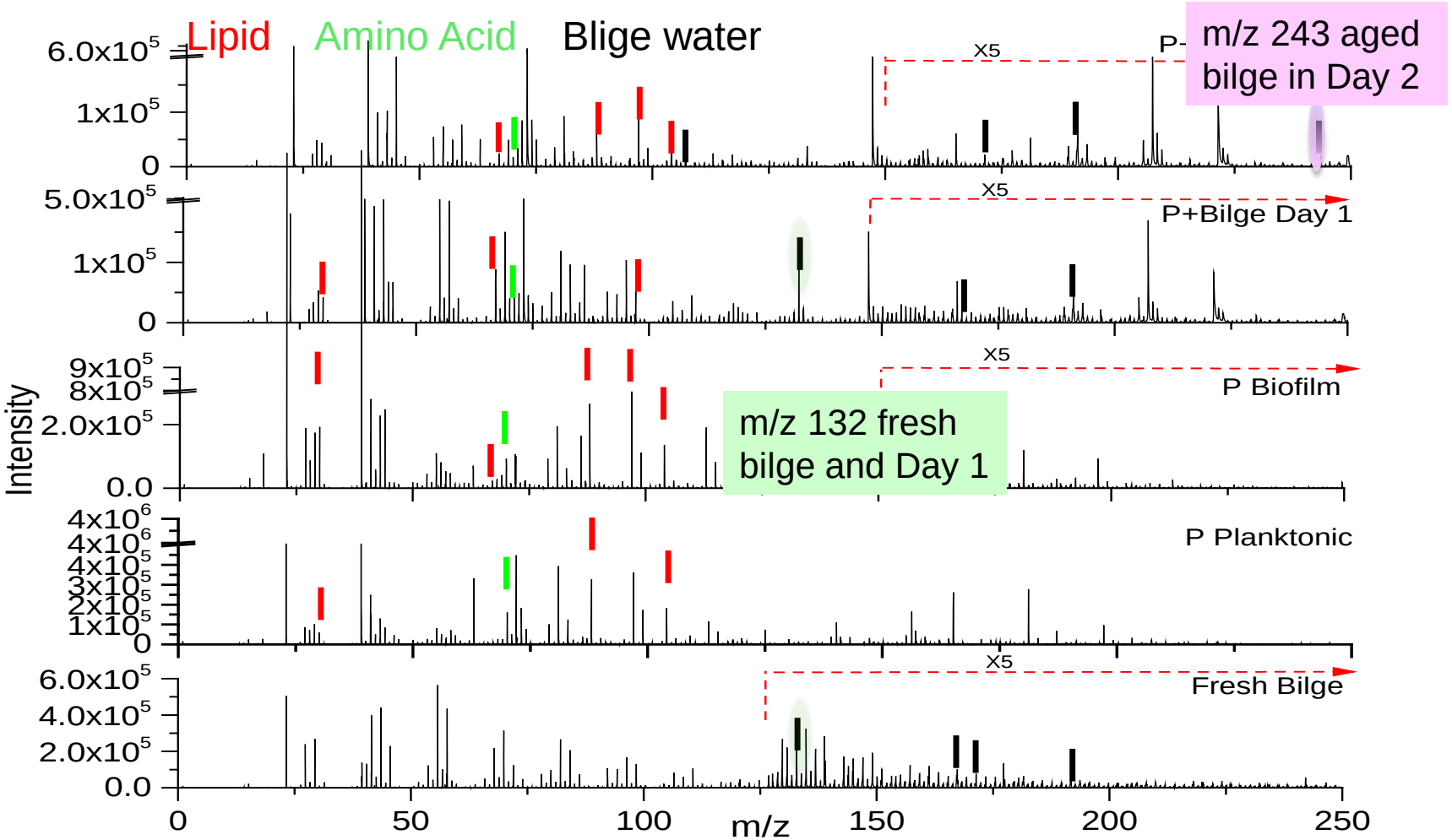
ToF-SIMS and CLSM Sample Matrix of Biofilm and Bilge Interactions

No.	Bacteria	Description	Status
1	Pseudomonas (*P.*)	Fresh bilge 0 day, *P.* Biofilm, *P.* Planktonic	Done
2		Bilge : Medium soln. (1:1) for 1 day	Done
3		Bilge : Medium soln. (1:1) for 2 days	Done
4		Growth media with bilge	Done
5	Arthrobacter (*A.*)	Fresh bilge 0 day, *P.* Biofilm, *P.* Planktonic	Done
6		Bilge : Medium soln. (1:1) for 1 day	Done
7		Bilge : Medium soln. (1:1) for 2 days	Done
8		Growth media with bilge	Done

- We first determined spectral signatures of each bacteria and biofilms
- Then we used our microreactor to culture biofilm with bilge emulsion mixed with the growth medium to simulate the bacteria and bilge interactions in the marine environment
- We compared the effect of bacteria and show their differences on bilge

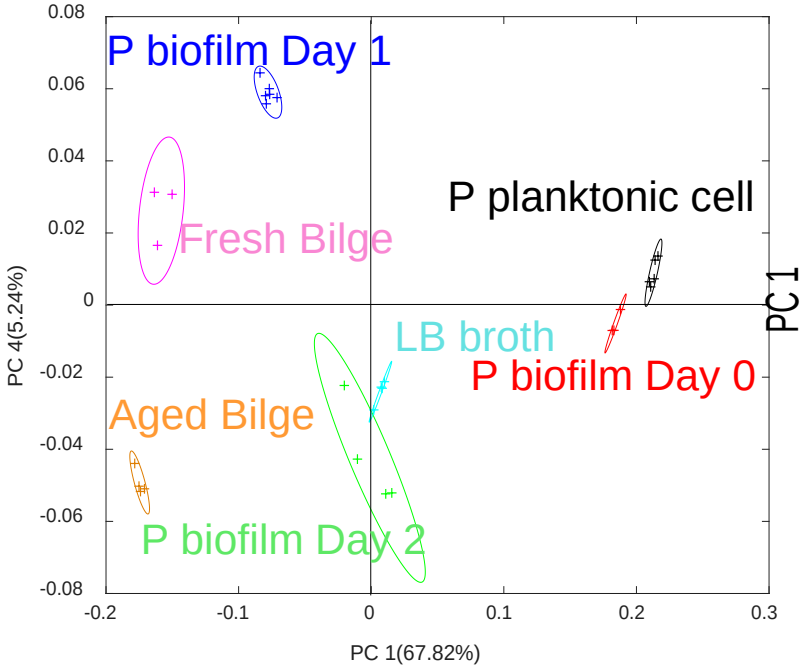
Son et al., in preparation, 2019

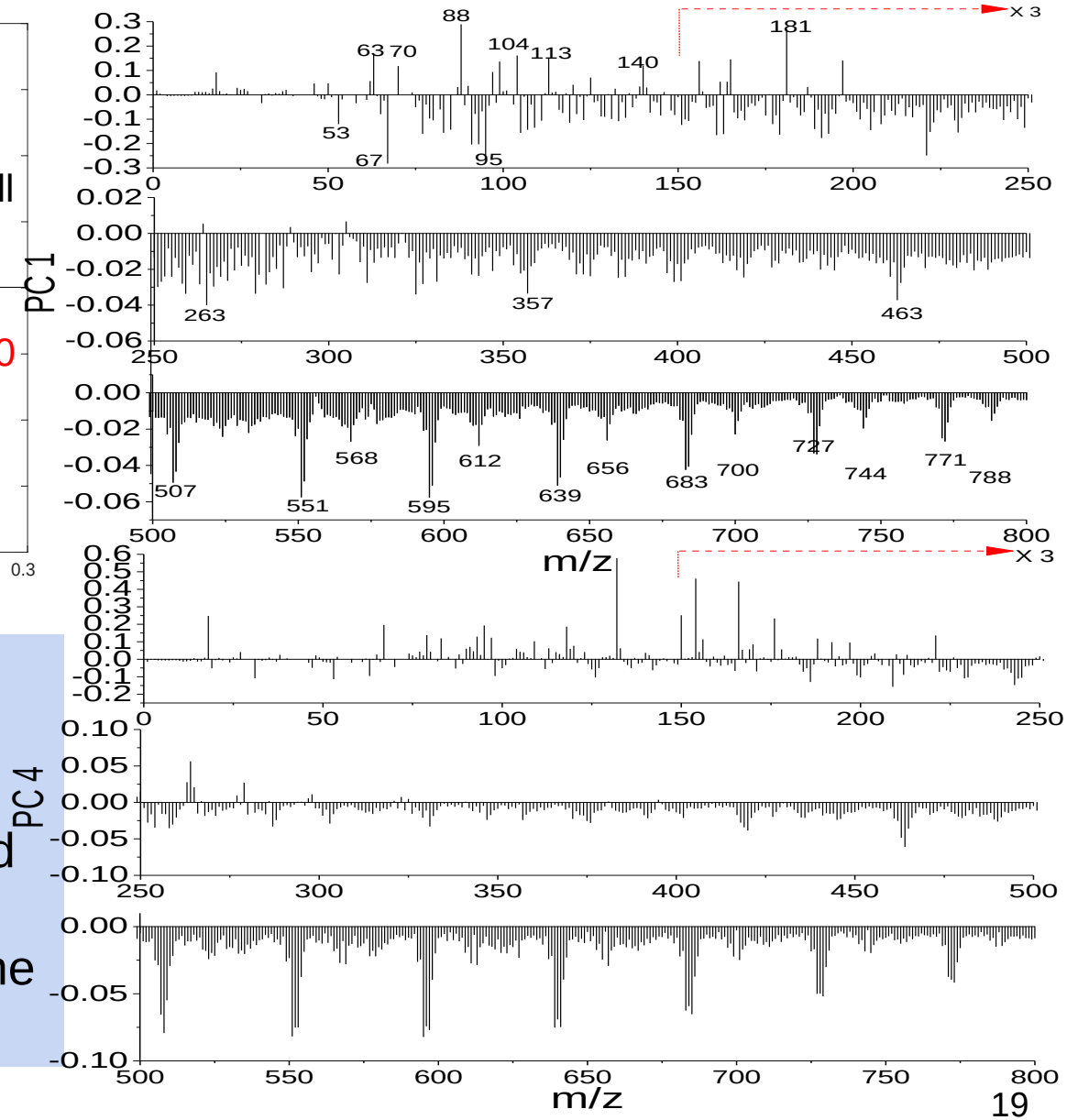
SIMS Spectral Analysis, Positive Mode



- Characteristic bilge peaks appear in Day 1 and Day 2 sample
- Blige exposed biofilm (Day 1 and Day 2) show new species compare to the P biofilm
- In mid mass (m/z 250-500), biofilm organic peaks are seen in Day 1 and Day 2 samples, indicating that biofilms are interacting with bilge emulsions.

Spectral PCA Confirms Bilge Surface Changes



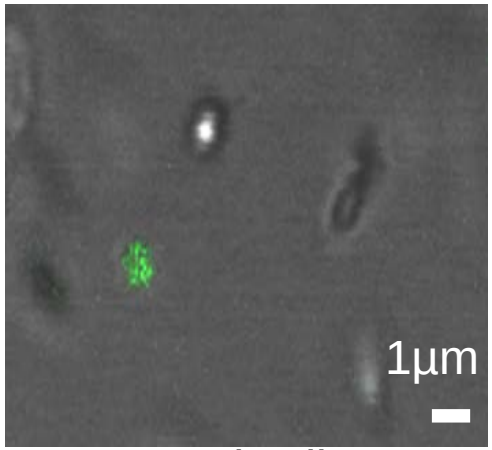


- PC1+: non bilgewater sample
- PC1-: bilgewater related sample
- PC4+: Day 1 is closely related to fresh bilgewater
- PC4-: Day 2 closely related the aged bilgewater

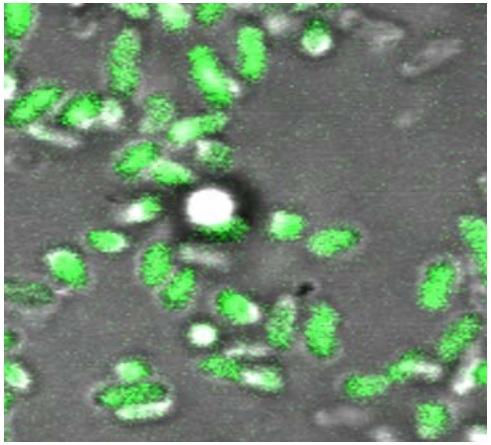
CLSM Imaging of Bilge and P. Biofilms

- CLSM shows larger bilge droplets surrounded by biofilms compared to smaller droplets surrounded by planktonic cells in a fresh bilge
- *P.* + fresh bilge DSD: 0.40 – 1.4 μm, *P.* + aged bilge DSD: 1.4 – 2.8 μm
- Measurements consistent in different samples
- Go/No Go: This result demonstrates the feasibility to study biofilm and bilge using multimodal imaging

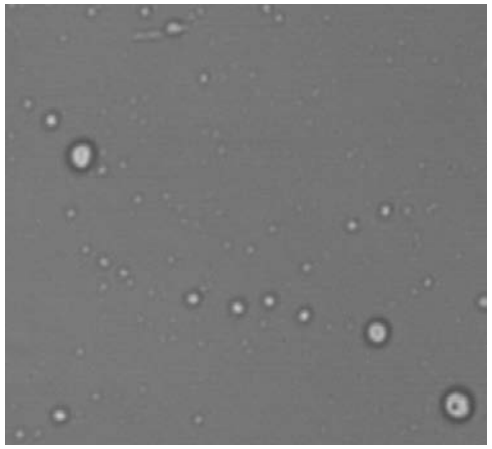
Fresh Bilge w/ *P.* biofilm



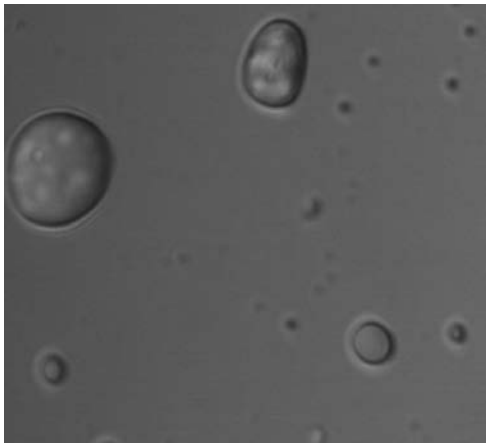
Aged Bilge w/ *P.* biofilm



Fresh Bilge



Aged Bilge



CLSM reveals biofilms promote bilge droplet growth in size

Next Steps

- Publication:
  - First bilge imaging by SIMS & optical microscopy published in Chemosphere
  - First paper of in situ multimodal imaging of bilge submitted to Talanta
  - Biofilm and bilge interaction manuscript in preparation for Env. Chem. Lett.

- Presentation:
  - In situ multimodal imaging talk in AVS and EMSL Workshop
  - Biofilm-bilge poster in the SERDP Symposium

- Collaboration:
  - Work with CARDROCK and other funded teams in an effort to summarize bilge emulsion properties
  - Make our data available to other funded PIs and teams

Technology Transfer

- Present scientific findings in the EMSL integration workshop, AVS, and SERDP Symposium.
- Started IP discussion of the concept of bilge droplet growth by biofilms for facilitated separation with the PNNL commercialization manager
- Plan the following:
  - Collaborate with CARDRCOK to verify new bilge treatment methods
  - Pursue copyrights of the microfluidic reactor design to process bilge and enhance bilge droplet size growth
  - Pursue a US patent with assistance from commercialization manager and supported by additional results
  - Develop a video and ppt for potential users of the technology, including EPA & State regulators, consultants, DoD Remedial Program Managers, and researchers, with guidance from SERDP program mangers.

Key Points

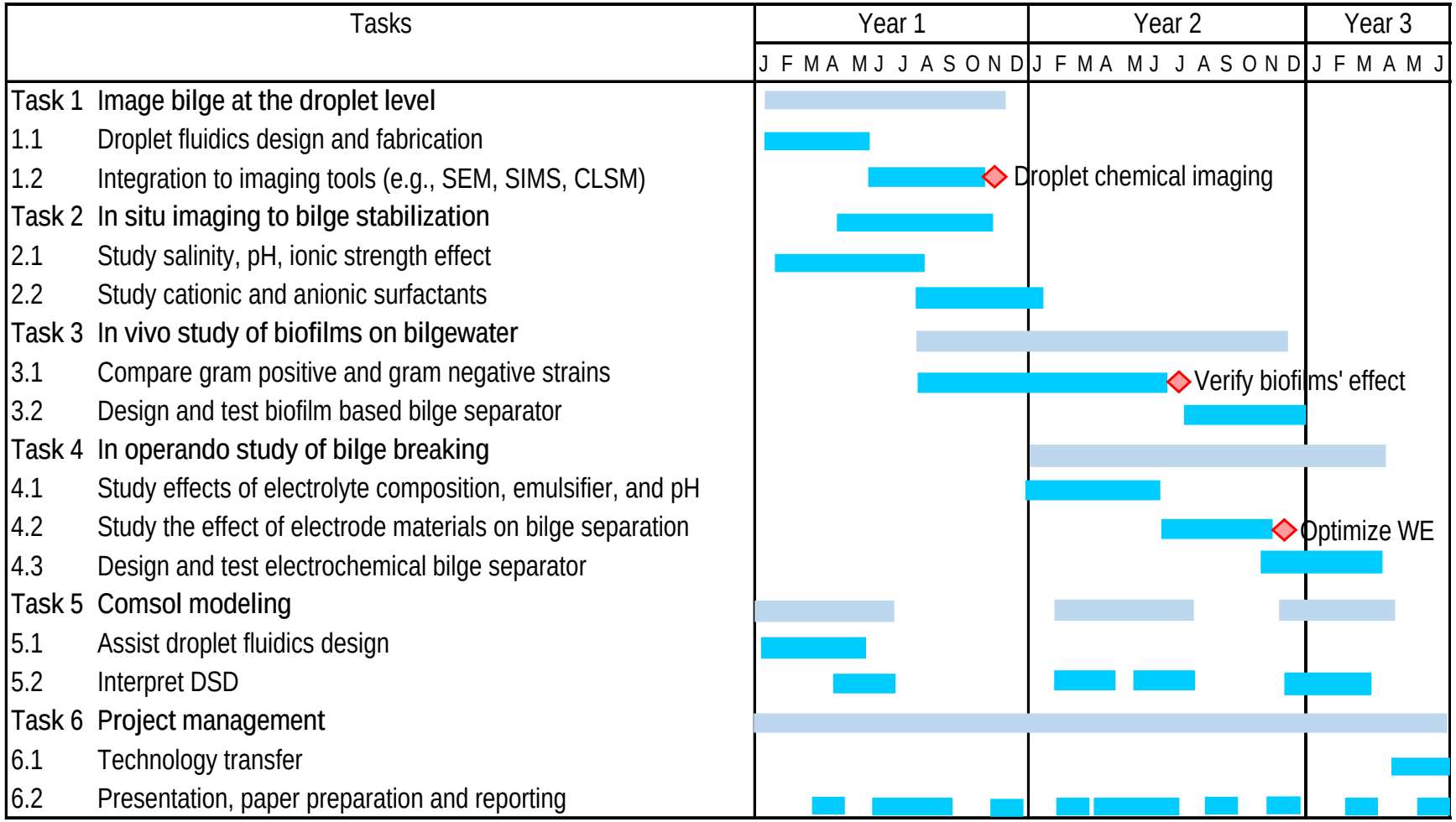
- The bilge oil-in-water l-l interface evolves over time.
  - Bilge DSD growth is accompanied with surface composition changes, first imaged by ToF-SIMS, SEM, CLSM, & optical microscopy.
  - In situ imaging further verifies that surfactants are important in stabilizing bilge droplet size growth over time
  - **We need to investigate the surfactants that stabilize bilge efficiently**
- Biofilms interact with bilge and promote droplet size growth
  - The oil mix in bilge acts a source of organic matter for biofilm culture
  - The biofilm secrets extracellular polymeric substance (EPS) as surfactants and promotes droplet growth and stabilization
  - **Biofilms offer a new way to grow bilge to a larger size for separation**
- In situ multiplexed imaging is powerful to study surface changes at the droplet level with molecular understanding
  - In vivo and in operando techniques will offer more insight into the physiochemical process at the microscale and provide fundamental guidance in bilge waste water treatment
  - Go/No Go

Future Research

- We propose the following research:
  - Continue collaboration with CARDROCK
  - Develop droplet fluidics to study the bilge l-l interface on the droplet level
  - Continue in situ imaging to study factors that affect bilge systematically (e.g., pH, salinity, ionic strength, surfactants)
  - Conduct in vivo study to further elucidate the effect of biofilms on bilge
  - Conduct in operando study to investigate the effect electric field on surface charges of bilge droplets and how it will facilitate separation
  - Use Comsol modeling as a tool to assist droplet design and interpret droplet data
- The cost estimate:

Funding ($K)	Year 1	Year 2	Year 3	Total
	$390K	$410K	$200K	$1000K

Project Plan



- **GO/NO GO Decision:**
  - Integration of the droplet microfluidics to vacuum analytical platforms
  - Verify the effects of biofilms on bilge stabilization prior to biofilm separator design
  - Optimization of working electrode (WE) materials in the EC separator

Deliverables

Key outcome and deliverables include the following:

- Novel droplet fluidics integral to multiple chemical imaging instruments for studying bilge at the droplet level
- Advance understanding of the liquid surfaces and l-l interfaces for emulsion reduction and prevention
- Mesofluidic biofilm bilge separator as a new method
- An electrochemical microreactor as a prototype for emulsion treatment
- Potential IP filings
- Collaboration with CARDROCK for enhanced bilge study
- Peer reviewed articles (4+)
- Presentations and reports
- Students: 1 Masters, 2 PhDs, and 1-2 Post Docs

BACKUP SLIDES

Publications

- Jiyoung Son, Yanjie Shen, Jenn Yao, Danielle Payton, and Xiao-Ying Yu, *Surface evolution of synthetic bilgewater emulsion*, **Chemosphere**, 236, 124345, 2019.

- Yanjie Shen, Jenn Yao, Jiyoung Son, Zihua Zhu, Danielle Payton, and Xiao-Ying Yu, *Surface composition change induced droplet size growth confirmed by in situ imaging of synthetic bilgewater*, **Talanta**, to be submitted in Oct., 2019.

- Jiyoung Son, Dehong Hu, Danielle Payton, and Xiao-Ying Yu, *Probing the effect of microbes on the oil-in-water bilgewater emulsion*, **Env. Chem. Lett.,** to be submitted in Dec., 2019.

Project Funding

	FY19	FYxx	FYxx
Funds received to date ($K)	200 K		
% Expended	92%		
Funds Remaining ($K)	18 K		

Status of Funds for Federal Performers

- Report on the status of funds for each MIPR received by a directly funded Federal performer. Provide information on each fiscal year for which there has not been 100% expenditure of funds.

FY2018 Funds			
Directly Funded Federal Performer(s)	**Funds Received**	**Funds Obligated***	**Percent Funding Obligated**
Federal Performer A - Direct Cite MIPR		$200,000	100%
Federal Performer A - Reimbursable MIPR		$200,000	100%
Federal Performer B - Direct Cite MIPR			
Federal Performer B - Reimbursable MIPR			

* Funds put on contracts and/or purchase orders that have been issued, and funds associated with internal labor or travel expenses that have been incurred.

WP-1660: Revealing Bilgewater Emulsion Formation and Breaking by In Situ Multiplexed Chemical Imaging and Microfluidics

Performers: Dr. Xiao-Ying Yu, Pacific Northwest National Laboratory, Richland, WA

Technology Focus

- *In situ multimodal imaging has been used, for the first time, to study bilge emulsion stabilization for the first time, revealing that surface composition change is indispensable to droplet size growth*
- *Biofilms can act as an efficient emulsifier to facilitate droplet growth to a larger size for waste water separation*

Research Objectives

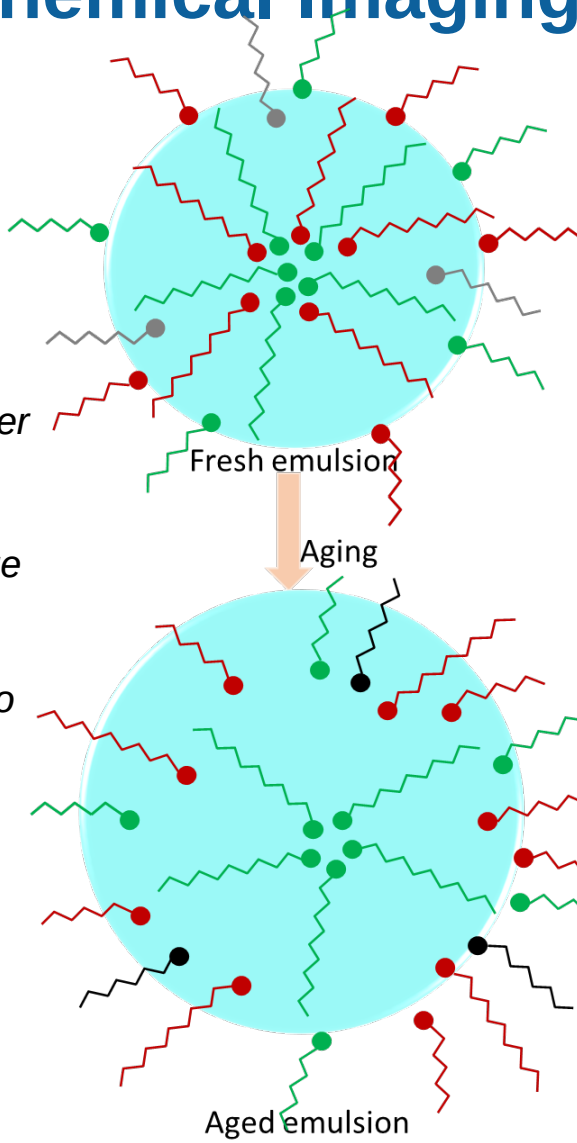
- *Study chemical changes at the l-l interface that is critical for mass and charge transfer leading to bilge formation, stabilization, and worsening*
- *Obtain distribution of key species at the droplet surface and the l-l interface*
- *Set the technical foundation for the development of cost-effective solutions to reduce, prevent, and treat ship-relevant emulsions*

Project Progress and Results

- *Demonstrate in situ multimodal imaging is viable to study bilgewater*
- *Oil and detergent components both appear on bilge surface, however, surfactants become more significant over time*
- *Biofilms use bilge as a source of OM and secret surfactants to help droplets grow*

Technology Transition

- *Seek copyright protection of the droplet fluidics design*
- *Validate the biofilm bilge separation concept with more strains prior to IP filing*
- *Publish peer-reviewed articles*



Bilgewater emulsion interfaces evolve over time