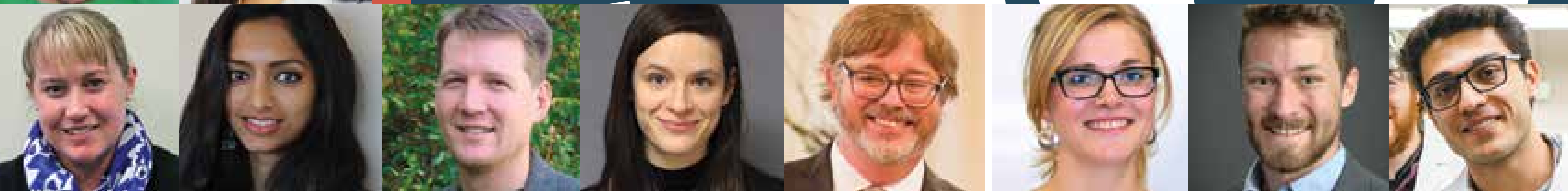
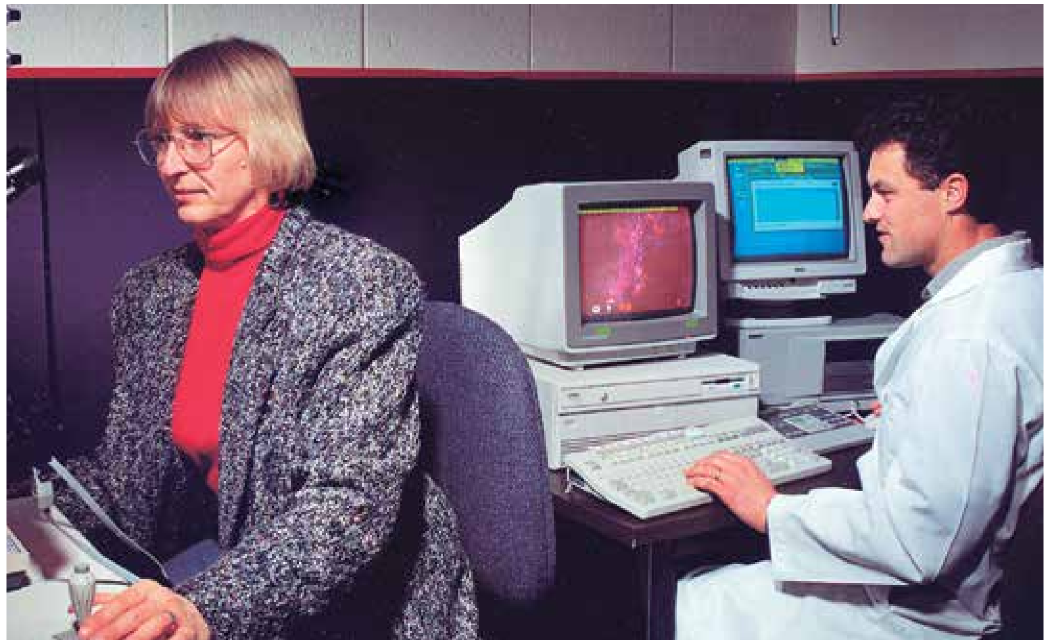
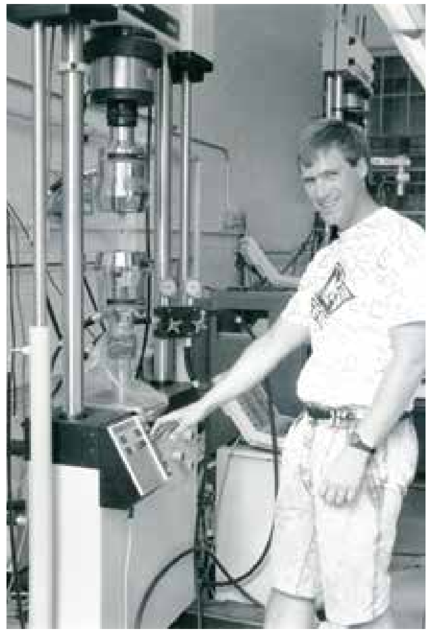
2020 ANNUAL REPORT

30 YEARS

CENTER FOR BIOFILM
CBE
ENGINEERING







Clockwise from top: Anne Camper and Paul Stoodley (1990s). Eric Wentland (date unknown). CBE Director Bill Costerton (left) and industrial associates (1990s).

30th ANNIVERSARY

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CBE DIRECTOR
Matthew W. Fields, PhD

INDUSTRIAL COORDINATOR
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DESIGN AND PRODUCTION
Jill Story

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On the Cover

Photos of selected faculty, staff, and students past and present from the CBE's first 30 years. *(Left to right from the top row. The year represents the photo was taken.)*

Row 1 Rob Sharp 1994
Zbigniew Lewandowski 1990
Bill Characklis 1980s
Paul Sturman 1991
Anne Camper 1980s
Robin Gerlach 2000
Mark Shirtliff 2002
Ross Carlson 2004

Row 2 Eric Wentland 1995
Bill Costerton 1995
Bryan Warward 1991
Brent Peyton 1980s
John Neuman 2005
Peg Dirckx 2002
Kelly Kirker 2008
Lindsey Lorenz 2008

Row 3 Diane Williams 1990
Paul Stoodley 1991
Warren Jones 1980s
Betsey Pitts 2002
Jayne (Billmayer) Morrow 2002
Phil Stewart 2007
Ellen (Swogger) Lauchnor 2005
Joe Seymour 2014

Row 4 Gill Geesey 1991
Al Cunningham 1992
Elinor Pulcini 1995
Kelli Buckingham Meyer 2002
Sarah Codd 2004
Heidi Smith 2009
Dana Skorupa 2012
Erika Espinosa-Ortiz 2018

Row 5 Nick Zelver 2000
Laura Boegli 1996
Marty Hamilton 2002
Matthew Fields 2007
Al Parker 2008
Diane Walker 2013
Steve Fisher 2018
Kristen Griffin 2017

Row 6 Mike Franklin 2000
Darla Goeres 2003
Garth James 2006
Kristen Brileya 2007
Jim Wilking 2013
Sobia Anjum 2015
Sarah Gorlitz-Burke 2016
Jill Story 2017

Row 7 Christine Foreman 2004
Otto Stein 2017

Row 8 Liz Sandvik 2007
Abigail Richards 2007
Markus Dieser 2007
Connie Chang 2016

Row 9 Adie Phillips 2013
Neerja Zambare 2015
Jeff Heys 2009
Sarah Huth 2016
Skip Anderson 2017
Shawna Pratt 2017
Brian Pettygrove 2017
Reha Abbasi 2018

30th ANNIVERSARY

In the year of COVID, we nixed celebrations to solve problems and keep people safe

The CBE turned 30 years old in 2020. That's truly a remarkable feat. We began with the then-largest grant in Montana State University's history from the National Science Foundation's innovative Engineering Research Center program (for more information see, *Agents of Change: NSF's Engineering Research Centers — A History*, by Lynn Preston and Courtland Lewis). The CBE is widely regarded among the most successful research centers borne from the ERC program. Nonetheless, it goes without saying that the COVID-19 pandemic forced us to reconsider our sizable plans to celebrate this milestone throughout 2020. As disappointed as we were not to host reunions, symposia, and panel discussions about how our efforts over the past 30 years have changed the world's understanding of biofilms, I am so very pleased that we are using our collective knowledge and skills to advance research into the novel coronavirus and COVID-19.

Take, for instance, CBE-affiliated faculty member Connie Chang, assistant professor of chemical and biological engineering. Together with MSU faculty across four colleges, Connie received funding from the CARES Act to optimize a potential Loop-mediated Isothermal Amplification (LAMP) test for asymptomatic COVID virus detection. She and her team are also developing a LAMP assay to test simultaneously for the influenza virus. Notably, the CBE bestowed the 2020 W.G. Characklis Award upon two PhD students, Tom LeFevre and Isaak Thornton from the Wilking Laboratory, in part for their efforts to fabricate lab supplies to support this critical research. And associate research professor of chemical and biological engineering Garth James, who is the principal investigator of the CBE Medical Biofilms Lab, has been testing surface disinfectants that specifically target a surrogate coronavirus. (See Page 10 for more information on these important COVID-related projects under way at the CBE.)

I would also like to bring your attention to the ever-widening vision for growth. We are building a regulatory science program from the ground up that our own Darla Goeres is heading. (See Page 21 for a Q&A with Darla Goeres.) When it launches in 2022, this academic curriculum will enable us to guide in new ways this exciting field as it continues to evolve rapidly. And I know I talk a great deal about the CBE Imaging Facility, which provides MSU's faculty, staff, and students access to state-of-the-art microscopy equipment. But that's because these tools reveal hidden worlds in ways that were hardly fathomable only a few years ago. And last year, Heidi Smith, the facility's manager, along with numerous CBE faculty, won a $1.1 million grant from the NSF to increase the capabilities of the facility that is unrivaled in the Pacific Northwest. We're actively collaborating with biofilm researchers around the world through several joint projects that will help define the field of biofilm research for the next decade and beyond. And, we're also working with the DoD through its Defense University Research Instrumentation Program in which the CBE was awarded funding for new epifluorscent microscopes. I am confident that you'll be hearing more about these exciting instruments in the coming months and years.

And finally, I'd like to thank the participants past and present in our innovative Industrial Associates program. Without you, this center would not be fiscally viable. Your membership not only funds many of our mission-critical administrative functions, it also ensures we dedicate much of our efforts to engineering practical, real-world solutions to real-world problems. I sincerely appreciate our collaborations.

Here's to a healthy 2021,

Matthew W. Fields
Director, Center for Biofilm Engineering
matthew.fields@biofilm.montana.edu

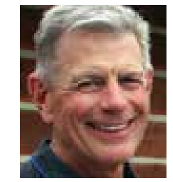
INDUSTRY

30th

INDUSTRY

PARTNERSHIPS WITH INDUSTRY KEEP CBE RESEARCH RELEVANT TO REAL-WORLD PROBLEMS

IA relationships, research thrive despite COVID-19 pandemic



The year 2020 started well, with a well-attended and very successful "Pathways to Product Development" meeting in Arlington, VA, in early February – our 7th annual meeting to engage regulators, industry, and the academic community. See highlights from this meeting on page 7.

Of course, shortly following this meeting, we have all been tasked with the challenge of maintaining contact with our colleagues, customers, friends, and family amid the restrictions of the pandemic. The challenges of 2020 have forced us all to seek out novel ways of staying connected. Here at the CBE, we have sought to meet this challenge by expanding our online communications efforts. We encourage each of our Industrial Associates to attend virtually our weekly research seminars, which feature CBE speakers as well as distinguished scientists from other institutions and industry. Our summer Montana Biofilm Meeting was also held virtually, a change that allowed us to reach more than 500 CBE members, potential members, academicians, and regulators. We are also eager to reach out to our members with targeted seminars from CBE researchers – an option that allows many attendees within a company to hear about a biofilm-related topic of their choice. While we continue to hope that in-person gatherings will again be possible later in 2021, we remain committed to helping our members with all things biofilm, including consulting projects helping to match our graduates with companies seeking biofilm-savvy employees, or simply answering a question.

Although many of our office staff are working remotely, our labs remain open and functional, albeit with strict safeguards to inhibit virus transmission. In 2020, we completed more than 50 sponsored projects for 25 different companies. In addition, we continue to offer small testing projects to our Industrial Associate members at no additional cost. Both the Medical Biofilms Laboratory and the Standardized Biofilm Methods Laboratory remain committed to helping industry conceive, develop, and commercialize biofilm-related technologies and products.

As we look forward to 2021 and beyond, we seek to integrate further our research and technology transfer efforts to advance biofilm science and help industry develop products and solve problems. We are committed to demonstrating the value of CBE membership to existing and new members alike. And as we celebrate the 30th anniversary of our original NSF Engineering Research Center award, biofilms are only increasing in relevance to industry, medicine, and consumers. The CBE is here to help.

Paul Sturman, PhD, is the industrial coordinator for the CBE. He is available at paul_s@montana.edu and 406-994-2102.

CBE by the Numbers

FY2020

53 TESTING PROJECTS

25 SPONSORS

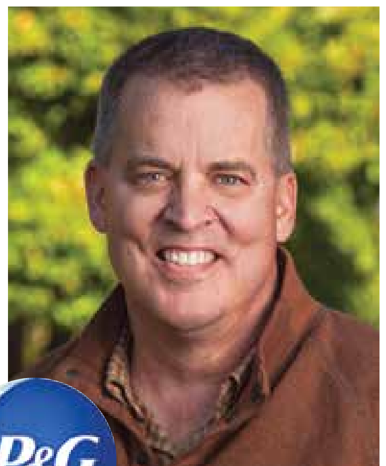
$770,000 GENERATED

Q&A

Partner Profile

Chuck Pettigrew of Procter & Gamble

By Skip Anderson



P&G has been a CBE Industrial Associate since 2008. What motivates P&G to renew membership year after year?

The CBE is an excellent biofilm research institute for interdisciplinary, collaborative research with a worldwide reputation in the field of biofilms. We believe this collaboration is a very good return on our annual investment. Specific examples of these services are:

- Regulatory interactions – CBE staff, maintain close ties with decision makers at EPA, FDA, and other government agencies concerned with biofilms.
- The two annual meetings the CBE convenes continue to grow in terms of the number of participants, the breadth of biofilm topics covered, and by attracting top investigators from around the world. We would be hard pressed to find access to this collection of individuals and data at another venue.
- When we have needed help to train microscopy staff, the CBE have been willing to help. In particular, the Biofilm Workshops provide an excellent opportunity for a younger staff member to train at the CBE.

P&G has sponsored multiple projects at the CBE over the years. Can you speak without revealing anything proprietary about the types of projects that have been undertaken?

Over the past several years the CBE has delivered quantitative biofilm test results on formulated products and multiple upstream technologies, a new biofilm imaging and statistical analysis tool, as well as qualitative microscopic images that help our microbiologists to explain the magic behind our science.

A collaborative P&G-CBE research team also studied two haloalkaliphilic bacteria via molecular, physiological, and *in silico* metabolic pathway analyses. Genomes from the organisms, designated *Halomonas BC1* and *BC2*, were sequenced; 16S ribosomal subunit-based phylogenetic analysis revealed a high level of similarity to each other and to *Halomonas meridiana.* The annotated *BC1* genome was used to build an *in silico*, genome-scale stoichiometric metabolic model to study catabolic energy strategies and compatible solute synthesis under gradients of oxygen and nutrient availability. The theoretical analysis identified energy metabolism challenges associated with acclimation to high salinity and high pH. This study documents central metabolism data for the industrially and scientifically important *haloalkaliphile* genus *Halomonas*, providing a knowledge base for research of applied relevance.

What do you think are the most important/useful takeaways from the CBE industrial meetings? Has the move to an online format due to COVID impacted this?

The CBE industrial meetings provide unique opportunities to foster new and ongoing relationships with CBE staff, other Industrial Associates, and with the federal regulators. In particular, the CBE provides connectivity with the U.S. Environmental Protection Agency on technical issues and concerns regarding biofilm test methods and biofilm guidance that the EPA is working to develop.

The CBE industrial meetings provide unique opportunities to foster new and ongoing relationships with CBE staff, other Industrial Associates, and with the federal regulators.

The CBE listened to Industrial Leadership on the Technical Advisory Committee at the CBE and in 2014 moved the Annual Winter Meeting to the Washington D.C. area in order to maximize the participation of federal regulators. More recently, in response to the COVID-19 pandemic, the move to an online format has greatly expanded the number of industrial associates that can participate in the meeting.

Is P&G active in other university/industry partnerships? If so, is there anything that makes the CBE unique in this regard?

The depth and breadth of expertise, across multiple disciplines is a unique attribute of the CBE. As a consequence, the CBE has consulted on numerous research programs over the years; they are trusted colleagues that respond to our needs on fast timing.

What would you say about the value of CBE membership to a scientist at a company considering joining?

In addition to having priority access to the bi-annual Workshops and Technology Meetings hosted by the CBE (numerous staff have participated), the CBE staff are available to consult and provide training.

BIOFILM TECHNOLOGIES PATHWAYS TO PRODUCT DEVELOPMENT

Highlights from the CBE's 2020 Biofilm Technologies meeting in DC

By Skip Anderson

The CBE hosted its annual regulatory-focused meeting Biofilm Technologies: Pathways to Product Development in the Washington, DC, area Feb. 4-5, just weeks before the COVID-19 pandemic shut down virtually all in-person conferences across the world. Scientists from the US EPA and FDA joined industry representatives and scientists at the Hyatt Regency Crystal City Hotel for the five-session, two-day conference. This year's meeting featured sessions on Perspectives on Biofilm, Regulation, and Research, Food-Related Biofilms, Biofilm Infection, Oral Biofilm, and Reusable Medical Devices. Twenty-two speakers presented at the meeting including five regulatory scientists, four CBE researchers, and prominent researchers from industry and academia. Robin Patel from the Mayo Clinic delivered the keynote presentation "Medical biofilms: Insights from the first two decades of the millennium." Agendas and proceedings for this meeting and others hosted by the CBE are available here.

Contact Paul Sturman for information about the CBE's innovative Industrial Associates Program.

NEXT SCIENCE®

CBE industrial associate earns antibiofilm efficacy claim

CBE industrial associate Next Science, a biotechnology company, received notification from the Environmental Protection Agency that its hospital cleaner surface disinfectant LMN8 has been accepted for registration. The product has also received clearance to include a claim for effectiveness against biofilm on its labeling. Unlike many disinfectants, LMN8 is a non-toxic product that uses patented technology to eliminate biofilm-based bacteria by attacking the biofilm matrix to expose the bacteria within, making it more vulnerable to attack and eradication. The product's effectiveness against many types of bacteria, including *S. aureus* and *E. coli*, has been validated by clinical testing, Food and Drug Administration clearances, and more than 130,000 patient treatments since 2017. The EPA requires antibiofilm products pass a standardized testing method developed by the CBE prior to including an efficacy claim on its label.

Clarification: The cover story of the CBE's 2019 *Annual Report* incorrectly reported that STERIS earned the US's first anti-biofilm claim with EPA. In fact, CBE member Sterilex Corporation was the first company to achieve this. The Sterilex claim dates back to 1999 for non-public health uses and 2005 for public health uses, following 10 years of specialized protocol development and field testing under a variety of biofilm growth conditions. Sterilex's claims include kills biofilm bacteria, removes biofilm, and prevents/suppresses formation of biofilm. While the STERIS claim is the first granted by EPA using Method MB-19 and/or MB-20 (dated July 2017), the Sterilex claim preceded this by 14 years and was achieved using biofilm testing protocols developed independent of the CBE. The CBE is happy to clarify this important distinction.

STERILEX

2020 INDUSTRIAL ASSOCIATES

In its formative years, companies from the energy sector were heavily represented in the CBE's Industrial Associate program. Corporate interest in biofilm research is constantly evolving. Today, our membership features companies from healthcare, disinfectant, and consumer marketplaces.

3M
new novozymes
American Chemet Corporation
OLYMPUS
ASP
new Perfectus Biomed
Baxter
PPG
P&G
new BioMendics
PureLine
Chem-Aqua
new Quest Medical, Inc.
Church & Dwight Co., Inc.
SANUWAVE
Clorox
Sharklet Technologies, Inc.
Decon Seven
Smith+Nephew
DeLaval
Solvay
icumedical
Sterilex
Lonza
STERIS
Masco
Sherwin-Williams
Next Science
Zimmer Biomet

RESEARCH AREAS

BIOFILM CONTROL STRATEGIES, ENERGY, ENVIRONMENTAL, INDUSTRIAL, STANDARDIZED METHODS, WATER SYSTEMS

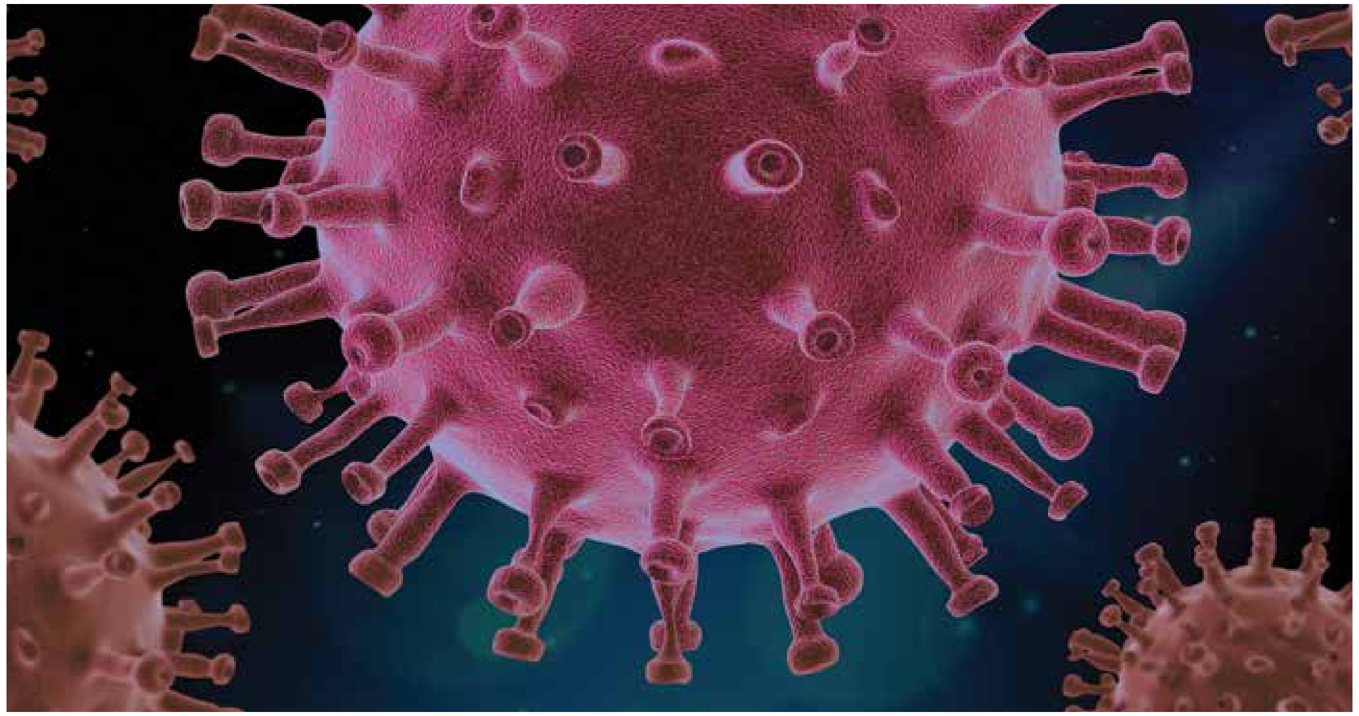
RESEARCH

30TH ANNIVERSARY FEATURE

30th

RESEARCH

THE CBE IS THE WORLD'S FIRST, LARGEST, AND BEST-KNOWN BIOFILM RESEARCH CENTER



CBE researchers working on multiple projects to Fight COVID-19 Pandemic

By Reagan Colyer

Several CBE faculty, staff, and students are working on projects fighting the COVID-19 pandemic.

A grant from the state of Montana supports Montana State University researchers as they explore a faster and less costly method for COVID-19 testing which, when used in conjunction with existing testing methods, could ultimately improve the access to and the speed of testing.

CBE-affiliated faculty member Connie Chang, an assistant professor of chemical and biological engineering, is leading a team of faculty members from the colleges of Agriculture, Education, Health and Human Development, Engineering, and Letters and Science. Chang specializes in microfluidics, the creation and scientific use of tiny droplets of fluid in genomic and bacterial studies.

The $776,000 grant is funding research on LAMP testing, short for loop-mediated isothermal amplification. LAMP testing is an emerging complement that could be used in conjunction with the standard COVID-19 test, which uses a method called quantitative polymerase chain reaction, or qPCR, Chang said. qPCR is the most widely used test in Montana and across the U.S. and is commonly administered using a nasal swab.

The funding comes from the federal Coronavirus Aid, Relief and Economic Security Act — or CARES Act. The grant was administered by the Montana governor's office and the Office of the Commissioner of Higher Education.

Rather than a nasal swab, LAMP testing can analyze saliva samples in as little as 30 minutes, Chang said. LAMP is compatible with "colorimetric" technology, meaning the sample changes color based on the presence of the virus: pink if the sample is negative for COVID-19, yellow if it's positive. LAMP testing doesn't require specialized training to use and attempts to avoid the supply-chain issues posed by the unique materials needed for qPCR tests.

LAMP testing doesn't require specialized training to use and attempts to avoid the supply-chain issues posed by the unique materials needed for qPCR tests.

"There's a lot of research exploring LAMP right now because of its speed," said Chang. "With the technology that we're developing, we think we can trim the result time even more. It's an exciting developing technology for point-of-care testing, and that's why a lot of people are working on it right now."

Several CBE faculty, staff, and students donated saliva samples to assist the project. The easy-to-use and rapid LAMP tests would allow public health officials to quickly identify those with a very high probability of having COVID-19 and then confirm those cases with a subsequent qPCR test.

"The bottom line is the addition of LAMP to our testing toolbox could help increase our ability to identify COVID-19 cases," said Jayne Morrow, MSU assistant vice president for Research, Economic Development and Graduate Education and a member of the project team. Morrow was an undergraduate researcher at the CBE while a student at MSU. (*See Page 16 of the 2019 CBE Annual Report for more on Morrow's work at the CBE and beyond.*)

Before LAMP technology can be made available for use, it must be validated to ensure that it is accurate at identifying COVID-19. To perform that validation, the team partnered with MSU's COVID-19 testing laboratory, which processes approximately 1,500 COVID-19 tests per day in partnership with Montana's Department of Public Health and Human Services. They compared the results of LAMP against the "gold standard" qPCR results and evaluate the new test's effectiveness.

In addition to validating LAMP technology, the team will develop epidemiological models to help determine vulnerable populations and the optimal rates of LAMP testing to best contain COVID-19 spread. The hope is to ultimately deploy LAMP testing in order to identify and contain outbreaks and identify groups that may need to quarantine in minutes rather than days.

Connie Chang and Matthew Fields

"This project takes a holistic approach not only to the scientific elements of exploring a new testing platform but also to the best practices for applying novel technologies to Montana's communities," said Jason Carter, Vice President for Research, Economic Development and Graduate Education. "Our researchers continue to live the land-grant mission of Montana State by pivoting their research expertise and talents to help our state and local public health agencies to make an immediate positive impact in the effort to slow the spread of COVID-19 and maximize the benefit to our most vulnerable communities."

Along with Chang and Morrow, collaborators on the project include CBE Director Matthew Fields of the Department of Microbiology and Immunology; associate professors Deborah Keil, Raina Plowright, Matthew Taylor, and Seth Walk of the Department of Microbiology and Immunology; CBE-affiliated faculty member James Wilking of the Department of Chemical and Biological Engineering; assistant professor David

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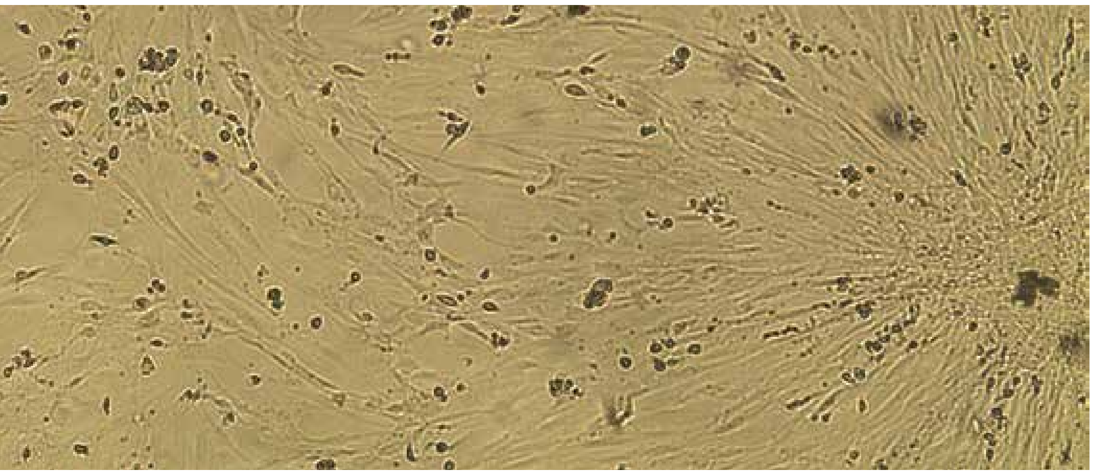
Research



Left: Cells infected with the canine coronavirus. Garth James' (right) lab is using canine coronavirus to investigate how surface disinfectants may or may not target the virus. The eventual goal is to investigate biofilm-coronavirus interactions. CBE microscopy image by Kelly Kirker

Millman of the Gianforte School of Computing; professor Kristen Intemann of the Department of History and Philosophy; associate professor Selena Ahmed of the Department of Health and Human Development; and Alex Adams, director of MSU's Center for American Indian and Rural Health Equity. Importantly, CBE PhD students Tom LeFevre and Isaak Thornton shared the CBE's 2020 W.G. Characklis Award in part for their work fabricating lab supplies using 3D printing to support this initiative, which they did without additional remuneration while continuing their duties as PhD students.

Garth James, a CBE-affiliated faculty member and principal investigator of the CBE's Medical Biofilms Lab, is also pursuing solutions for the pandemic by testing surface disinfectants that specifically target a surrogate coronavirus.

"We have a lot of requests from companies to do virus testing, including from CBE Industrial Associates," James says. "So, we're interested in basic questions on how these disinfectants might affect the infectivity and persistence of coronaviruses."

James says the surrogate coronavirus his lab is using for the testing projects is a variety found in canines, which is safer to work with than the novel coronavirus that is the cause of COVID-19.

"We have experiments running right now," he says. "It's complicated work. We use cell cultures to propagate and enumerate infective viruses as well as quantitative PCR to determine the total number of viral genomes. For disinfectant testing, viral suspensions are dried on to surfaces."

Once his lab perfects these techniques, they'll apply them to a human coronavirus that is in the same group as the novel coronavirus at the root of the pandemic.

"If a disinfectant inactivates the canine coronavirus, it may or may not work against the human virus," James says. "But, if it doesn't, it's not likely to inactivate human coronaviruses."

James says these initial experiments do not directly relate to biofilms, but plans to investigate biofilm-coronavirus interactions in future work.

"We're going to see what happens when coronaviruses get embedded into biofilms," he says. "That's one of the reasons we're getting this capability up and running as quickly as possible." ■

Skip Anderson contributed to this story.

CBE by the Numbers

FY2020

NEW RESEARCH GRANTS

$1.9 million

46
PUBLICATIONS

40
JOURNALS

Journal highlights CBE history, biofilm research

The open-source research journal *Biofilm* highlights the history of the Center for Biofilm Engineering in a recent comprehensive article, "The establishment of the CBE launched biofilms as a field of specialized research." The article focuses on the history of biofilm research, which began in earnest in 1990 when the National Science Foundation awarded Montana State University a $7.2 million grant to establish a biofilm-focused Engineering Research Center on its campus. The NSF ERC grant was, at the time, the largest grant in the school's history. ■

46 CBE AFFILIATED FACULTY

Roberta Amendola
Mechanical & Industrial Engineering
Material science and technology

Elliott Barnhart
Center for Biofilm Engineering
Environmental biotechnology

Iwona Beech
Center for Biofilm Engineering
Biocorrosion and metal-microbe interactions

Diane Bimczok
Microbiology & Immunology
Immunology

Jennifer Brown
Chemical & Biological Engineering
Rheology and biofilm mechanics

Ross Carlson
Chemical & Biological Engineering
Metabolic eng., metabolic networks; chronic wounds

Connie Chang
Chemical & Biological Engineering
Microfluidics

Sarah Codd
Mechanical & Industrial Engineering
Magnetic resonance imaging

Kevin Cook
Mechanical & Industrial Engineering
Tool and machine design

Lewis Cox
Mechanical & Industrial Engineering
Polymer science; scanning probe microscopy

Al Cunningham
Civil Engineering
Subsurface biotechnology and bioremediation

Markus Dieser
Chemical & Biological Engineering
Ecology

Erika Espinosa-Ortiz
Chemical & Biological Engineering
Environmental technologies

Matthew Fields
Microbiology & Immunology
Environmental biofilms

Christine Foreman
Chemical & Biological Engineering
Microbial ecology in cold temperature environments

Michael Franklin
Microbiology & Immunology
Molecular genetics, gene expression, alginate biosynthesis; *Pseudomonas*

Robin Gerlach
Chemical & Biological Engineering
Environmental biotechnology and bioremediation

Darla Goeres
Chemical & Biological Engineering
Standardized biofilm methods

Martin Hamilton
Mathematical Sciences
Mathematics and statistics

Roland Hatzenpichler
Chemistry & Biochemistry
Microbial activity

Chelsea Heveran
Mechanical & Industrial Engineering
Biomechanics; biomimetic materials

Jeffrey Heys
Chemical & Biological Engineering
Fluid-structure interactions

Garth James
Chemical & Biological Engineering
Medical biofilms

Kelly Kirker
Chemical & Biological Engineering
Medical biofilms

Catherine Kirkland
Civil Engineering
Environmental technologies

Ellen Lauchnor
Civil Engineering
Wastewater Systems

Zbigniew Lewandowski
Civil Engineering
Microsensors, chemical gradients, biofilm structure

Scott McCalla
Mathematical Sciences
Applied dynamic systems

Luke McKay
Land Resources and Environ. Sciences
Biofilms in extreme environments, metagenomics

Rebecca Mueller
Chemical & Biological Engineering
Microbial ecology

Albert Parker
Mathematical Sciences
Mathematics and statistics

Brent Peyton
Chemical & Biological Engineering
Environmental biotechnology and bioremediation

Adrienne Phillips
Civil Engineering
Environmental biotechnology

Elinor Pulcini
Chemical & Biological Engineering
Medical biofilms

Abbie Richards
Chemical & Biological Engineering
Environmental biotechnology

Cecily Ryan
Mechanical & Industrial Engineering
Polymers & composites

Joseph Seymour
Chemical & Biological Engineering
Magnetic resonance imaging

Dana Skorupa
Chemical & Biological Engineering
Microbes in extreme environments

Heidi Smith
Microbiology & Immunology
Biology, imaging

Otto Stein
Civil Engineering
Engineered waste remediation

Phil Stewart
Chemical & Biological Engineering
Biofilm control strategies

Paul Sturman
Civil Engineering
Biofilms in waste remediation, industrial systems

Seth Walk
Microbiology & Immunology
Infectious diseases, microbial ecology and evolution

Stephan Warnat
Mechanical & Industrial Engineering
MEMS, sensors, and actuators

James Wilking
Chemical & Biological Engineering
Physical and material biofilm properties

Tianyu Zhang
Mathematical Sciences
Mathematical modeling

7 DISCIPLINES

CHEMICAL & BIOLOGICAL ENGINEERING

CHEMISTRY & BIOCHEMISTRY

CIVIL ENGINEERING

LAND RESOURCES & ENVIRONMENTAL SCIENCES

MATHEMATICAL SCIENCES

MICROBIOLOGY/ IMMUNOLOGY

MECHANICAL & INDUSTRIAL ENGINEERING

MSU's Phil Stewart named Montana University System

Regents Professor

By Marshall Swearingen

Widely respected biofilm researcher Phil Stewart of Montana State University was named a Montana University System Regents Professor, the most prestigious designation attainable by a professor in the system. Stewart is a CBE-affiliated faculty member and past director, and professor in MSU's Norm Asbjornson College of Engineering.

According to MSU President Waded Cruzado, Stewart has played a central role in making MSU a world leader in the study of biofilms.

"Dr. Stewart has long been at the forefront of this work and is internationally recognized for his leadership and high-impact publications," Cruzado wrote in her nomination letter to Clayton Christian, Montana Commissioner of Higher Education. "I am deeply proud to have him as a faculty member at Montana State."

Stewart, the longest serving faculty member in the Department of Chemical and Biological Engineering, says the nomination was a "total surprise."

"It's a tremendous honor," he says. "I feel very much supported by the MSU community."

Stewart was recruited to MSU in 1991 after earning his doctorate at Stanford University and working in Switzerland, Paris and San Francisco. It was the year after MSU received a major grant from the National Science Foundation to launch what would become the Center for Biofilm Engineering. The goal of the funding was to work with private industry to solve real-world problems through a better understanding of how biofilms coat surfaces and cost billions of dollars annually in maintenance and lost efficiency. Stewart says it was a "natural fit" for his interests and background.

"When we were starting out, biofilms were a black box," he says. "We just didn't know that much about what the microorganisms were doing."

In its first years, the Center for Biofilm Engineering plunged into researching a host of biofilm-caused problems: corrosion on oil and gas infrastructure, slime in sink drains, and infections forming on replacement joint implants. Many

In 2019, the Montana University System Board of Regents named Phil Stewart a Regents Professor, the system's highest honor for a faculty. CBE photo by Skip Anderson

of the studies revolved around questions of how to treat or contain problematic biofilms. The MSU researchers built bioreactors that helped companies make standardized studies of how their products reacted with biofilms.

"At the time, there was this big, open question about why biofilms are so hard to kill," Stewart says. "That has been the biggest single problem that I've chewed on during my career: trying to understand the physics and chemistry and biology of how biofilms protect themselves from all kinds of disinfectants and antibiotics."

In a groundbreaking paper Stewart published in 1994, he showed that bacteria on the surface of a biofilm could neutralize chlorine — the active ingredient in bleach — before it could penetrate further into the microbial community. Another of Stewart's papers, published in 2001 and now cited some 3,000 times, established the foundations of the entire field of medical biofilms by showing how some bacteria that infect wounds and medical equipment resist antimicrobials by going into a dormant state.

"When we start to uncover the details of how biofilms behave, you see that they are so much more complex than we ever imagined," says Stewart, whose more than 186 peer-reviewed publications have been cited more than 41,000 times, making him the most-cited researcher at MSU.

Serving as director of the Center for Biofilm Engineering from 2004 to 2015 and as deputy director for eight years prior to that, Stewart led the center through a critical period following its 11-year NSF funding as it became self-sustaining. By continuing outreach to industry as well as regulators such as the Environmental Protection Agency, the Center for Biofilm Engineering rose to international prominence in a field it helped to bring into the mainstream. Stewart's own grant proposals have generated nearly $20 million in funding at MSU.

"As director of the Center for Biofilm Engineering, Dr. Stewart led an extraordinary group of students and faculty that pioneered this emerging field, while at the same time making major scientific contributions of his own," says Brett Gunnink, dean of MSU's engineering college. "That speaks volumes to why he is so deserving of this recognition."

"The Center for Biofilm Engineering has been a really dynamic, fun place to work with great colleagues, with interesting connections to industry and with excellent facilities," Stewart says. "I'm also part of an academic department that has been collegial and supportive, and I really appreciate MSU's commitment to teaching."

Jeff Heys, once a student of Stewart's and now the head of the chemical and biological engineering department, traces his decision to become a chemical engineer to Stewart's influence as a teacher and mentor. Heys had planned to change to another major at the end of his freshman year but changed his mind by the end of Stewart's first lecture in the first class he ever taught at MSU, "Elementary Principles of Chemical Engineering." Heys recalled how Stewart eloquently explained how elementary principles could be applied to something like eating food, which is both a mechanical and chemical process.

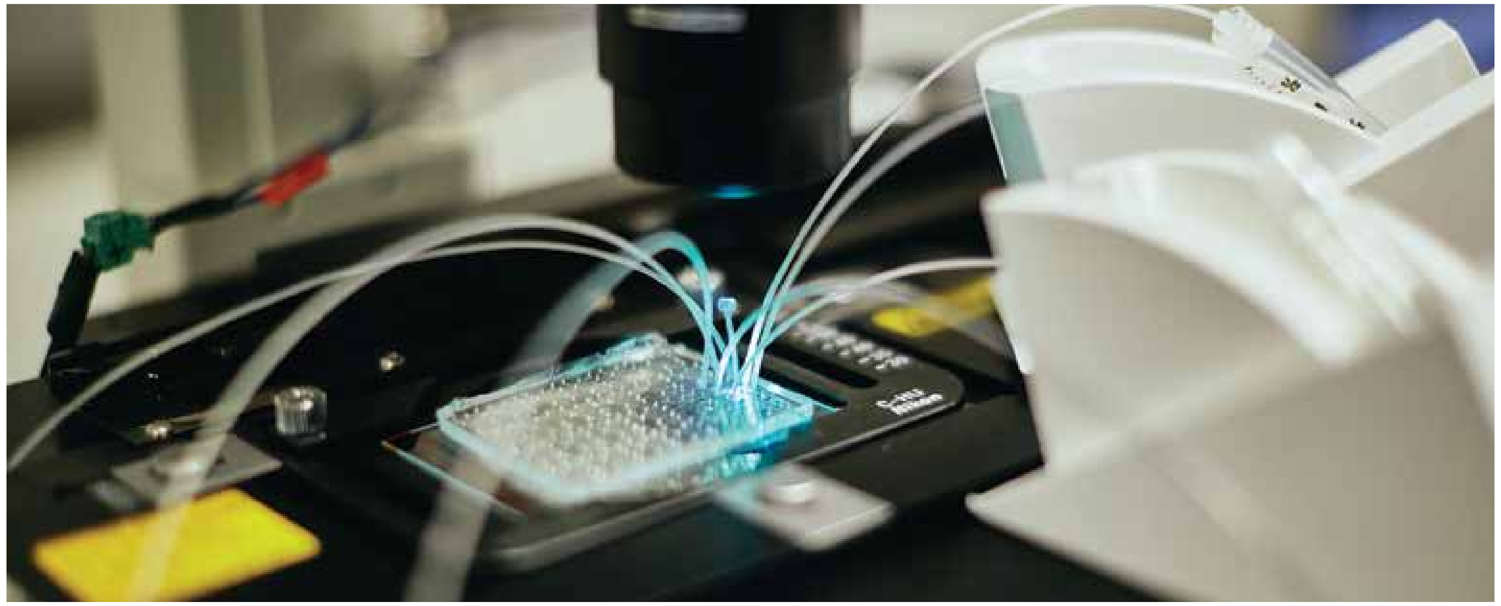
"He made a very, very positive impression," says Heys, who noted that Stewart has supervised more than 30 undergraduate students on research projects and served as graduate adviser for 32 students, including 14 doctoral students from across many departments.

Being named a Regents Professor comes on the heels of a sabbatical to the University of Copenhagen and the University of Calgary to develop new partnerships. Stewart says he is turning his focus to researching why biofilm infections on medical implants like artificial hip joints are so recalcitrant to treatment. "It's still a vexing problem," he says, and finding a solution will require that he push the boundaries of the field of biofilm engineering still further by working closely with immunologists.

"I've been able to be successful by having collaborations all across campus as well as around the country and globe. I really appreciate that the Center for Biofilm Engineering has allowed me to do that," Stewart says. "I'm a real believer in the center, its people and its mission."

Stewart joins 13 other previously named Regents Professors from MSU, including CBE-affiliated faculty member Anne Camper.

Another of Stewart's papers, published in 2001 and now cited some 3,000 times, established the foundations of the entire field of medical biofilms by showing how some bacteria that infect wounds and medical equipment resist antimicrobials by going into a dormant state.



Tiny tubes carry liquids into a microfluidics device in the Chang Lab. MSU photo by Adrian Sanchez-Gonzalez

Small, Beautiful Things

Connie Chang uses tiny drops in wide-ranging research

By Rachel Hergett

CBE-affiliated faculty member Connie Chang sees a world of possibilities in the steady stream of identical dots crossing her computer screen.

Those dots are actually a video of tiny droplets of fluid, roughly the diameter of a human hair, created at a rate of up to millions per hour. They are the building blocks of drop-based microfluidics, which has become the core of Chang's varied and interdisciplinary research at Montana State University.

In the motion of the drops on screen, slowed by high-tech cameras attached to a microscope, Chang, an assistant professor in the Department of Chemical and Biological Engineering in MSU's Norm Asbjornson College of Engineering, sees beauty, both in the flow and in the ability of the drops to streamline laboratory science across disciplines.

Microfluidics can take many forms, but all those forms manipulate fluids on a microscale. It is both a science in itself and an emerging technology built around that science. What once required a lab full of equipment now can be achieved inside 3-D channels in a clear rubber block that would fit in the palm of your hand, technology often referred to as a "lab on a chip." These microfluidics devices—chips with tiny channels—enable scientists such as Chang to perform chemical reactions, assay molecules for diagnostics or complete a multitude of other processes cheaper and faster than their full-scale counterparts.

"It's an exciting time when you can miniaturize everything," says Chang. "Being able to expand what is happening technologically is really exciting for me."

Chang's work lives in the intersection between disciplines, a place that some say is the future of the sciences.

The emergent nature of microfluidics raises a host of questions for Chang. For example, can she create devices to make certain drops merge, and others split in ways she wants? Can she make drops out of different materials? Inject them with viruses or bacteria? Make defined shapes or larger drops? Thus, Chang is simultaneously exploring the capabilities of drop-based microfluidics and using it as a tool for research into other questions.

Connie Chang, left, examines a Petri dish while PhD student Shawna Pratt removes excess silicone from a new microfluidics chip. MSU photo by Adrian Sanchez-Gonzalez

Chang's path to microfluidics began at Wellesley College in Massachusetts, a women's liberal arts school where she was inspired by the field of biophysical chemistry, which applies chemistry tools to the study of biology. A desire to live in the West led Chang to UCLA. There, Chang searched for a biophysics research direction in graduate school, before finding a collaborative project led by advisers Thomas Mason, William Gelbart and Chuck Knobler focused on viruses and nanoemulsions.

Nanoemulsions are tiny drops of water or oil inside of drops of water or oil. We see emulsions in everyday substances like milk or lotion. Chang keeps a bottled red watermelon drink with floating basil seeds on a shelf in her office at MSU. She likens emulsions, the suspension of one type of fluid within another, to the drink. Emulsions are the basis of drop-based microfluidics, which seeks to manipulate the suspended liquid.

Chang's introduction to microfluidics came at Harvard, where she was a postdoctoral researcher in David Weitz' lab. Weitz, a physicist studying soft matter, was the first person to use microfluidic drops to sequence the genome of single cells. Now, all of Chang's research uses the technology. In microfluidics, she found a new way of exploring the world.

"I've been wondering what I would do if I didn't have drop-based microfluidics, but for now I will run with it," Chang says.

Apart from her pervasive interest in microfluidics, it is next to impossible to put Chang and her work into a nice, tidy box encompassing a single science. Her PhD from UCLA is in chemistry, and her postdoctoral work at Harvard was in physics.

Chang joined the university as an assistant research professor in 2013. Within two years, she was granted a tenure-track position, bringing expertise in two important areas for MSU: microfluidics and biofilms, continuing the work of the CBE, the first research center of its kind focusing on the study of those resilient, ubiquitous microbial communities.

Her studies dip into virology, biology and materials science. Chang's work lives in the intersection between disciplines, a place that some say is the future of the sciences. Her research draws from new projects and new ideas gleaned through collaboration and extensive reading of scientific works.

When researchers stick within single disciplines, the science becomes limited, explained Jeff Heys, associate dean of research, economic development, and graduate education in the Department of Chemical and Biological Engineering. He imagines the scope of human knowledge as a blob resting on a flat surface, growing larger and larger while the boundaries of what is known are pushed ever outward. But when this knowledge is only advanced in narrow fields, the limits of that knowledge aren't flowing and curved. Instead,

continued on next page

Research

they end up looking like something of a spiky cartoon hairstyle. Large chasms exist between the advanced points, and single points of study can only advance so far. Chang's work, Heys says, is filling those knowledge gaps between existing fields.

The manifold applications of microfluidics have enabled Chang to take on an incredible variety of studies, bridging departments and disciplines and expanding what multidisciplinary research looks like at the university.

"Connie is one of a new generation of engineers," says Brett Gunnink, dean of the Norm Asbjornson College of Engineering. "The most challenging problems we have today need expertise across disciplines to advance solutions."

Chang's work has been paying big dividends in terms of bringing research funding to the university. She was awarded a share of a $5.2 million Defense Advanced Research Projects Agency grant in 2017 to study methods of treating vaccine-resistant viruses. In 2018, she received a National Science Foundation CAREER Award for research into the nature of biofilms. Both projects use drop-based microfluidics.

Chang also encourages cross-disciplinary research in microfluidics, allowing those in her lab to explore different facets of the groundbreaking technology. For example, postdoctoral researcher Dimitri Bikos is focusing on incubating cells and viruses, delving into some of the materials science behind the processes, and chemical and biological engineering PhD candidate Jake Fredrikson is using microfluidic technology to make hydrogel beads that mimic human tissue. He will work with MSU professor James Wilking, Chang's husband, to organize cells into 3-D structures once stabilized within individual beads.

"Microfluidics is an endless field," Fredrikson says. "If we can expand it in our group, we can push the interdisciplinary fields of research."

Another potential application of Chang's research is the creation of rapid diagnostic methods. Imagine a person is admitted to the hospital with sepsis, where chemicals produced by the body to overcome an infection themselves become toxic. Many modern diagnostic tests to determine the cause of the infection take days. But a rapid diagnostic method using microfluidics can run all the same tests in a couple hours.

Chang's current projects include a collaboration with microbiology professor Mike Franklin growing and sorting bacterial biofilms, one of several studies with other researchers in the Center for Biofilm Engineering. She's also looking into anaerobic microbes with Robin Gerlach and interactions between bacteria with Ross Carlson, both professors in MSU's Department of Chemical and Biological Engineering.

The many applications of microfluidics have enabled Chang to take on an incredible variety of studies, bridging departments and disciplines and expanding what multidisciplinary research looks like at the university.

"Is it normal? No," Heys says of Chang's immense research scope. "Is it the future? Absolutely yes."

The range of Chang's research is mirrored in her desire to promote diversity. Chang says she actively cultivates collaboration. She seeks perspectives from a range of backgrounds and experiences, creating a rich environment for science.

"That's where the most exciting science happens," Chang says.

Until this summer, the Chang Lab existed in three different spaces. Microfluidics and biofilms were studied in Barnard Hall, in the Center for Biofilm Engineering. Molecular biology, viruses and cell culturing were split between two labs in the Chemistry and Biochemistry Building. Having her team in one space, now a single lab in the Chemistry and Biochemistry Building, was necessary for Chang to encourage collaboration and exploration, she says.

Members of the Chang Lab range from undergraduates to post doctoral researchers. They represent a variety of cultures, genders and fields of study. Beyond a simple desire for more representation within the lab, Chang has found creating that diversity changes the dynamics in the lab.

"When we come together with a diverse group, there are more creative solutions and a friendlier environment," she says.

Chang's encouragement of underrepresented groups, such as women and minorities, stems from her own upbringing. Her parents met at Princeton University, where they married in the school chapel. Her father received his doctorate in chemical engineering from Princeton and went on to work in the Union Carbide pilot plant in Cleveland, Ohio. Chang and her two younger sisters were raised in Strongsville, a suburb of Cleveland, where Chang says she felt isolated as the daughter of Taiwanese immigrants in a predominantly white community.

When Chang entered her undergraduate years, she found a new community through Asian student groups and diversity coalitions to combat those feelings of seclusion. At MSU, she has made it a mission to show students of all backgrounds that people like them have achieved the highest levels of success, something gleaned from her days at Wellesley and crystallized as her own young daughters enter grade school. Now, when she returns to Wellesley for reunions, such role models are everywhere.

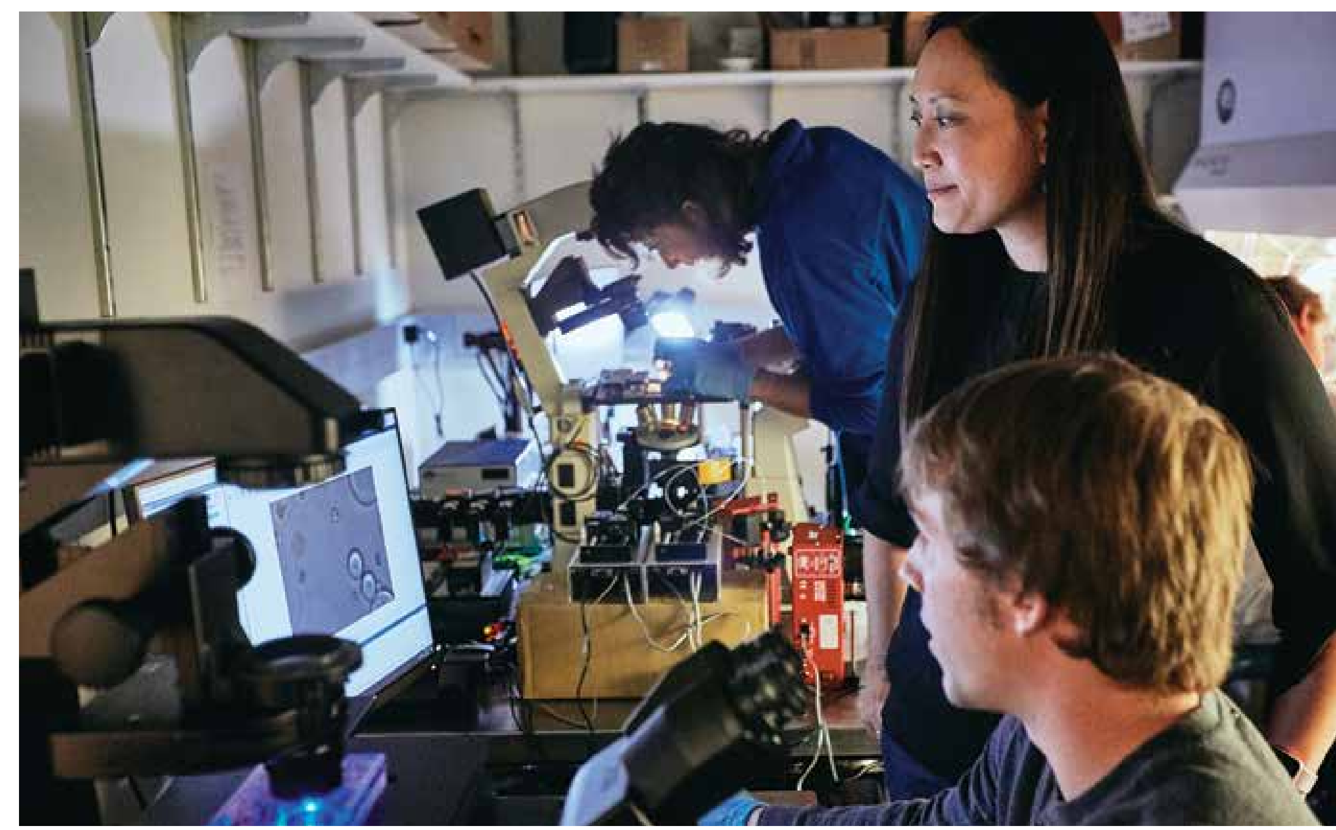
"You see women who can do anything," Chang says. "You see women who have broken glass ceilings, especially in the sciences."

Chang may be a woman in a traditionally male-dominated field, but the scope of her work has placed her at the forefront of microfluidics research and applications. She is simultaneously studying gene sequencing, drug delivery methods, biofilm creation and the capabilities of microfluidics itself. With a love for new ideas, projects and collaborations, Chang uses the tiny particles to ask and answer some big questions.

"How can we use this to be able to understand nature, to make something good, to be able to help?" Chang asked.

Right now, microfluidics is a young science and technology. But that only opens up the directions in which researchers like Chang can take it. Heys recalls a talk Chang gave on microfluidics attended by former MSU Center for Biofilm Engineering director Phil Stewart.

"She's going to have to beat off collaborators with a stick," Stewart says after hearing about Chang's research. "We're just scratching the surface."



Connie Chang won a prestigious CAREER Award from the NSF in 2018. MSU photo by Adrian Sanchez-Gonzalez

Chang Featured in Book about Women in Workforce

When Connie Chang was in graduate school, a senior colleague told her to not expect to become a professor. He may have thought he was giving her a friendly reality check, Chang says, but the comment nonetheless illustrated the persistence of gender stereotypes that all too often undercut women's aspirations.

Now an accomplished researcher, Chang is featured on the cover of a new book titled "Women's Work: Stories From Pioneering Women Shaping Our Workforce," which highlights women defying expectations to lead careers shaped by their persistence and talent.

"I had no idea I would be on the cover," says Chang, assistant professor in the Department of Chemical and Biological Engineering. "I thought the book was a really neat idea, and I've been happy to be part of it."

A project of award-winning photographer Chris Crisman, the book pairs his photos of 56 women —including a blacksmith, a vice president at Google and a rancher who lives near Livingston — with short essays authored by each woman about their upbringing and the challenges they've overcome.

The cover photo shows Chang in her lab in MSU's Center for Biofilm Engineering, seated in front of a microscope and surrounded by a variety of chemistry glassware. "Forging a path as a woman in science is challenging, but each experience has helped me to grow and gain confidence in my abilities," she writes in the two-page essay about her parents, job at MSU and roles as mentor and mother of two young daughters.

Crisman invited Chang to participate in the project about two years ago, when he told her he would be in Montana for another photo shoot, according to Chang. A prominent scientist at MSU, Chang won a $500,000 CAREER grant, the National Science Foundation's highest honor in support of early-career faculty, in 2018. Her work focuses on new applications of microfluidics, in which tiny drops of fluid are manipulated in channels the size of a human hair in order to rapidly sort different microorganisms to help create vaccines, and test antibiotics, among other applications.

"I'm really proud to have Connie as a colleague," says Abbie Richards, head of the Chemical and Biological Engineering Department. "Her research is truly cutting-edge. She's very creative and is a top performer in our department. She's also an excellent mentor to the students in her lab, both graduate and undergraduate, and through her mentorship, she provides opportunities for others to follow in her successes."

Richards, who was appointed in January as the engineering college's first female department head, serves with Chang on MSU's committee for the IChange initiative. MSU is one of 15 public research universities participating in the inaugural cohort of the IChange Network, which is designed to increase the diversity of faculty in science, technology, engineering and math.

IChange comes as the latest chapter in an effort, going back at least a decade, to increase the number of women faculty in STEM fields at MSU, according to Richards. In 2012, MSU won a $3.4 million NSF grant called ADVANCE Institutional Transformation, designed to improved gender equity, job satisfaction, inclusiveness and diversity. "It has been really transformative for our college," Richards says. Both Chang and Richards are featured in a recent video highlighting women in engineering at MSU.

Over the past decade, the number of female tenure-track faculty has climbed from 9% to 24% in the engineering college and from 18 percent to 38 percent in the chemical and biological engineering department. Lloyd Berg, who served as the department's head for 33 years until 1979, pioneered efforts to recruit women students into the field. Chang recalls her father, who earned a doctorate in chemical engineering, coming across an old article in an engineering publication about MSU setting national records in 1972 for its number of female graduates. "I'm incredibly fortunate to be a member of a department that has a long history of supporting women students and faculty," Chang says.

"In our college, we're very motivated to change the culture of engineering to one that is inviting to all people," says dean Brett Gunnink, who serves on the IChange committee as well as the President's Commission on the Status of University Women at MSU. "When new, talented faculty join our community and excel, we aren't surprised. Dr. Chang is an outstanding example."

Chang says she is awed by the variety of paths forged by all the women featured in the book. "There are no limits to what women can do," she says.

The book is available for sale online from publisher Simon and Schuster.

Q&A

Setting the Standard

Goeres expands mission of the Standardized Biofilm Methods Lab

By Skip Anderson

When Darla Goeres, a research professor of regulatory science, joined the CBE in 1997, standardized methods for testing the efficacy of chemicals, compounds, and mechanical processes in killing and/or removing biofilms did not exist. Fast forward to 2017 when the US Environmental Protection Agency adopted the standardized testing methods Goeres, et al, developed at the CBE. This led to consumer and commercial products being able to include data-driven biofilm efficacy claims on their products. Goeres has held numerous leadership positions at ASTM International, and currently serves as the Chair of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents. Goeres is also a founding editor of the open-source research journal *Biofilm.*

You've made some exciting changes to the Standardized Biofilm Methods Laboratory in the past few months that will affect the CBE. What's the new emphasis?

The CBE has embraced the need to expand methods development into the broader topic of regulatory science, which is the next logical progression for standard methods. For over 20 years, the CBE and the Industrial Associates were supportive of our work to develop standard methods. Now industry and the biofilm community at large have a significant number of testing methods that we can reference in our discussions with the federal regulators. These methods have been validated and so we are at a place where we can have constructive discussions about bringing products tested using these methods to the marketplace.

What does that mean for the SBML?

New opportunities. We have a chance to put science into action which will continue to bridge the gap between federal regulatory agencies, industry, and academia. We have created a critical mass of resources for when we're talking to federal regulators. We used to say, "How do we test this?" And now we have those tests that can be used as is or modified for specific applications. For a long time, we were in a narrow and winding canyon. But that canyon has recently opened to a huge lake allowing us to see the potential of these methods and how they will enable innovative products to reach the marketplace.

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Q&A

What does this mean for the CBE's Industrial Associates program?

Our industrial associates were key influencers in making this process happen. Many of the current standard methods were born out of projects originally sponsored by our industry partners. What we have is a mechanism to engage industry and regulators. Standard methods have provided a common language to communicate and allowed for everybody to be on the same page. If you allow me to mix metaphors, standard methods leveled the playing field, so to speak, which is important when you are in a canyon. There is now general agreement as to why biofilms are important and why we need standard test methods. And what is even more important, is now we have momentum. The number of validated biofilm tests will increase as our knowledge about how biofilm grows and thrives increases and new technology becomes available. The whole field is opening up, and the possibilities are expansive. We no longer need to explain what a biofilm is. The "B word" is thrown around all the time with industry and regulators now, and that was a *huge* hurdle to clear. What's exciting is that we have developed the process for people to use whether they are from industry, academia, and regulatory agencies.

We have a chance to put science into action which will continue to bridge the gap between federal regulatory agencies, industry, and academia.

Can we expect more anti-biofilm products to be on the shelves soon?

I do expect there to be more products on the shelves soon. Of course, everyone pivoted this last year to deal with the coronavirus pandemic, but because of this, consumers are much more aware of the importance of surface disinfection, which includes biofilm. I also expect there may also be a rebranding of products that have been cleaning surfaces for years, and perhaps they've been saying that their products clean slime, grunge, etc. But, people are more conscious of microorganisms living on the surfaces in their homes and work spaces. So, companies will likely take some of these products that have been working for years and will seek a biofilm claim so the consumer understands more specifically what they have been cleaning.

CBE research assistant Kelli Buckingham-Meyer works with a CDC Biofilm Reactor, which was designed by the CBE in conjunction with Bozeman-based BioSurface Technologies, Inc. MSU photo by Kelly Gorham

That, of course, will drive additional biofilm awareness.

COVID has really caused people to be more aware of microbes in general. The general consumer may now take the time to understand what the biofilm claim on the product means. This is exciting, and an opportunity for all of us in science to work to educate the general consumer.

The CBE turned 30 in 2020. What do you consider the landmark achievements of the SBML?

Wow, it is challenging to capture all of the accomplishments of the last 30 years. From my perspective, getting those biofilm [testing] methods developed and standardized was huge. We were the first to get those on the books, which provided industry a pathway. It was an incredibly long process with a lot of moving parts and yet we persisted. I also think having the US Environmental Protection Agency recognize those ASTM biofilm methods in their guidance documents is huge.

The bottom line is that more biofilm research centers and international collaboration are very positive things. It creates momentum to move forward. And that's a good thing.

Another significant accomplishment of the SBML is the educational component. I'm proud that the SBML promoted these methods in an effective way; they are now in the public domain. We have training videos and we offer workshops. We've been very active in educating industries, regulators, and academics, and that's huge. This sharing of knowledge has enabled a critical number of methods to be developed and we're just at the beginning.

Another contribution we've made is the collaboration with BioSurface Technologies, Inc., to develop a collection of biofilm reactors that are used in laboratories around the world. We've provided the biofilm community a collection of tools they can use and adapt for their own purposes.

The field of biofilm research has surged over the past decade. New research centers have opened around the world, and the number of biofilm-related papers published annually have grown from around 3,000 to more than 6,000. How is the global interest affecting what you're doing at the SBML?

It's a huge opportunity to collaborate with our colleagues in the US and overseas to work towards international standardization for biofilm methods that describe how to grow, treat, analyze, and sample biofilm bacteria. Once you have multiple entities working from the same vocabulary and working towards the same goals, it will expedite the potential for innovation in science and in the marketplace. We live in a global market.

But there are growing pains, too. Biofilm research has gone from being a pretty small field to one of international scientific inquiry. It's like when a company grows from a small, family-owned business where everyone is related in some way or another and is fully invested, and suddenly, the company is so large, you don't even know the person in the office in the second location and they have a completely different idea for what the business plan should look like. Biofilm research has grown into this large global entity and we all view the biofilm paradigm through different lenses. And, as with anything when you go from small to global, there will be growing pains. The leading biofilm researchers from around the world gathered in 2019 at the Biofilm Bash to recognize those growing pains and ask how best to grow the field through collaboration in a productive way.

It's critical that the CBE move in a global direction. If we want to continue to be biofilm influencers, and I think we do, then we need to direct the conversation so we can grow our organization while also expanding biofilm science and technology as it relates to biofilm products. The bottom line is that more biofilm research centers and international collaboration are very positive. It creates momentum to move forward. And that's a good thing.

Research

Three research teams set to

Improve Water Quality

A National Science Foundation EPSCoR award of $20 million to a consortium of Montana colleges and universities aims to advance research and education in water quality. MSU photo by Kelly Gorham

By Marshall Swearingen

As part of a statewide effort to advance research and education in water quality, three Montana State University research teams have won funding for projects that could lead to more affordable water monitoring, new treatment methods and better understanding of contamination sources.

The roughly $50,000 "seed" grants were awarded to the MSU teams in December through the Consortium for Research on Environmental Water Systems, or CREWS, which received $20 million from the National Science Foundation earlier in 2019. The CREWS Research Seed Award Program is designed to fund innovative research related to water quality and build research capacity across Montana's higher education institutions, according to MSU's Todd Kipfer, associate director of the project.

"These are three fantastic projects," Kipfer says. "We had a lot of strong proposals, and these projects really showcase MSU's strengths in water quality research."

Leading each of the three projects are John Dore, associate research professor in the Department of Land Resources and Environmental Sciences in MSU's College of Agriculture; Erika Espinosa-Ortiz, research assistant professor at the Center for Biofilm Engineering; and Stephan Warnat, assistant professor in the Department of Mechanical and Industrial Engineering in MSU's Norm Asbjornson College of Engineering.

Warnat draws from his experience in electrical engineering and material science to develop a new, more affordable micro-sensor technology for measuring water quality in streams and soils. The standard equipment used to gather a suite of water quality data is often prohibitively expensive, he says. By engineering smaller, cheaper sensors that measure individual water properties and then integrating them with electronics, "we think we can manufacture these sensors at a much lower cost," he says.

According to Warnat, bringing down the cost allows multiple sensors to be used with each device, so that researchers can more easily measure water quality across multiple soil locations, for instance. Using multiple, redundant sensors also improves the reliability of the equipment in the field. The seed grant provides an opportunity to prove the concept of the technology, which could lead to additional funding for refining the accuracy of the sensors, he says.

Espinosa-Ortiz is leading a team of Center for Biofilm Engineering researchers that will develop and test new biofilms for treating water contaminants commonly associated with coal mining. Some biofilms are known for their ability to transform selenium, nitrate and sulfate into less harmful compounds. Espinosa-Ortiz's team will attempt to isolate new bacteria and fungi from streams and soils and compare their treatment abilities with known biofilms in the lab.

Erika Espinosa-Ortiz is leading a team to develop new biofilms to treat water contaminants commonly associated with coal mining. MSU photo by Adrian Sanchez-Gonzalez

Espinosa-Ortiz says that previous work has focused on using various bacteria-dominated biofilms to treat selenium, nitrate and sulfate individually, but that the team's explorations could produce biofilms that are more effective at treating the contaminants together. "We think there is a lot more potential for using fungi," she says. The researchers will also analyze which genes are turned on when the biofilms form, which could help refine treatment methods. The team includes several CBE-affiliated researchers, including Robin Gerlach and Brent Peyton, in the Department of Chemical and Biological Engineering; Ellen Lauchnor, assistant professor in the Department of Civil Engineering; and Rebecca Mueller, research assistant professor.

Dore, working with Eric Boyd, associate professor in the Department of Microbiology and Immunology in MSU's College of Agriculture, will explore a new theory on the origins of mercury detected in wildlife of the Upper Clark Fork River below its confluence with Flint Creek. By sampling water and particulates in Georgetown Lake, which sits in the upper reaches of the Flint Creek watershed, the researchers hope to learn whether microorganisms in the reservoir are converting inorganic mercury from old gold and silver mines into an organic form called methylmercury that is more easily absorbed by body tissues when ingested. Maria Clara Fernandes-Martins, graduate student in the Molecular Biosciences program, will identify and quantify the responsible microbes through analysis of their DNA.

Dore says microbes produce methylmercury in oxygen-depleted conditions, such as those are common in Georgetown Lake in winter, when ice cover allows oxygen to be completely consumed in its deeper waters. The dam at Georgetown Lake drains water from near the reservoir's bottom, potentially delivering methylmercury produced in the low-oxygen bottom waters downstream, where it could enter the food web.

"Accumulation of methylmercury is a particular concern for top predators," like osprey, whose diet is primarily fish, Dore says. Montana's interagency sport fish consumption guidelines for the Upper Clark Fork River and Flint Creek acknowledge the presence of mercury and recommend limits to dietary fish intake. "There's so little that's actually known about how mercury makes its way from mines into the ecosystem, and greater understanding of these mechanisms may lead to improved strategies for mitigation of the potential hazard," Dore says.

The three MSU projects were among seven selected for CREWS seed grants in the research category. A total of 14 projects, including for workforce development and tribal college programs, were selected from 30 proposals submitted statewide. MSU's Office of Research, Economic Development and Graduate Education contributed supporting funds for the MSU research projects. CREWS is a partnership between MSU, the University of Montana, Montana Tech, Salish Kootenai College and Little Big Horn College. CREWS is an RII Track-1 project of NSF's Established Program to Stimulate Competitive Research (EPSCoR).

CBE, MSU ramp up $6 million project seeking

Effective Solutions to Biofilm Corrosion

By Marshall Swearingen

The microbial gunk that forms in bathroom sink drains everywhere can be a slimy nuisance, but it pales in comparison to similar, more corrosive biofilms that cause billions of dollars in damage each year to oil pipelines and other infrastructure.

That's why Montana State University researchers and their colleagues are taking a new, multi-disciplinary approach to developing coatings that can prevent or hinder the metal-eating microbes.

"This is a huge problem," says Matthew Fields, director of the Center for Biofilm Engineering. Dealing with metal corrosion consumes at least 2 percent of U.S. gross domestic product each year for maintenance and other costs, and it has been estimated that biofilms are responsible for about half of that corrosion, he says.

Backed by a $6 million National Science Foundation grant that the team recently received, the four-year project combines materials science, microbial chemistry, gene sequencing and cutting-edge computer science in an attempt to unravel how the microbial mats interact with the surfaces they feed on.

"Our question really is, could we start to amass enough data that we could anticipate how a biofilm would respond to a new coating," says Fields, who is leading the $1.4 million MSU portion of the project. A total of 21 researchers at MSU as well as South Dakota School of Mines and Technology, University of Nebraska Omaha, and the University of South Dakota are involved.

The challenge is not only that the biofilms are made up of diverse communities of bacteria and other microorganisms, but that they display a range of behaviors. "Sometimes, under the same conditions, the material resists for years and other times it fails in weeks," says researcher Roberta Amendola, CBE-affiliated assistant professor in the Department of Mechanical and Industrial Engineering in MSU's Norm Asbjornson College of Engineering. "It's not clear what is triggering what."

The MSU team is culturing hundreds of biofilms on metal samples, some with special coatings, and observing the corrosion using specialized microscopes in MSU's Imaging and Chemical Analysis Laboratory directed by project member Recep Avci, research professor in the Department of Physics in MSU's College of Letters and Science. Avci's research focuses on microscopic techniques that can predict where corrosion will start and grow, and how that relates to the properties of metals and coatings.

> Dealing with metal corrosion consumes at least 2 percent of U.S. gross domestic product each year for maintenance and other costs, and it has been estimated that biofilms are responsible for about half of that corrosion.
>
> — Matthew Fields, director of MSU's Center for Biofilm Engineering

Laura Camilleri, who earned her doctorate at MSU in 2019, handles samples in the Matthew Fields' Lab. Camilleri's work contributed to a project to study how biofilms respond to surface coatings. MSU photo by Kelly Gorham

"You can see the material being consumed, sometimes very quickly," Amendola says. "But there's still a lot that's not known about the interaction between the material and the biofilm. It's very dynamic."

That's where the gene sequencing comes in. By mapping the genetic material of the microbes, the team, which includes Robin Gerlach and Brent Peyton, CBE-affiliated professors in the Department of Chemical and Biological Engineering, can see which genes are turned on or off and correlate those to the observed corrosion behavior.

As the different kinds of data pile up, the team will turn to new computing techniques to blend the information together and search for patterns that lurk below the obvious — patterns that may hold the key to predicting how a certain corrosive biofilm might respond to a particular coating.

"We'll be able to computationally advance experiments in ways that are faster and more efficient than just trying out combination after combination in the lab," says Brendan Mumey, professor in the Gianforte School of Computing. The technique, called data fusion, "is a relatively new area in computer science, and I think this project is a good test bed for it."

"We're hoping to find that some of these new coatings are more effective against biofilms," Amendola says. A few decades ago, research on microbial corrosion was focused on the chemical interaction between the biofilm and metal, and then shifted to focus on the biology, but both approaches fell short of insight that could produce effective coatings, she says.

"This is the first time we're bringing together so many different disciplines to get a better picture of what's happening," Amendola says. "I'm excited to see where it goes."

Left: Members of Anne Camper's lab in the late-1990s, including Camper (left to right), Calvin Abernathy, Phil Butterfield, Brian Ellis, and Laura Boegli (right). Above: Frank Roe 1990.

30 Years Later

CBE Leads the World in Biofilm Research

By Marshall Swearingen

Biofilm causes hundreds of billions of dollars in metal corrosion to pipelines and other infrastructure annually. Biofilm in urinary catheters accounts for an estimated 30 percent of all hospital-related infections, resulting in as many as 13,000 deaths per year. Biofilm is a factor in outbreaks of foodborne *E. coli*, as well as a routine source of household frustration, clogging sink drains everywhere.

Yet until relatively recently there wasn't much of a concept of — or even a name for — the diverse microbial communities that form on surfaces. People in industry called the stuff some variant of the word "gunk," microbiologists studied the organisms but didn't view them as fundamentally different from microbes growing individually, much less understand how to control them.

Three decades ago, when Montana State University applied for a National Science Foundation grant to start a biofilm-focused research center, "the scientific community hadn't really accepted biofilms yet — their existence let alone their importance," says Al Cunningham, CBE-affiliated professor in the Department of Civil Engineering in the Norm Asbjornson College of Engineering. Applying for the prestigious grant was "a total long shot," he says.

But MSU had Bill Characklis, a charismatic engineer who had recognized the untapped possibilities of studying biofilm. And in 1990, MSU was selected as one of only three recipients nationwide for the more than $7 million grant — the largest MSU had ever received at that time.

Characklis helped launch what would become known as MSU's Center for Biofilm Engineering. "He basically brought together the small biofilm community, with industry, and said to NSF, 'This field has huge potential.' And time has proven that to be the case," Cunningham says.

As the CBE is now in its 31st year, MSU researchers say the center remains true to Characklis' vision of an interdisciplinary research community dedicated to solving real-world problems. The CBE has overcome obstacles and evolved to tackle new challenges, and it continues to provide meaningful educational opportunities for students while pushing the boundaries of biofilm research.

The founding of the CBE in 1990 "was paradigm-changing," says Matthew Fields, the center's current director. "What it said was, 'Biofilms are unique, and they deserve their own field of study.'"

Pretty Radical at the Time

When the CBE launched, scientists tended to view bacteria primarily as organisms suspended in liquid. Even if microbiologists knew that bacterial slime clogged pipes, traditional views distanced them from the idea that bacteria could actually attach to surfaces. Meanwhile, engineers designed things like pipes and had complex mathematics to explain fluid interaction with surfaces but seldom studied the microbes that interfered with their plans. Characklis, who had witnessed pipe-clogging microbes while working as a chemical engineer, saw the opportunity to mesh the two fields.

"Bringing in engineering as a way of looking at microbiology was pretty radical at the time," says Nick Zelver, an early Characklis collaborator and now the senior technology manager in MSU's Technology Transfer Office. "Engineering was required to understand what was happening. Why don't the bacteria just get washed off? These were hard problems that nobody really understood."

In an era when academic disciplines were more siloed than they are today, understanding biofilm meant engineers had to learn microbiology, and microbiologists had to learn engineering. Cunningham "was an engineer who had never done anything with microbiology, ever," he recalled. "I was learning about microbes from the ground up."

Anne Camper, a Montana University System Regents Professor in the civil engineering department, earned her bachelor's and master's in microbiology at MSU

continued on next page

CBE's 30th Anniversary

before switching to engineering to earn a doctorate while conducting research at the CBE about microbial buildup in drinking water pipes. Like many of the researchers advancing the new biofilm field, Camper found the cross-disciplinary work to be a challenge. Attending seminars where whiteboards were filled with complex engineering math "made my head hurt," she says. "But it was fascinating."

Characklis deemed it a practical necessity to bring together scientists from different disciplines, but that approach also stemmed from his open, curious and energetic personality, according to Brent Peyton, who came to the CBE in 1986 as doctoral student. Characklis drew people to him and made them excited to work toward common goals, he says.

"He put into place such a good culture of collegiality that it has just carried on since," Peyton says.

Characklis also thought real-world applications should drive MSU biofilm research, Peyton says. When Characklis came to MSU in 1979 from Rice University, he brought contacts with industry, mostly oil companies trying to keep biofilms from clogging and corroding their pipelines. Their support had been critical to MSU getting the NSF grant, Peyton recalled, and for Characklis, "everything we did had to have an industrial impact."

Frontier Spirit

Characklis recruited Phil Stewart to the CBE in 1991 after the latter earned his doctorate at Stanford University and worked in Switzerland, Paris and San Francisco. The open-endedness of the new biofilm field was exhilarating, Stewart says.

"There was a very creative, almost frontier, spirit," says Stewart, now a Regents Professor in the Department of Chemical and Biological Engineering. "There was this sense of, 'Here we go, we can do anything.'"

That was tempered, however, by Characklis' death from cancer in 1992 at age 50. Characklis had been so central to the CBE's founding that there was talk at NSF of withdrawing the MSU grant altogether. It took reassurance from MSU leadership and the hiring of a new director, accomplished Canadian microbiologist Bill Costerton, to keep the CBE alive, Stewart says.

But the interdisciplinary, practical approach to biofilm was already paying off. MSU researchers using fluid dynamics models, powerful microscopes and other advanced tools were unraveling the fundamentals of how biofilms structured themselves into complex communities with properties far beyond what the single-celled organisms could muster individually.

"We were constantly adapting and bringing in new ideas," says Stewart, who would serve as CBE director from 2004 until 2015. "We were also realizing how widespread biofilms could be." Biofilm wasn't just gunk that clogged pipes, but invisibly thin layers of bacteria on medical equipment and countertops, festering wounds that didn't respond to antibiotics, and more.

Stewart published a groundbreaking paper in 1994 showing that bacteria on the surface of a biofilm could neutralize chlorine — the active ingredient in bleach — before the chemical could penetrate the microbial layers. The finding helped explain why biofilms resisted traditional disinfectants and drew interest from companies focused on developing improved cleaning products for households and health care.

Stewart's discovery, along with subsequent studies, helped launch the CBE into a new role of conducting research that assists regulatory agencies like the U.S. Environmental Protection Agency. As companies sought to develop new, biofilm-specific products like hospital antiseptics, a need arose for science to guide how the agencies test product effectiveness.

"We are really committed to this idea of translating research into a form that enables something — to developing fundamental knowledge and then taking it a step further," says CBE researcher Darla Goeres, whose lab developed methods that were adopted by the EPA in 2017 to create one of the first-ever biofilm-specific testing standards. The standards are what allow companies to accurately claim that their products, such as hospital disinfectants, are effective against biofilms.

Did you know?

TO DATE, PHIL STEWART'S WORK HAS BEEN CITED MORE THAN

48,200 TIMES

THAT'S EQUIVALENT TO ONCE PER DAY FOR

132 YEARS

Support from a widening range of industries, especially health care, helped the CBE become self-sustaining as the original NSF grant ran its 11-year course. Today, the center's industrial associates program includes nearly 30 companies, including 3M, SC Johnson and Procter & Gamble. Interaction with industry, including at the annual biofilm meeting the CBE hosts each year, allows CBE scientists to share the latest in biofilm science while gaining ideas for new research that can solve problems.

"These problems have always been there, but now we have more of an ability to understand what's causing them," says Paul Sturman, the CBE's industrial program coordinator. For instance, there's growing awareness that many instances of foodborne illness are caused by biofilm-forming bacteria. "What we've seen is an increasing recognition that problems that were thought of as just bacteria problems are actually biofilm problems."

CBE-affiliated faculty member James Wilking uses a 3D printer as part of a project to better understand their behavior and properties. MSU photo by Adrian Sanchez-Gonzalez

The Staying Power of Biofilm

Although much progress has been made in the past 30 years, many unknowns remain, says microbiologist Fields, who became CBE director in 2015. Scientists have a fairly good understanding of a handful of biofilms, but even with those, the closer researchers look, the more complexity they find — the way the microbes attach to materials, what happens in the space around the aggregated bacterial cells. That's why solutions to many of the CBE's earliest challenges have proven elusive. Biofilm corrosion of oil and gas pipelines, for example, remains a persistent problem. Fields is leading a team that was recently awarded a $6 million NSF grant to apply cutting-edge technologies — including gene sequencing, powerful imaging techniques and nanomaterials — to develop coatings that could prevent biofilms from latching onto surfaces.

Another advancing technology, 3D printing, has opened the possibility of not just culturing biofilms in the lab but actually assembling bacterial layers from scratch to better manipulate and understand biofilm behavior. "It's exciting to think we could get to a point where we live up to our namesake in a whole new way, that we could actually begin to engineer biofilms," says Fields, who is partnering on a 3D printing project with James Wilking, associate professor in the chemical and biological engineering department.

Wilking is part of a new generation of biofilm scientists at MSU who have gravitated toward the CBE's scientific legacy and collegial reputation. "You can talk to anybody here about a problem or idea, and they're willing to listen and collaborate," he says. The CBE's research endeavors involve around 30 affiliated faculty from departments across campus, as well as roughly 50 graduate students and 60 undergraduates, giving them valuable research experience.

The CBE also increasingly collaborates with scientists around the world who study biofilm, which has gone from an obscure term to a field that's the focus of multiple research centers.

"If you ask anyone in the world who works on biofilm, they've heard of Bozeman," Wilking says.

"One of our goals is to make biofilm a household word," says Adrienne Phillips, associate professor in the civil engineering department. Although most CBE research involves controlling harmful biofilms, hers focuses on using the microbes beneficially, to seal cracks in oil and gas wells that would otherwise be difficult or impossible to repair. Phillips is also involved in projects to use biofilm to cement recyclable building materials. "I get excited about finding ways to use biofilm to improve the environment," she says.

According to Cunningham, the growing recognition that biofilm can be understood in order to reduce its worst effects and even harnessed for good is a tribute to the vision that founded the center three decades ago.

Not unlike the persistent nature of the microbes themselves, "it's a testimony to the staying power of the biofilm concept," he says. "And it's what has allowed the Center for Biofilm Engineering to continue and to flourish."

CBE's 30th Anniversary

1980s

Before launching the CBE, founder Bill Characklis developed a 12-member industrial associate program to support an indirect predecessor to the CBE. It would later serve as the model for CBE's longstanding IA program.

1990 ▶▶

1990

NSF awards MSU $7.5 million ERC grant to launch the Center for Interfacial Microbial Process Engineering, later to be known as the CBE. Bill Characklis is its director.

1991

REU summer program begins. DOW Chemical and Conoco support fellowships for graduate students.

Cunningham, Characklis, Abedeen, and Crawford publish "Influence of biofilm accumulation on porous media hydrodynamics" in *Environmental Science & Technology*.

The center acquires its first Confocal Scanning Laser Microscope (CSLM) at a cost of $234,000.

1992

Founding director Bill Characklis dies.

Founder of the NSF's ERC program Lynn Preston, and others, successfully persuade NSF not to pull funding for the center in the wake of Characklis' death.

1993

Bill Costerton named center's second director.

Center renamed the Center for Biofilm Engineering.

MSU enters agreement with BioSurface Technologies Corporation to manufacture, market, and sell the Annular Reactor developed at the center.

MSU now offers four biofilm engineering courses and one biofilm microbiology course.

1994

Researchers use CSLM microscope to demonstrate proof that water flows through channels in biofilm communities.

Costerton, Lewanowski, deBeer, Caldwell, Korber, and James publish "Biofilms-the customized microniche" in *Journal of Bacteriology*.

1995

24 INDUSTRIAL ASSOCIATES

CBE now has 24 Industrial Associates.

CBE conducts bioremediation education workshops for the US EPA.

CBE develops the rotating disk reactor, a predecessor of the CDC reactor.

Hamilton, Johnson, Camper, Stoodley, Harkin, Gillis, and Shope publish "Analysis of bacterial spatial patterns at the initial stage of biofilm formation" in *Biometrics*.

1996

Science features CBE in article titled "Biofilms Invade Microbiology."

1997

CBE develops the drip flow reactor

CBE acquires $400,000 in microscopy equipment via NSF-ERC grant.

1998

The CBE was the first to show, in an April 1998 *Science* paper (Davies, Parsek, Pearson, Iglewski, Costerton, and Greenberg) that biofilm formation in Gram-negative bacteria is controlled by chemical signals that also control quorum sensing processes by which bacteria "sense" the number of cells present in a given ecosystem. Subsequently, the CBE described many different signals of this type. Because of this discovery, the medical research community began to realize that many chronic diseases, such as cystic fibrosis, prostatitis, and chronic wounds, are the result of biofilm formation.

CBE undertakes promising new initiative: Biomineralization.

1999

Bill Costerton and Phil Stewart co-author a review article in *Science* that reveals biofilms to be composed of cells in matrix-enclosed colonies, which form "towers" with open-water channels that enable the flow of nutrients. They conclude that a system of chemical signals must control the development of these complex communities.

2000

CBE develops new research emphasis: Standardized Biofilm Methods.

2001

Lancet publishes the influential paper "Antibiotic resistance of bacteria in biofilms," authored by Phil Stewart and Bill Costerton.

Geesey publishes "Bacterial behavior at surfaces" in *Current Opinions in Microbiology*.

2002

ASTM approves the E2196 Rotating Disk Reactor Method developed at the CBE.

2003

CBE establishes a Microscope Resource Room, acquires new confocal microscope, flow cytometer, and image analysis facilities.

2004

CBE established its Medical Biofilm Laboratory.

Bill Costerton retires from MSU.

2004 ▶▶

* listed papers for particular years are representative of the numerous influential papers produced each year by our faculty, staff, and students along with their co-authors

CBE's 30th Anniversary

▶▶ 2005 ▶▶ 2020

2005

Phil Stewart named CBE's third director.

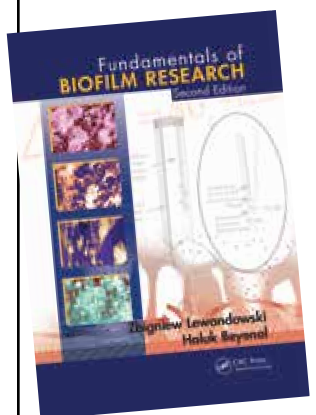
Shirtliff, Bargmeyer, and Camper publish "Assessment of the ability of the bioelectric effect to eliminate mixed-species biofilms" in *Applied and Environmental Microbiology*.

2006

NIH awards Chronic Wound grant to CBE.

2007

Fundamentals of Biofilm Research by Zbigniew Lewandowski & Haluk Beyenal, is published.



2008

ASTM approves Drip Flow Reactor Model.

Stewart and Franklin publish "Physiological heterogeneity in biofilms" in *Nature Reviews Microbiology*.

2009

CBE-hosts Wound Biofilm Retreat, drawing scientists, clinicians, and industry representatives.

2010

MJ Murdoch Charitable Trust and NSF award combined grants of $1 million to provide CBE with Leica confocal microscopes.

Together with the Southwest Regional Wound Care Clinic, the CBE showed that chronic wounds, such as diabetic lower extremity ulcers, are due to persistent biofilm infections. Preliminary work in this field led to the award of an NIH grant to the CBE to continue to develop models for the in vitro study of chronic wounds and assess the efficacy of anti-biofilm agents.

2011

James is a co-author for the publication "New horizons for cutaneous microbiology: the role of biofilms in dermatological disease" in the *British Journal of Dermatology*.

CBE Industrial Associates Program now has 33 members.

33 INDUSTRIAL ASSOCIATES

2012

ASTM approves the standard test method for evaluating disinfectant efficacy for *Pseudomonas aeruginosa* biofilm grown in a CDC biofilm reactor using the single tube method.

2013

Khan, Lewandowski, Camper, and co-authors publish "Combined effects of EPS and HRT enhanced biofouling on a submerged and hybrid PAC-MF membrane bioreactor" in *Water Research*.

Montana University System names Anne Camper a Regents Professor.

2014

CBE cosponsors the US FDA's first public biofilm workshop.

2015

CBE celebrates 25th anniversary. Cumulative data include:

- 1,024 peer-reviewed papers published
- More than 730 undergraduates (including 7 Goldwater Scholars) conducted biofilm research
- More than 235 graduate students awarded advanced degrees
- Funded interaction with more than 100 companies
- ASTM accepts five biofilm testing methods developed at CBE
- 270 visiting researchers have studied biofilm at CBE
- CBE conceptual graphics downloaded by researchers in all 50 states and 59 countries.

2016

Matthew Fields named the CBE's fourth director after Phil Stewart steps down to focus on research and teaching.

Phillips, Cunningham, Gerlach, Hiebert, Hwang, Spangler, and co-authors publish "Fracture sealing with micobially-induced calcium carbonate precipitation: a field study" in *Environmental Science & Technology*.

CBE Bioprocessing Lab marks significant milestone toward commercialization of fracture-sealing process for oil and gas wells using biofilm-forming bacteria and the process of MICP.

2017

Garth James participated in an international expert panel that called for physicians to abandon the longstanding practice of gradually escalating treatment of patients with chronic wounds for aggressive treatment as soon as possible.

2018

CBE wins $7.8 million in new research grants.

US EPA adopts standard testing methods for biocide efficacy developed at CBE.

Parker, Hamilton, and Goeres publish "Reproducibility of antimicrobial test methods" in *Scientific Reports*.

2019

Montana University System names Phil Stewart a Regents Professor.

Stewart and Parker publish "Measuring antimicrobial efficacy against biofilms: a meta-analysis" in *Antimicrobial Agents & Chemotherapy*.

CBE Industrial Associate STERIS earns an anti-biofilm claim from the US EPA using the standard method dated 2017, which was developed at CBE.

Ad hoc Biofilm "Bash" brings together international biofilm researchers in Washington state for informal meeting to strengthen interactions and collaborations.

NASA solicits ideas from CBE to solve biofilm issues associated to longer-term space travel in a joint workshop in Bozeman.

2020

CBE is featured in a ~700-page history of the research centers funded through the NSF-ERC program, written by Lynn Preston and Courtland Lewis.

CBE researchers work to develop viral efficacy testing for SARS CoV-2 as well as a saliva-based detection assay.

CBE researchers are coordinating with international biofilm centers and groups to initiate an International Standards Task Group

The risk of COVID-19 forces CBE to host its Montana Biofilm Meeting online for the first time.

CBE works with biofilm centers and research groups from around the world to identify growth areas for biofilm research.

CBE's 30th Anniversary

Q&A

CBE founder Bill Characklis recruited him, now Phil Stewart is

The Vanguard of Biofilm Research

Phil Stewart's lab circa 1994.

By Skip Anderson

Shortly after Bill Characklis founded the CBE in 1990, he recruited Phil Stewart from Stanford University to join the team. Stewart, who was named Regents Professor of Chemical and Biological Engineering in 2019, is now one of the most prolific and well-known researchers in the field of biofilms. Stewart's work has been cited more than 47,000 times. Stewart served as CBE Director from 2004-2015. He answered questions about his career, the field, and areas of opportunity that excite him today.

There is huge potential for such immunotherapeutic approaches, and also opportunities for innovative and integrative science along the way.

You joined the CBE within a year of its founding. What interested you in the nascent field of biofilm research?

As a graduate student at Stanford, I worked in a lab that packed bacteria and yeast into reactors to make high-value microbial products. Looking back, those were examples of engineered biofilms, though we didn't use that term at the time. The engineering science behind those reactors is exactly the same as the science underpinning the diverse biofilms we study at CBE, so the transition was a very natural one for me. And it was exciting to have my eyes opened to a much larger universe of applications that spanned from dental plaque to oilfield pipelines and so many other areas.

Was there an "aha" moment that fueled your interest in biofilm?

In my first year at CBE, I was given the opportunity to lead the biofilm-control research area. We asked the question: Why are biofilms so hard to kill with antimicrobial agents? This was an inspiring problem because it had not been tackled from an engineering perspective before, and it definitely required an integration of physics, chemistry, and biology. This challenge quickly became the central focus of my academic career. I also found the real-world relevance of the infection and fouling problems caused by biofilm motivating.

Looking back on the past 31 years since the CBE was founded, how has awareness of biofilm changed?

The term "biofilm" first appeared in technical journals related to wastewater treatment in the mid-1970s. Biological processes for treating dirty water remain the largest engineered use of biofilms. During the 1980s, biofilm terminology was picked up by other research specializations. The CBE's arrival in 1990 came on the cusp of a full expansion of biofilm concepts into a remarkable diversity of disciplines and fields. This timing, and CBE's deliberate investments in education and communication, surely helped create and unify the biofilm field and of course raised this center to prominence.

Do we understand the processes and mechanisms of a biofilm better than we did back then?

For sure. One giant transformation in the field has followed the revolution in molecular biology, which has allowed researchers to give names to the molecules and genes that matter in a biological system, for example, by sequencing DNA or analyzing metabolites. When I first started, this detail was mostly invisible to us. Now we can read the genetic and biochemical intricacies and work out mechanisms about how a microbe sticks to a surface, builds a multicellular structure, and interacts with other species.

Where does your most-cited paper come into play?

The 1999 *Science* article really expanded the reach of biofilm into medicine. It connected many seemingly disparate chronic infections as having a likely biofilm etiology. There are strong parallels between the infections on a urinary catheter, a pacemaker, and a knee joint. Recognizing this commonality took the biofilm

continued on next page

Q&A

Left to right: Phil Stewart, Jeff Anderl, and Karen Xu, circa 1994.

infection beyond being a niche concept to having relevance across virtually every anatomical site and medical specialty.

Why is it important that scientific inquiry into biofilm continues?

For all that we have learned about biofilms, we still have not realized the full promise of alternative ways to solve problems caused by biofilms or better engineer beneficial biofilms. Take dental plaque as an example. If you ask me what you should do to manage biofilm on your teeth, I would counsel you to brush your teeth. Not fresh advice, but it works. I envision a day when there will be a mouthwash that, rather than killing bacteria, works by weakening the extracellular matrix of dental biofilm making it easier to remove with a brush. Or a mouthrinse that contains a probiotic bacterium that helps keep pathogenic organisms, such as those associated with periodontitis, from taking hold in the biofilm. In this example, the strategy is to shift the ecology of the system from detrimental to pro-health. There are so many new ways that we can imagine, based on the basic science, to come at these problems.

Over the past decade, biofilm research has truly boomed. The number of biofilm-related papers published annually has more than doubled from 3,000 to 6,000, and research centers dedicated to studying biofilm have sprouted up globally. Why do you think this is?

It is surely because biofilms matter; they are not just an academic curiosity. Biofilms are integral to so many real-world systems from every aquatic natural environment, to the numerous industrial processes that use water, to dentistry and medicine.

What are some of the big questions regarding biofilm you'd like to see answered?

Well, let me tell you about the one that I am personally working on these days: Can we learn to prevent biofilm infections by engaging constructively with our body's own immune system? So far clinicians have fallen back on surgeries and antibiotics to deal with infections on implanted medical devices. Surgery is traumatic and expensive, and antibiotics often don't do the job. What if we could jump start the immune system to clear contaminating bacteria from a biomaterial before a biofilm can get started? There is huge potential for such immunotherapeutic approaches, and also opportunities for innovative and integrative science along the way. I am super-excited about this direction.

> It is surely because biofilms matter; they are not just an academic curiosity

Could you speculate as to what might surprise Bill Characklis about the CBE and the field of biofilm research some 30 years later?

I am guessing he would be pleasantly surprised that the biofilm field has grown so much, and that the center he started remains an internationally recognized leader three decades later. In the faddish world of academia, such endurance is remarkable. ■

EDUCATION

30th

EDUCATION

MORE THAN 1,100 STUDENTS HAVE RECEIVED HANDS-ON TRAINING IN RESEARCH AND PRESENTATIONS AT THE CBE

Q&A

Madelyn Mettler stays at CBE for PhD to work on

Biofilms in Space

By Skip Anderson

Madelyn Mettler joined the CBE in 2016 as an undergraduate researcher. A native of Littleton, Colorado, Mettler initially worked in the Standardized Biofilm Methods Lab, headed by Darla Goeres. There she learned the critical importance of meticulous recordkeeping when planning and conducting experiments. For her senior year, she joined Brent Peyton's lab where she worked exclusively on a NASA project to address a biofilm problem that has presented itself within the water-recycling system on the International Space Station. "If you can't have fresh water in space, you can't have astronauts in space," she says. Mettler graduated MSU in May 2020 with a degree in biological engineering. The COVID-19 pandemic cost her a post-graduation internship at NASA's Jet Propulsion Laboratory in Pasadena, California. But she's not through with NASA. Mettler stayed on at MSU to pursue a PhD in chemical engineering where she's continuing her work in the Peyton Lab on NASA's biofilm project, which if it remains unsolved, would likely make manned missions to Mars an impossibility.

Madelyn Mettler joined the CBE as an undergraduate researcher. CBE photo by Skip Anderson

Why did you decide to become an undergraduate researcher at the CBE?

When I visited MSU in high school, I walked around the halls of the CBE and I saw all the posters from the Montana Biofilm Meeting and all that research on biofilm, which I had never even heard of at the time. It stayed in my mind that the CBE would be an interesting place to work. So, when I got to campus, I had heard you could go to the front office and ask if any of the labs were hiring, and Standardized Biofilm Methods Lab had an opening. I sent in my resume and cover letter that same day. I got an interview the next day.

I think back to all of the individual projects I've worked on over the years. And when I do that, I know I am where I am because I deserve to be here.

What did you learn from working with Darla Goeres in the SBML?

I learned the importance of being precise and consistent with your work, and writing everything down as you do it so you can replicate it. Standard methods, obviously, are critical in a lab. And that hit home with me.

You were in Brent Peyton's lab for your senior year. What types of projects did you work on as an undergrad?

I was there for one year, and the whole project was working with NASA's Jet Propulsion Laboratory. We were trying to figure out what type of coating we could use on surfaces to reduce biofilm growth on the International Space Station. They're particularly worried about the water system. Biofilms can clog the plumbing and stop the recycling system from working. And if you can't have fresh water in space, you can't have astronauts in space. As a girl, I wanted to be an astronaut. And working to solve a biofilm problem on the International Space Station is as close as I'm going to get to that. It can be really intimidating to be in meetings with the head honchos of the ISS. It can be paralyzing if you let it.

How do you overcome that?

You have to be confident in your training. I think back to all of the individual projects I've worked on over the years. And when I do that, I know I am where I am because I deserve to be here.

The COVID-19 pandemic sent a wrecking ball through your internship plans. What were you going to be doing?

I was going to NASA in Pasadena at the JPL continuing my research in the water systems on the ISS. That may still end up happening this summer or the next, depending upon how COVID looks.

How did you decide to stay at Montana State for your PhD?

I already had this great relationship with Brent and everybody in the lab, and I still had all these questions about biofilms in space. So, it made sense for me to stay here.

Do you think you'll stay in academia after you graduate?

Initially, I had written off academia. But after working as a TA last semester, I'm more open to that now.

Education

CBE undergraduate researcher documents

Microplastic in Precipitation

By Marshall Swearingen

A mountain meadow glittering with fresh snow may seem a picture of purity. But, for Bekah Anderson, an MSU senior majoring in chemical engineering and an undergraduate researcher at the CBE, the picture is more complicated.

Working with dozens of snow samples taken over the past year from Big Sky Resort, Teton Pass and other Rocky Mountain sites, Anderson uses microscopes and other specialized laboratory tools in the CBE to peer into the world of tiny particles that mix with airborne water that then falls from the sky as precipitation.

"There's all sorts of stuff in there," including plant pollen and dust, Anderson says, but of greatest interest to her are fibers of polyester and other pieces of microplastic.

"All the pieces I've found so far have been small fibers that seem to be from fabrics like fleece," Anderson says, noting that many kinds of outdoor clothing are made of finely spun plastic fibers. "We think that's because they're fine enough to get whisked up into the atmosphere."

Previously, scientists have documented the presence of microplastic in streams and other water bodies, but the MSU study is among the first to examine the manmade particles directly in precipitation, according to the project's leader, CBE-affiliated faculty member Christine Foreman, an associate professor in the Department of Chemical and Biological Engineering in MSU's Norm Asbjornson College of Engineering.

"It's been exciting, but also saddening, to find so much microplastic in snow," Foreman says.

The team's preliminary results are significant because they suggest the synthetic fibers are prevalent throughout the water cycle and not just in certain waterways.

Microplastics are "an emerging concern," Foreman says. Not much is known about how they affect ecosystems, but it's reasonable to suspect the petrochemical particles are, for example, being consumed by some aquatic organisms, she says. Scientists have warned that microplastics have a number of impacts on insects and fish, including clogging up digestion and disrupting hormones that regulate body functions.

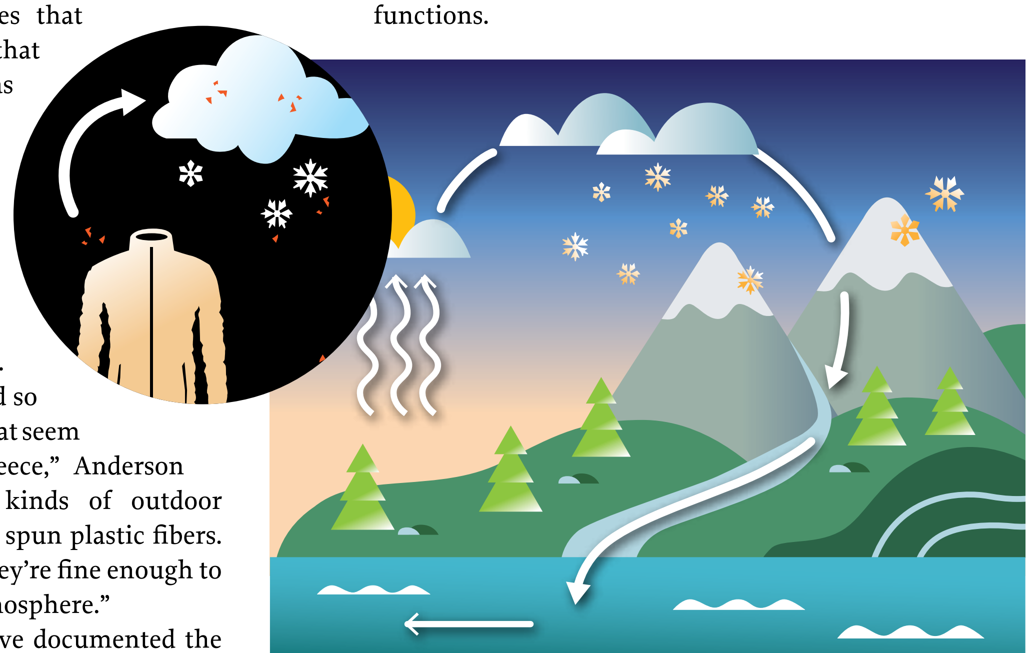


Illustration by Jill Story

In the lab, Anderson passes each sample of melted snow, as well as rain, through a fine filter that collects any particles. Then she applies a dye that binds to plastic. A specific type of light applied under the microscope causes the dye to fluoresce, making any microplastic stand out. Using another technique called Raman spectroscopy, which measures how light interacts with a material's molecular composition, Anderson can determine what kind of plastic each particle is made of.

"I'm passionate about it," says Anderson, who is from Golden, Colorado. "It's important for us to understand the consequences of our plastic use."

> "All the pieces I've found so far have been small fibers that seem to be from fabrics like fleece," Anderson says, noting that many kinds of outdoor clothing are made of finely spun plastic fibers. "We think that's because they're fine enough to get whisked up into the atmosphere."

When she presented early results from the project in 2020 at the Western Regional Honors Conference, which brought top students from across the western US to MSU to present their research, Anderson won the award for the best poster presentation.

"She's incredibly motivated," Foreman says. "You can tell she's really excited about this project, and she brings that excitement to the rest of our research group," which focuses on studying microbes in glacial environments.

Anderson, whose work in Foreman's lab is funded by MSU's Undergraduate Scholars Program, also presented at the 2019 National Conference on Undergraduate Research in Kennesaw, Georgia.

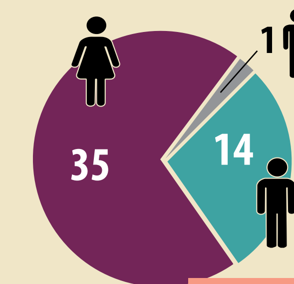
"I loved being around other students who were so passionate about their projects," Anderson says. "Research has a strong community." ■

EDUCATION

1,173 STUDENTS TRAINED SINCE 1990

BY THE NUMBERS fy2020

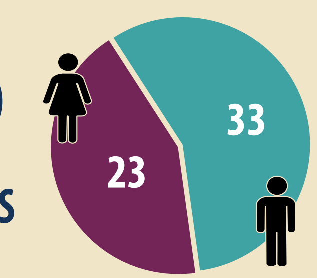
46 INTERDISCIPLINARY FACULTY



50 UNDERGRADUATE STUDENTS

34 international undergraduate students in the past five years.

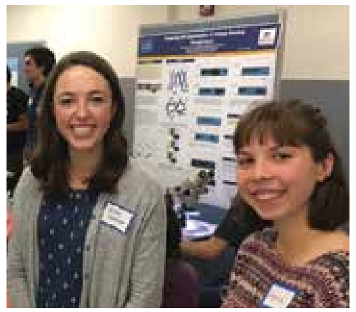
56 GRADUATE STUDENTS



10 DEPARTMENTS

Cell Biology & Neuroscience
Chemical & Biological Engineering
Chemistry & Biochemistry
Civil Engineering
Directed Interdisciplinary Studies
Ecology
Mathematical Sciences
Mechanical & Industrial Engineering
Microbiology & Immunology
Plant Sciences & Plant Pathology

MSU 97th Annual Day of Student Recognition

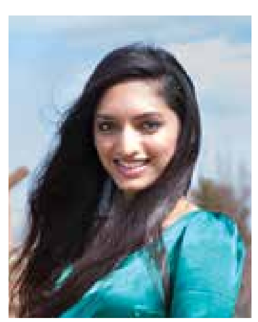


Undergraduate students Ellie Jackson (left) and Petria Russell participated in MSU's Crossroads of Discovery research event in 2019.

Ellie Jackson, a CBE undergraduate student in cell biology and neuroscience, was among the students honored in MSU's 97th Annual Day of Student Recognition. The event recognized student achievement in leadership, involvement in various campus-wide activities, and community service. Jackson received the Outstanding Sophomore Leadership Award, which recognizes a sophomore who demonstrates notable leadership skills and values. Jackson, a Bozeman native, is active in several service organizations in the community and on campus. She holds leadership roles in the HEART Initiative and the Health Professions Club. She also serves as a Child Advancement Project mentor, has volunteered at Bozeman Health, and has engaged in service activities with Alpha Omicron Pi and Breaks Away trips. Her CBE advisor is CBE-affiliated faculty member Connie Chang, an assistant professor in chemical and biological engineering. Due to the coronavirus pandemic, an in-person ceremony for this event was not possible but a video honoring the awardees is available on the MSU Office of Student Engagement Facebook page and on its website.

CBE PhD Alumna Earns Prestigious Fellowship at Pacific Northwest National Laboratory

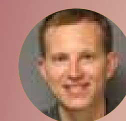
By Skip Anderson



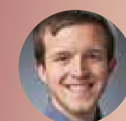
Neerja Zambare, a former CBE student in MSU's Department of Chemical and Biological Engineering who successfully defended her PhD dissertation in April, has accepted the distinguished Linus Pauling Fellowship at the Pacific Northwest National Laboratory. "I knew [the fellowship] was highly competitive, and if I got it, I would get to work with some of the leading microscopes and experts in the world, which would be a fantastic way to start a career in microscopy and microanalysis," she says. Zambare's dissertation was titled "Microbially induced calcium carbonate precipitation: Meso-scale optimization and micro-scale characterization." While at the CBE, Zambare worked in Robin Gerlach's lab where her research exposed her to advanced microscopy techniques and microanalysis, which she says will play an integral part in her career.

CBE Awards

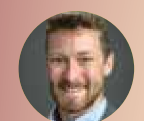
CBE STUDENT AWARDS



TOM LEFEVRE
W.G. Characklis Award
Center for Biofilm Engineering
Montana State University

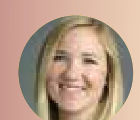


ISAAK THORNTON
W.G. Characklis Award
Center for Biofilm Engineering
Montana State University

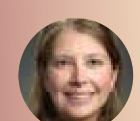


BRIAN PETTYGROVE
John Neuman Lab Citizen Award
Center for Biofilm Engineering
Montana State University

FACULTY AWARDS

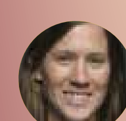


HEIDI SMITH
Outstanding CBE Faculty Award
Center for Biofilm Engineering
Montana State University



KRISTEN BRILEYA
Outstanding CBE Researcher Award
Center for Biofilm Engineering
Montana State University

ADRIANNE PHILLIPS
Excellence in Research Award
Norm Asbjornson College of Engineering, Montana State University



ELLEN LAUCHNOR
Excellence in Outreach
Norm Asbjornson College of Engineering, Montana State University

OUTREACH

30th OUTREACH

CBE THRIVES ON COLLABORATIONS WITH RESEARCHERS AND INDUSTRY REPRESENTATIVES WORLDWIDE

CBE builds on collaboration with University of Stuttgart

By Skip Anderson

CBE-affiliated faculty member Al Cunningham, professor emeritus in the Department of Civil Engineering, was recently awarded a Mercator Fellowship from SFB 1313 at the University of Stuttgart. Cunningham is a founding member of the CBE and serves on the CBE Executive Committee, which is responsible for coordinating center research, education, and industry projects.

Al Cunningham, CBE professor emeritus of civil engineering, received the Mercator Fellowship from his collaborators at the University of Stuttgart in 2019.

SFB 1313 is an interdisciplinary Collaborative Research Centre at Stuttgart, consisting of four major project areas—free flow and porous-media flow, fracture propagation and fluid flow, fluid-solid phase change and benchmarks, computing, and visualization. The center is funded by the by the German Research Foundation (DFG). The SFB 1313 team consists of 48 researchers from 18 different institutes at Stuttgart and partner universities.

Mercator Fellowships enable an intensive, long-term project-based collaboration between researchers from both domestic and foreign institutions. Cunningham, along with CBE-affiliated faculty members Robin Gerlach in MSU's Chemical and Biological Engineering Department and Adie Phillips in MSU's Civil Engineering Department, have been instrumental in the long standing collaboration between the work groups of Holger Class and Rainer Helmig at Stuttgart and the center. The intensive scientific exchange has resulted in a number of high-ranked joint publications.

Cunningham's fellowship focuses on porosity-permeability relations and experiments will partly be conducted at the CBE. The team will investigate dynamic changes in porosity and associated changes in pore-water-velocity distributions with MRI and XRCT in order to determine permeability changes. Experiments on salt precipitation during evaporation and microbially-induced calcite precipitation in porous glass-bead systems will be carried out using microfluidic cells.

Picture This

FY2020

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- Naval Information Warfare Center
- US Army Research Office
- Olympus
- Idaho National Laboratory
- US Environmental Protection Agency

VISITS TO INDUSTRY AND GOVERNMENT

Paul Sturman & Heidi Smith
Sherwin Williams

Paul Sturman
L'Oreal

Matthew Fields
Argonne National Lab

VISITING RESEARCHERS

Hakan Armagan
Visiting High School Teacher
Burke High School,
Omaha, NE
CBE Host: Adie Phillips

Ondřej Chlumský
Visiting PhD Student
University of Chemistry & Technology, Prague, Czech Republic CBE Host: Jim Wilking

Mathieu Devos
Visiting Masters Student
University of Antwerp
Belgium
CBE Host: Darla Goeres

Nicolas Forquet
Visiting Faculty
National Institute for Environmental and Agricultural Science and Research
Lyon, France
CBE Hosts: Otto Stein & Ellen Lauchnor

Emily Gan
Visiting High School Student
Jericho School District
Jericho, NY
CBE Host: Robin Gerlach

Rachel Kleiman
Visiting Masters Student
University of North Carolina at Chapel Hill
Chapel Hill, NC
CBE Host: Robin Gerlach

Kendra Lunday
Visiting High School Teacher
Capital High School
Helena, MT
CBE Host: Adie Phillips

Scott Wade
Visiting Faculty
Swinburne University of Technology
Victoria, Australia
CBE Host: Matthew Fields

NBIC's interdisciplinary workshop addresses biocorrosion

By Kristen Griffin

CBE and the National Biofilm Innovation Centre hosted the Microbe-Metal Interactions Workshop held in conjunction with the 25th Reservoir Microbiology Forum in London in November 2019. The workshop brought together key academic expertise and thought leaders in biofilms and contamination of metal surfaces and biocorrosion processes across the physical sciences and engineering in order to identify the key knowledge gaps and research challenges for future projects and research collaboration. The goal of the workshop was to develop a shared understanding of current state of knowledge and the unmet needs of interactions between complex microbial communities and metals, with a focus on the challenge of microbially-influenced corrosion (MIC), and to identify projects and actionable ways forward. MSU and CBE were represented by faculty members Roberta Amendola (mechanical engineering), Matthew Fields (microbiology and immunology), Robin Gerlach (chemical and biological engineering), and Recep Avci, director of MSU's Imaging and Chemical Analysis Laboratory. As a result of the workshop, several working groups have formed to explore the development of standard methods for MIC and biocide testing.

Biofilm Bash: researchers publish paper on 'the future of biofilm research'

Twenty-nine of the world's top biofilm researchers convened in 2019 to identify areas for future research. Photo submitted

In 2019, 29 of the world's leading biofilm researchers met in Leavenworth, Washington, to identify the most fertile areas for future research. Invited researchers from Australia, Belgium, Denmark, the Netherlands, Portugal, the United Kingdom, and the United States were present, including Matthew Fields, Darla Goeres, and Phil Stewart from the Center for Biofilm Engineering. A full account of the proceedings, including attendee list, is available at Science Direct, courtesy of the open-source research journal *Biofilm*.

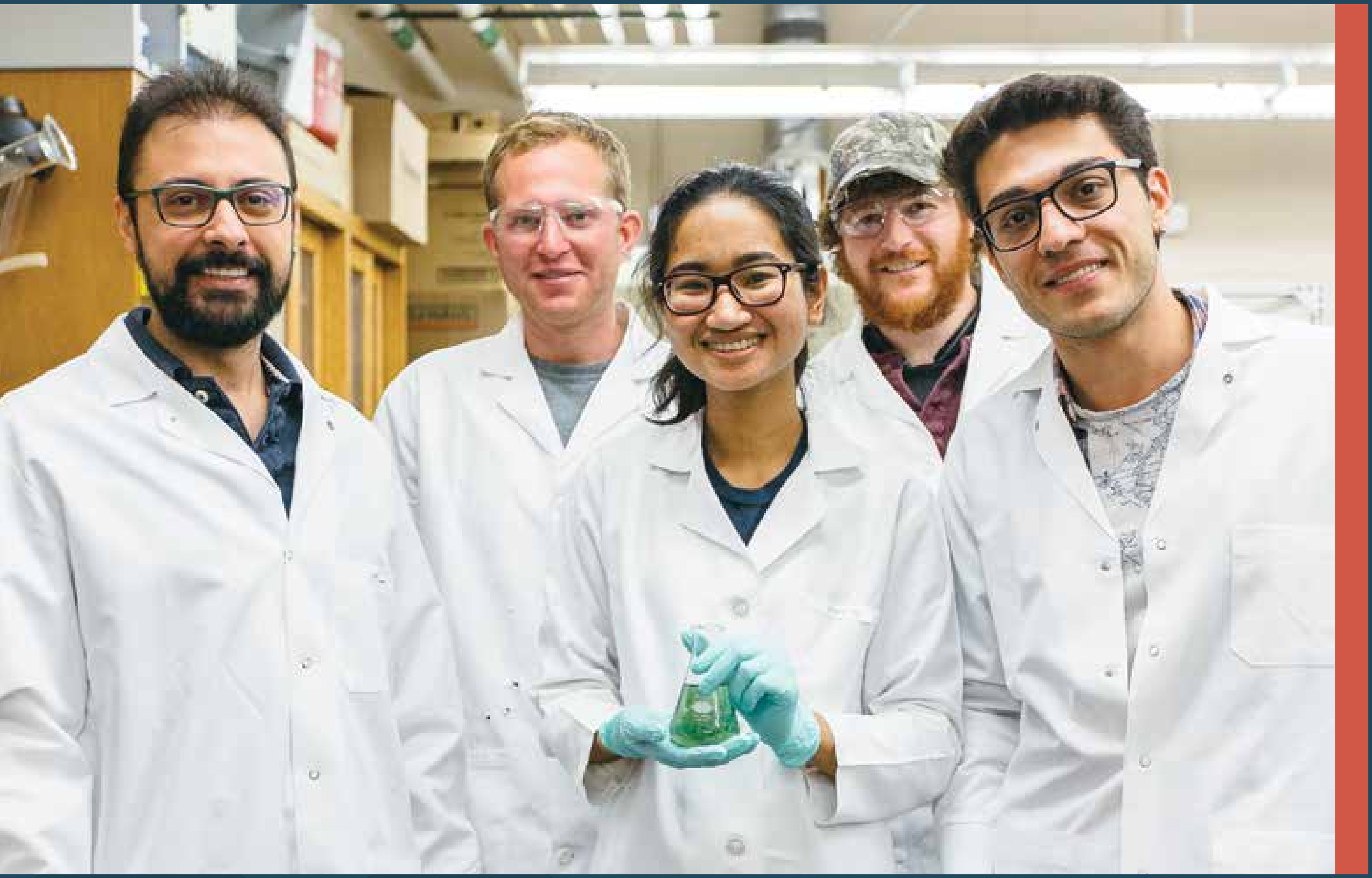
CBE produces biofilm primer video

During the 2019 Montana Biofilm Meeting, the CBE premiered a 6-minute video designed to put into context the myriad ways biofilms shape our world. From primordial ooze to primary culprit, interviews with six CBE researchers explain the many ways biofilms wreak costly – and sometimes deadly – havoc in industrial, residential, infrastructure and healthcare settings. The video is available on the CBE's YouTube channel.

IN MEMORIAM

CBE alumnus Robert Gardner passed away Oct. 24, 2019. A graduate student of Brent Peyton, Gardner graduated from MSU in 2012. Gardner, who was instrumental in establishing algae research at Montana State University, later became an assistant professor at the University of Minnesota. Gardner received CBE's Characklis Award in 2012.

Lynn Preston, who led the Engineering Research Center program for the National Science Foundation, died Oct. 26, 2020. Preston's enthusiasm for the CBE, in part, helped sway the NSF not to withdraw funding after the death of CBE founder Bill Characklis in 1992. She received numerous prestigious awards for her 42 years at the NSF, including President Bill Clinton conferring upon her the rank of Meritorious Executive in the Senior Executive Service.



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