

Center for
Biofilm Engineering
25 years & growing



MONTANA
STATE UNIVERSITY

College of
ENGINEERING

Fundamental & Applied RESEARCH

25-YEAR RESEARCH RECAP



Over the years CBE research teams have been part of numerous groundbreaking advances in biofilm science and technology that have propelled and transformed the field. These seminal contributions include those related to quorum sensing, porous media permeability, mineral precipitation, antimicrobial tolerance, viscoelasticity, detachment, standardized biofilm methods, chronic wounds, algal biofuels, corrosion, souring, and biofilm structure and function.

Since 1990 CBE has published 1,024 papers including articles in such prestigious journals as *Nature*, *Lancet*, *Science*, *JAMA*, *PNAS*, *Journal of Clinical Investigation*, *Developmental Cell*, *EMBO Journal*, *ISME Journal*, and *Physical Review Letters*. Collectively, these papers have been cited more than 40,000 times—exceeding the biofilm-specific portfolio of any other institution, anywhere.

How has CBE attained this impressive record? Part of the answer lies in broad participation of faculty from 9 departments across the Montana State University campus who have combined their expertise to solve biofilm problems.

The breadth of expertise has helped CBE continue to compete successfully for research dollars. CBE principal investigators frequently win funding from NIH, NSF, DOE, DOD and EPA, as well as private foundations and industrial funding for specific projects and testing.

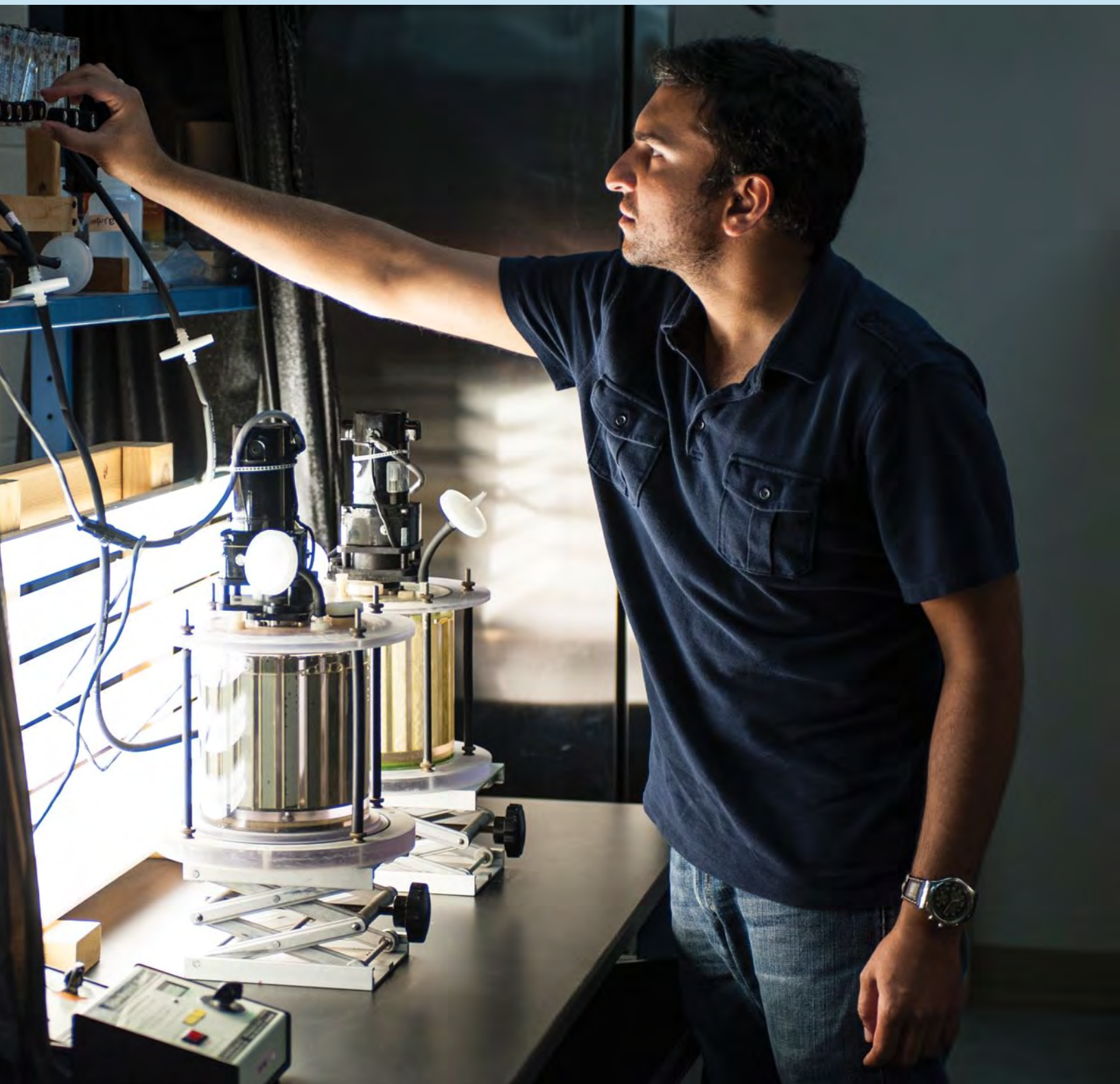
CBE has also influenced the future of biofilm research through students and post-docs who have gone on to faculty positions elsewhere and continue to do biofilm work. Our alumni can be found in universities and research institutes from New York to Bremen and from Colorado to Sapporo.

CBE research has been broadly disseminated and its impact has been heightened through video microscopy as well as striking graphic art and microscope images that have been reproduced on 17 journal covers and in many textbooks, science encyclopedias and magazines.

With new tools and analytical techniques at our disposal, we look forward to exciting advances in biofilm science to come.

In 2015 the Center for Biofilm Engineering celebrates the 25th anniversary of its inception as a National Science Foundation Engineering Research Center (NSF ERC). We are proud that CBE continues to successfully integrate all three of its original NSF missions: cutting-edge interdisciplinary research, innovative education, and effective technology transfer. This special edition of our annual report combines highlights of recent activity with retrospectives on CBE's impact since our founding.

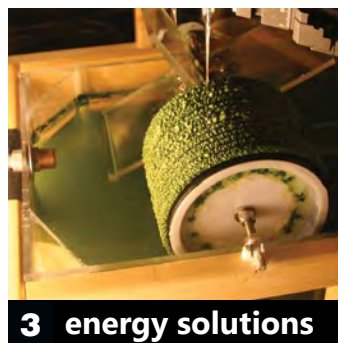
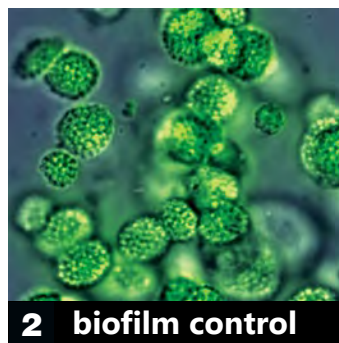
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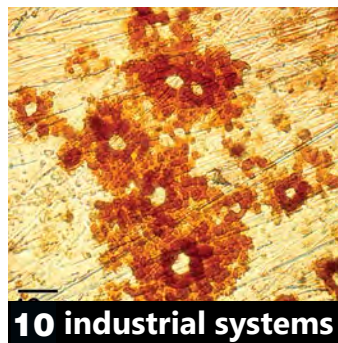
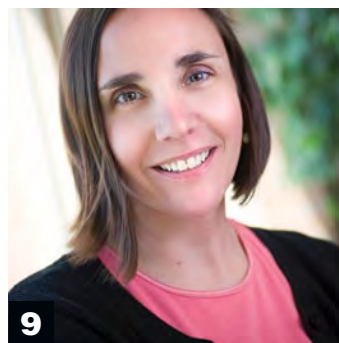
Muneeb Rathore, a PhD student in chemical and biological engineering attending MSU on a Fulbright scholarship, studied algal biofuels under the guidance of CBE affiliated professor Brent Peyton. Photo by K Gorham, MSU News.

2014 | Research Snapshots: CBE faculty, research areas, and sample publications

1. In late 2013 **Anne Camper** became the first woman and first engineer at MSU to be **appointed Regents Professor**—the Montana University System’s top honor for faculty of distinction. In early 2014 Camper was also inducted as a Fellow of the National Academy of Inventors (photo, D Brown). **2.** *Staphylococcus aureus* tagged with green fluorescent protein embedded in agarose gel beads (B Pitts & B Pabst). **Recent publication:** Stewart PS. Biophysics of **biofilm infection**. *Pathog Dis.* 2014;70:212–218. **3.** A **microsensor** is used to measure oxygen levels in a lab-scale rotating algal biofilm reactor at CBE (H Bernstein). **Recent publication:** Bernstein HC et al. Direct measurement and characterization of active photosynthesis zones inside **wastewater remediating** and **biofuel** producing microalgal biofilms. *Bioresour Technol.* 2014 Mar;156:206–215. **4.** Microbially induced calcite precipitation sealing a fracture in a sandstone core sample (A Phillips). **Recent publication:** Phillips AJ et al. Engineered applications of **ureolytic biomineralization**: A review. *Biofouling.* 2013;29(6):715–733. **5.** SEM of wound tissue, with activated white blood cell (yellow) and bacterial cells (green) (ES Lauchnor). **Recent publication:** Zhao G et al. Biofilms and **inflammation in chronic wounds**. *Adv Wound Care* (New Rochelle). 2013 Sep;2(7):389–399. **6.** Reverse osmosis membrane cut open to reveal microbial biofouling (C Wend). **Recent publication:** Khan M et al. Enzymatic cleaning of biofouled thin-film composite **reverse osmosis (RO) membrane** operated in a biofilm membrane reactor. *Biofouling.* 2014;30(2):153–167. **7.** Confocal microscopy of dual-species anaerobic biofilm for research on **subsurface bioremediation** (K Brileya). **Recent publication:** DeLeón KB et al. **Archaeal and bacterial communities** in three alkaline hot springs in Heart Lake Geysir Basin, Yellowstone National Park. *Front Microbiol.* 2013 Nov 12;4:330. **8.** Disk removal from a rotating annular reactor. **Recent publication:** Hamilton MA et al. Guidelines for the statistical analysis of a collaborative study of a laboratory method for testing **disinfectant product performance**. *J AOAC Int.* 2013;96(5):1138–1151. **9.** **Fulbright Scholar Darla Goeres** collaborated on international studies of standardized methods at Åbo Akademi University in Finland (photo, K Gorham). **10.** **Pitting corrosion** of 316S stainless steel (W Dickinson & Z Lewandowski). **Recent publication:** Donovan C et al. Sediment **microbial fuel cell** powering a submersible ultrasonic receiver: New approach to remote monitoring. *J Power Sources.* 2013 Jul;233:79–85.

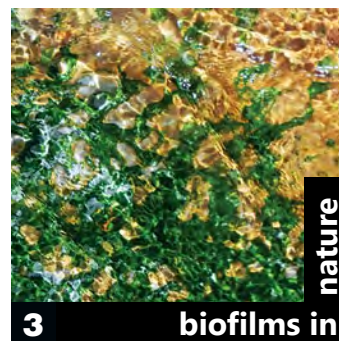
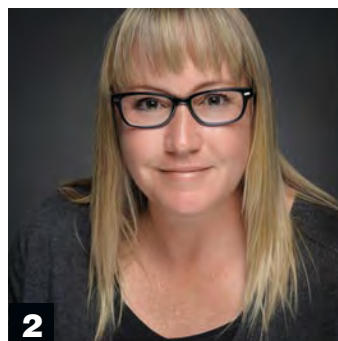


36 affiliated faculty from 10 MSU departments participate in biofilm research.

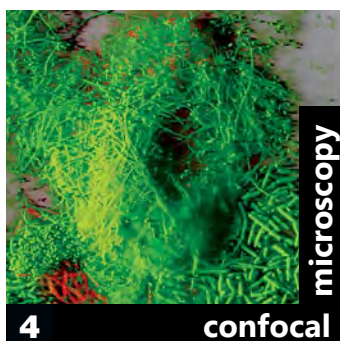


73 projects were sponsored by 48 companies, for a total of \$1.3 million.

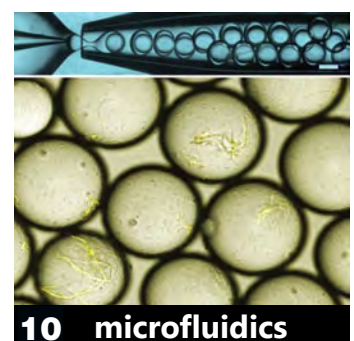
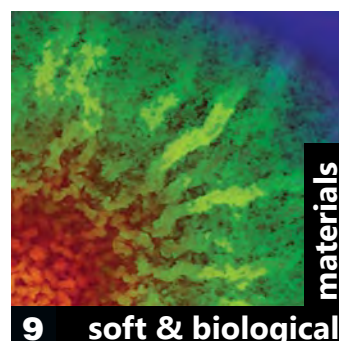
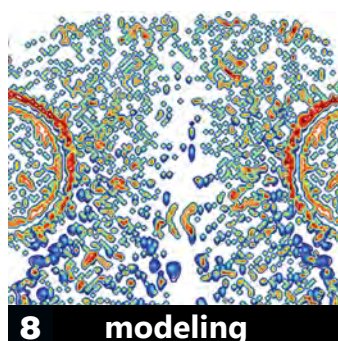
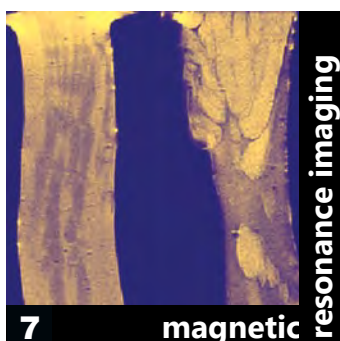
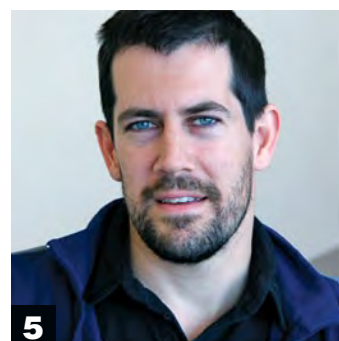
1 and 2. Two CBE biofilm experts, **Ellen Lauchnor** and **Adie Phillips**, joined the Department of Civil Engineering as assistant professors in 2014. Lauchnor's research areas are **bioremediation**, nitrification, and wastewater biofilms. Phillips' research interests include **biofilms in energy** and bioremediation-related technologies (K Gorham). **3. Thermophiles** in a Yellowstone National Park stream. CBE conducts research in both extreme **hot and cold** environments. **Recent publication:** D'Andrilli J et al. Characterization of IHSS Pony Lake fulvic acid dissolved organic matter by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry and fluorescence spectroscopy. *Org Geochem*. 2013;65:19–28. **4.** Confocal microscopy of a microbial consortium (L Lorenz). **Recent publication:** Briley KA et al. **3D-fluorescence in situ hybridization** of intact, anaerobic biofilm (book chapter). *Methods Mol Biol*. 2014;1151:189–197. **5 and 6.** CBE welcomed **Jim Wilking**, assistant professor, and **Connie Chang**, assistant research professor, who joined MSU's Department of Chemical and Biological Engineering from postdoctoral positions in the David Weitz laboratory at the School of Engineering and Applied Sciences, Harvard University (K Griffin). Wilking's areas of interest include **physical and material properties** of microbial biofilms, mechanics of soft materials, and colloidal and nanomaterials synthesis. Chang's area of interest is droplet-based **microfluidics** for use in such applications as **biomaterials**, ultra high-throughput screening of bacterial biofilm formation, single cell genomics, and directed evolution in biology. **7.** MRI imaging of microbial alginate gels (H Fabich). **Recent publication:** Yang X et al. Direct numerical simulation of pore-scale flow in a bead pack: Comparison with **magnetic resonance imaging** observations. *Adv Water Resour*. 2013;54:228–241. **8.** Single frame from an animated **mathematical model** of biofilm mechanics (T Zhang). **Recent publication:** Zhang T et al. General theory for integrated analysis of growth, gene, and **protein expression in biofilms**. *PLOS ONE*. 2013;8:12.e83626. **9.** Microscopic image of *Bacillus subtilis* biofilm with motile cells (red), sporulating cells (green), and matrix producers (blue) (J Wilking). **Recent publication:** Wilking JN et al. Liquid **transport facilitated by channels** in *Bacillus subtilis* biofilms. *PNAS*. 2013 Jan 15;110(3):848–852. **10.** Microfluidics droplet formation (top) and cell culturing (bottom) (C Chang). **Recent publication:** Chang CB et al. Monodisperse emulsion drop **microenvironments for bacterial biofilm growth**. *Small*, in revision.



CBE authors published 50 papers in 39 different journals.



New grants in FY 2014 totaled \$4.6 million.



CBE's biofilm infection message resonates with clinicians and industry

For healthcare companies, the interplay of industry and academic science showcased by Montana State University's Center for Biofilm Engineering is a critical ingredient in the development of new medical technologies, particularly in one area of human health: biofilm-related infections.

CBE's twice-yearly meetings bring members from CBE's Industrial Associates program—currently numbering 33 companies—together with dozens of MSU faculty and students to discuss the latest science regarding biofilms.

The summer 2014 meeting featured talks by several distinguished physician/researchers from across the USA. **Thomas Mustoe, MD**, the former chief of plastic surgery at Northwestern University Hospital in Chicago and a leader in medical biofilm research, was one of the featured speakers. Mustoe, who has spent years exploring *in vivo* models for testing the fundamentals and response to drugs of biofilms in chronic infections, said his interest in biofilms can be traced to a talk given by Phil Stewart, CBE director and MSU professor of chemical and biological engineering.

"That talk really triggered a light bulb in my head—I understood for the first time that bacteria didn't just exist in wounds" in a free-floating form, but were configured in complex biofilms, Mustoe said. "I think the group at Montana State has shown they are pioneers in the field and these meetings put that leadership on display. . . and there's a huge value to the community by holding these meetings. That's how you advance research."

The three-day meeting also featured: **Pradeep Singh, MD**, professor of medicine and microbiology at the University of Washington, who presented work he has done on the bacteria *Pseudomonas aeruginosa* in relation to lung infections; **Robin Patel, MD**, professor of medicine and microbiology based at the Mayo Clinic in Rochester, Minnesota, who discussed her findings on the challenges that biofilm-related infections present for patients receiving prosthetic joint replacements; and **Randall Wolcott, MD**, a wound specialist with a private practice in Lubbock, Texas, who discussed the ways he tailors his treatments by using genetic testing to determine what kind of biofilm is present in a given wound.

"These meetings are a great way for us to connect the dots between what we do here in our labs at the Center for Biofilm Engineering and what is happening in the clinics," Stewart said. "We are able to hear from a speaker like Randy Wolcott, who operates one of the only biofilm-centric wound care clinics in the country, as well as from those who have conducted preliminary research here at MSU on some of the products that he uses to successfully treat chronic wounds."

For his part, Wolcott said he's another member of the medical profession who can trace his focus on biofilms to MSU. This time it was a lecture by Garth James, who is a CBE scientist and MSU research professor of chemical and biological engineering. Wolcott, James and Stewart went on to be among the co-authors of a groundbreaking paper on the nature of biofilms in chronic wounds (see 2008 citation below).

"CBE has been the center of the biofilm universe for more than 20 years," said Wolcott, "and they are the group that gets together every year to try and get out front and push this idea."

That's why Wolcott said it was a very positive sign that the CBE was a co-host with the FDA last winter for a conference dedicated to the subject of biofilms (see page 18).

Stewart said that biofilm approaches taken by the medical establishment and pharmaceutical industry continue to evolve. He added that the cutting-edge nature of the field underscores the importance of partnerships between academic science and industry that are fundamental to CBE.

Adapted from a 2014 story by Sepp Jannotta, MSU News Service



Industry attendees of CBE's summer 2014 meeting followed up with questions after a presentation by CBE's Garth James, Medical Biofilms Project Manager.

CBE medical biofilm publications for further reading:

Zhao G, Usui ML, Lippman SI, James GA, Stewart PS, Fleckman P, Olerud JE. Biofilms and inflammation in chronic wounds. *Adv Wound Care* (New Rochelle). 2013 Sep;2(7):389–399.

Ryder M, James G, deLancey Pulcini E, Parker A. An *in vitro* comparison of intraluminal biofilm bacteria transfer of three peripheral intravenous valved blood control catheters. *Am J Infect Control*. 2013 Jun;41 Supplement: S127–S128.

Secor PR, Jennings LK, James GA, Kirker KR, deLancey Pulcini E, McInerney K, Gerlach R, Livinghouse T, Hilmer JK, Bothner B, Fleckman P, Olerud JE, Stewart PS. Phevalin (aureusimine B) production by *Staphylococcus aureus* biofilm and impacts on human keratinocyte gene expression. *PLOS ONE*. 2012;7(7):1–10.

James GA, Swogger E, Wolcott R, deLancey Pulcini E, Secor P, Sestrich J, Costerton JW, Stewart PS. Biofilms in chronic wounds. *Wound Repair Regen*. 2008;16:37–44.

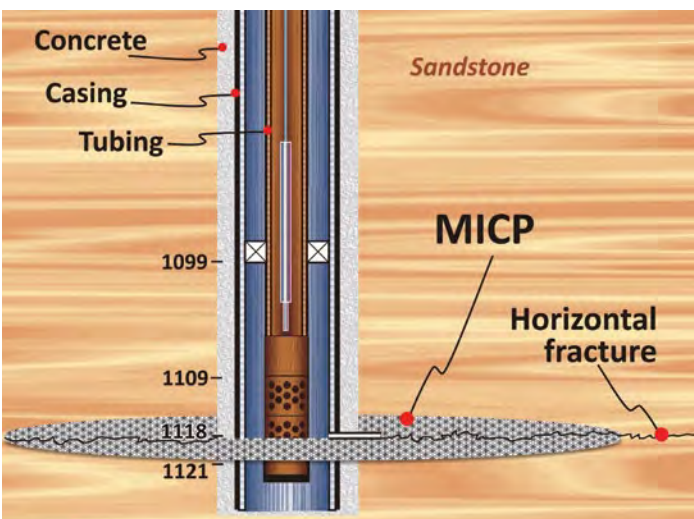
Proven in the field: Microbially induced fracture sealing



A recent successful field-scale experiment marked a significant milestone in CBE's development and control of biofilm processes that mitigate unwanted leakage pathways near wells. The potential for upward leakage of gas and fluids through wells poses a major environmental risk, especially in depleted oil and gas reservoirs where large numbers of abandoned wellbores are often present. When compromised, the near-wellbore environment can become a key leakage pathway for CO₂, methane and fluids to migrate to the surface or into functional aquifers above, leading to potential atmospheric release of greenhouse gases or damage to drinking water aquifers.

In response to concerns about this environmental risk, CBE established a major research program centered on microbial biofilms that enzymatically hydrolyze urea, resulting in the precipitation of crystalline calcium carbonate (calcite)—a process referred to as microbially induced calcite precipitation (MICP). The use of ureolytic, biofilm-forming bacteria (i.e., *Sporosarcina pasteurii*) allows control over the distribution of the catalyst that induces calcite formation. This method has the potential to reduce near-wellbore permeability, coat cement to reduce CO₂-related corrosion, and lower the risk of unwanted migration of CO₂, methane or fluids.

In April 2014 MSU-CBE researchers and their collaborators used MICP to seal a hydraulically fractured Fayette Sandstone formation 341 meters (1,118 feet) below ground surface in the Gorgas #1 well at the Southern Company Gorgas Power Plant in Walker County, Alabama. A combination of *S. pasteurii* cultures and urea, calcium and growth-media solutions was delivered to the fracture region by an injection bailer. The injection of MICP-promoting solutions resulted in fracture plugging and reduced instant shut-in pressure decay, suggesting improved wellbore integrity after MICP treatment. The field test was accomplished in collaboration with MSU Energy Research Institute, Schlumberger Carbon Services, Southern Company Generation, Shell International Exploration and Production B.V. Major funding was provided by the U.S. Department of Energy (Project numbers DE-FE000478 and DE-FE000959).



At the Gorgas #1 well in Walker County, Alabama, (top) a combination of microbial cultures and solutions was injected into a well to demonstrate the use of microbially induced calcite precipitation (MICP) to seal hydraulic fractures. Calcite precipitation induced by biofilm formation resulted in fracture plugging (diagram, bottom).



MSU-CBE team members Adie Phillips, Al Cunningham, and Robin Gerlach pose by the well with Jim Kirksey and Billy Hancock of Schlumberger Carbon Services.



McNair Scholar Emily Bermel, an undergraduate in chemical and biological engineering, studied Antarctic bacteria under the direction of faculty member Christine Foreman. Photo by K Gorham, MSU News.

Interdisciplinary EDUCATION



25-YEAR EDUCATION RECAP



Back in 1990, interdisciplinary science and engineering education for undergraduate and graduate students was little more than a dream. But it was a dream that Montana State University encouraged by providing the newly founded CBE a location in the heart of campus.

25 years later, CBE is fully integrated with the educational mission of the university and has been a tremendously successful leader in interdisciplinary education through research.

Consider the numbers. In its first year of NSF funding, CBE had a total of 31 students—including both graduate and undergraduates. By 2014, the total number of active students had risen to 110.

Since 1990, 143 master's and 89 PhD students have received degrees based on their work at CBE. Center investigators have sustained an excellent track record of incorporating students in their research projects. This can be made concrete by examining the ratio of PhD productivity to research funding for different units in science and engineering at MSU; CBE is a leader by this metric.

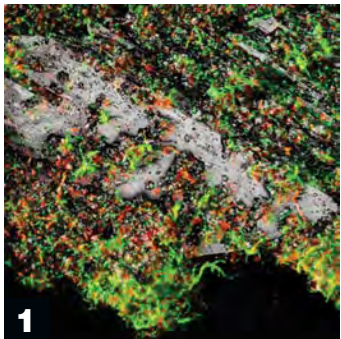



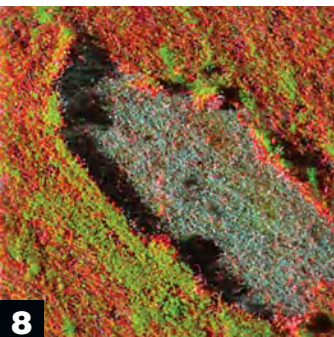

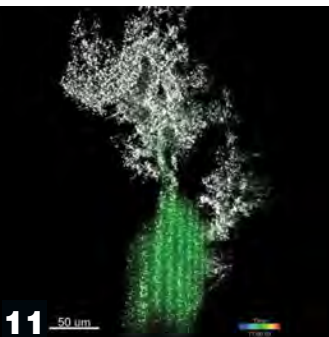
Over 730 undergraduates from disciplines as varied as chemical and biological engineering, nursing, physics, and microbiology (as well as seven others) have studied and worked in CBE laboratories—many of them for several semesters.

The quality of Center students, as scholars and volunteers, has often been inspiring. Many have won departmental and MSU awards. Seven undergraduate students have been named Goldwater Scholars. Six additional students have received NSF Graduate Research Fellowships.

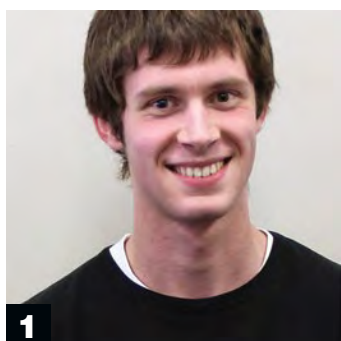
Students who join CBE receive training in experimental design and laboratory research under the direction and mentorship of world-renowned faculty researchers. Our students are exposed to real-world problems and industrial perspectives that help them to integrate their classroom learning and place their own research activity in context. These experiences are also excellent preparation for careers, whether in academia or industry.

2014 | Education Snapshots: Student news and research activities

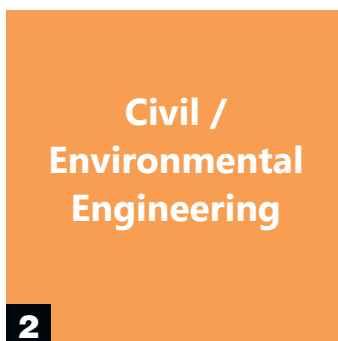
1. Undergraduate **Trace Hobbs** investigated the **role of microbes in the formation of kidney stones** with his project: "Association of struvite formation with *Proteus mirabilis* biofilm." Faculty mentor: Robin Gerlach. **2.** LRES PhD student **Heidi Smith** published "Draft genome sequence and description of *Janthinobacterium* sp. strain CG3, a psychrotolerant **Antarctic supraglacial stream bacterium**," in *Genome Announcements*. Faculty mentor: Christine Foreman. **3.** Chemical and biological engineering senior **Steven Davis** graduated with **highest honors**, played three years of basketball for MSU, and was invited to offer a Blackfeet prayer to open the university's Honors College ceremony. Faculty mentor: Ross Carlson. **4.** **Ashley Beck**, PhD student in microbiology, received the CBE **John Neuman Memorial Student Citizen Award**. Beck was recognized for being a conscientious scientist and researcher with an eye for detail and analytic precision. Faculty mentor: Ross Carlson. **5.** The affiliated program Graduate Education in Health for Minority Scholars (GEhMS) graduated three masters students in 2014: **Yanet Eudave**, **Lidice Tobar Quezada**, and **Nathaniel Tucker**. CBE mentor: Mari Eggers. **6.** Recent doctoral student recipients of the **W.G. Characklis Outstanding Student Award**, given in memory of CBE's founding director, were **Heidi Smith**, land resources and environmental sciences, and **James Connolly**, chemical and biological engineering. **7.** **Ben Jackson**, PhD student in mathematics, presented "Biomining using biofilms: Estimating kinetic parameters using a simple **flow channel model**" at the Society for Mathematical Biology Annual Meeting, Tempe, AZ, USA. Faculty mentors: Isaac Klapper, Robin Gerlach. **8.** Junior **Amanda Richards** studied stains that reveal the **structure of *Pseudomonas aeruginosa* biofilms**. Faculty mentor: Michael Franklin. **9.** **Catherine Kirkland**, PhD student in civil engineering, was one of seven MSU recipients of a **2013 National Science Foundation Graduate Research Fellowship**. Faculty mentor: Sarah Codd. **10.** **Michelle Tigges**, PhD student in chemistry and biochemistry, presented her research "Understanding the microbially mediated **transformation of dissolved organic carbon**: An "omics" approach," at the 247th ACS National Meeting and Exposition, Dallas, TX, USA. Faculty mentor: Christine Foreman. **11.** **James Connolly** created a movie documenting **microbially induced calcite precipitation** for his article published in the *Journal of Microbiological Methods*. Faculty mentor: Robin Gerlach.

<p>Recent CBE student research participation reached 110: 55 graduates and 55 undergraduates.</p>	 <p>1</p>	<p>Land Resources and Environmental Sciences</p>	 <p>3</p>
 <p>4</p>	<p>Health and Human Development Nursing</p>	 <p>6</p>	<p>Mathematical Sciences</p>
 <p>8</p>	 <p>9</p>	<p>Chemistry and Biochemistry</p>	 <p>11</p>

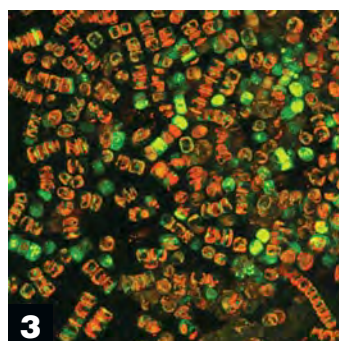
1. Chemistry senior **Blaine Fritz** received a Lundbeck Foundation International Masters Scholarship to pursue studies at the University of Copenhagen, Denmark, on the physiology of bacteria in **acute and chronic infections**. CBE mentor: Diane Walker. **2.** **Chris Allen**, PhD student in civil engineering, presented his research on **wetlands wastewater remediation** at a breakout session of the WETPOL 5th International Symposium on Wetland Pollutant Dynamics and Control, Nantes, France. Faculty mentor: Otto Stein. **3.** A recent publication by **Karen Moll**, PhD student in microbiology, found its way into the **top twenty most downloaded algal research articles** from ScienceDirect in a 90-day period. Faculty mentor: Brent Peyton. "Combining multiple nutrient stresses and bicarbonate addition to promote lipid accumulation in the diatom RGd-1," *Algal Research*. July 2014;5:7–15. **4.** **Anna Zelaya**, PhD student in **microbiology**, was invited to present "Particle campaign: Lab and field experiments" at a recent Ecosystems and Networks Integrated with Genes and Molecular Assemblies (ENIGMA) Annual Meeting in Berkeley, California. Faculty mentor: Matthew Fields. **5.** ChBE undergraduate **Kylie Bodle** investigated the ability of naturally occurring microorganisms to **remediate coal waste rock at mining sites**. Faculty mentor: Rich Macur. **6.** **Matthew Sherick** was one of seven MSU seniors to win a Torliel Aasheim Community Involvement Award—the **university's top award for student service**. In the lab, he used magnetic resonance imaging to study **microcapillary formation in microbial alginate gels**. Faculty mentor: Joe Seymour. **7.** Senior **Varsha Rao** supplemented her **magnetic resonance studies** with participation in the 2014 Summer Research Experience in Experimental Physics 5 Laboratory at Julius-Maximilians-Universität Würzburg, Germany. Faculty mentor: Sarah Codd. **8.** Undergraduate **Isaac Miller** was introduced to **microbial ecology** in his first year with the Ecology & Physiology research team. Faculty mentor: Matthew Fields. **9.** Undergraduate **Emily Bermel** studied *Janthinobacterium* for **bacterial mechanisms to protect themselves from UV exposure**. Faculty mentor: Christine Foreman. **10.** M&IE major **Andrew Bender** investigated variables affecting the growth of **biofilm in porous media** and served as an MSU Engineering Ambassador, introducing scores of prospective students to MSU research opportunities. Faculty mentor: Sarah Codd. **11.** CBN undergraduate **Erika Avera** was able to increase her knowledge of **oral biofilms** in preparation for future work in dental school. CBE mentor: Laura Boegli.



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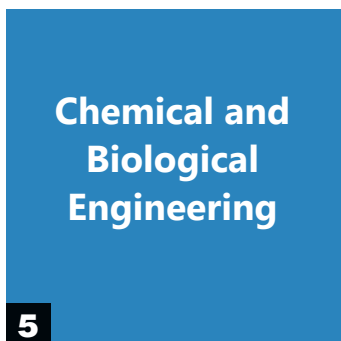
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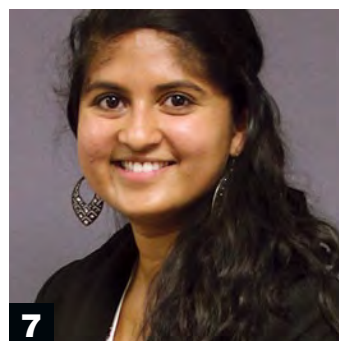
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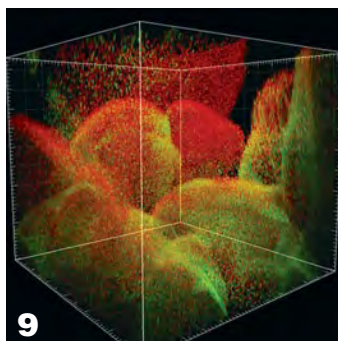
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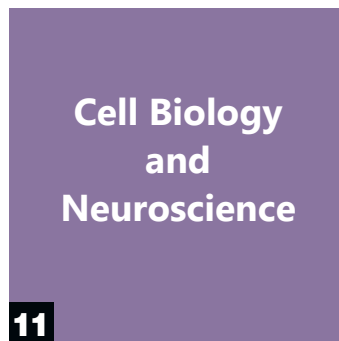
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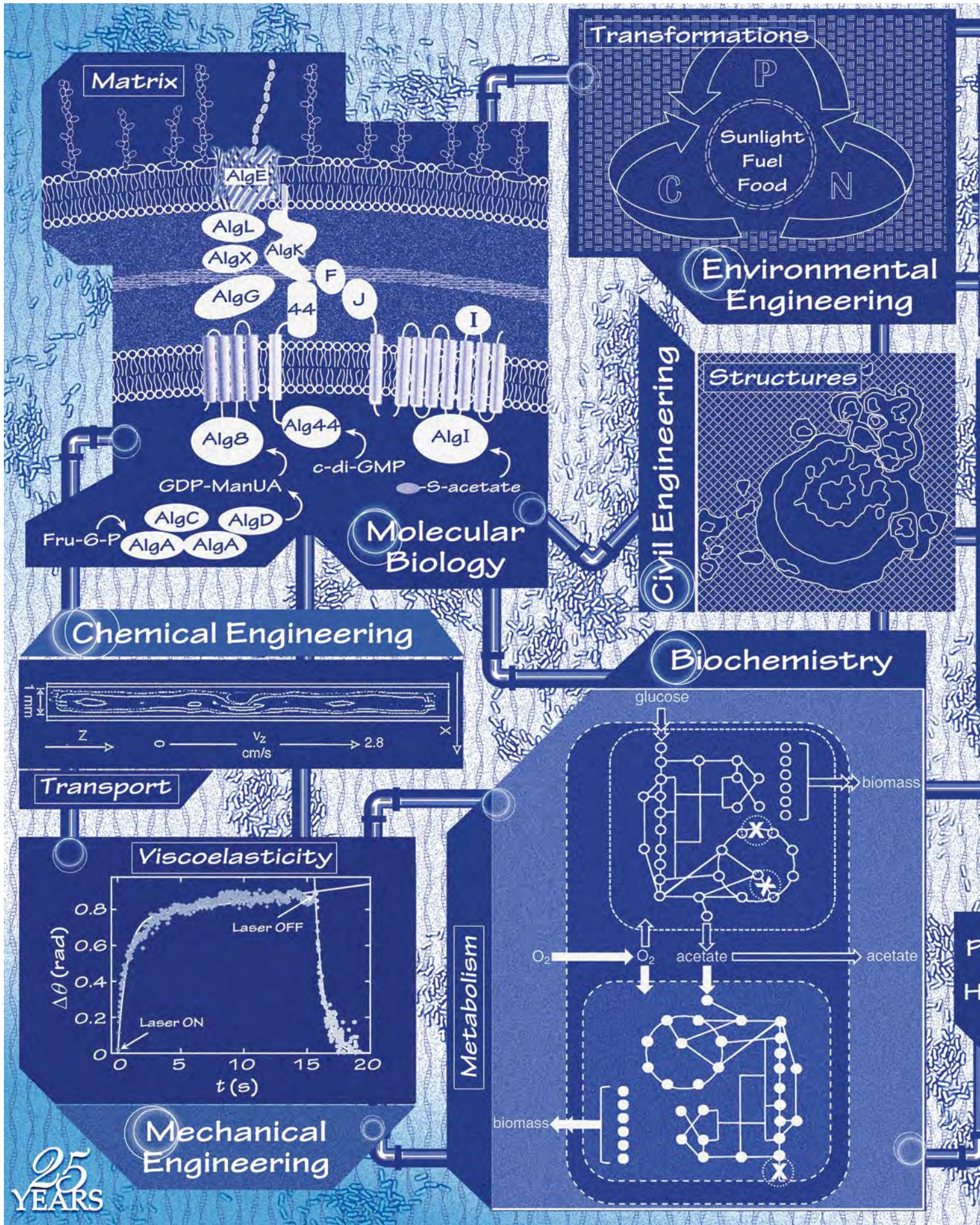
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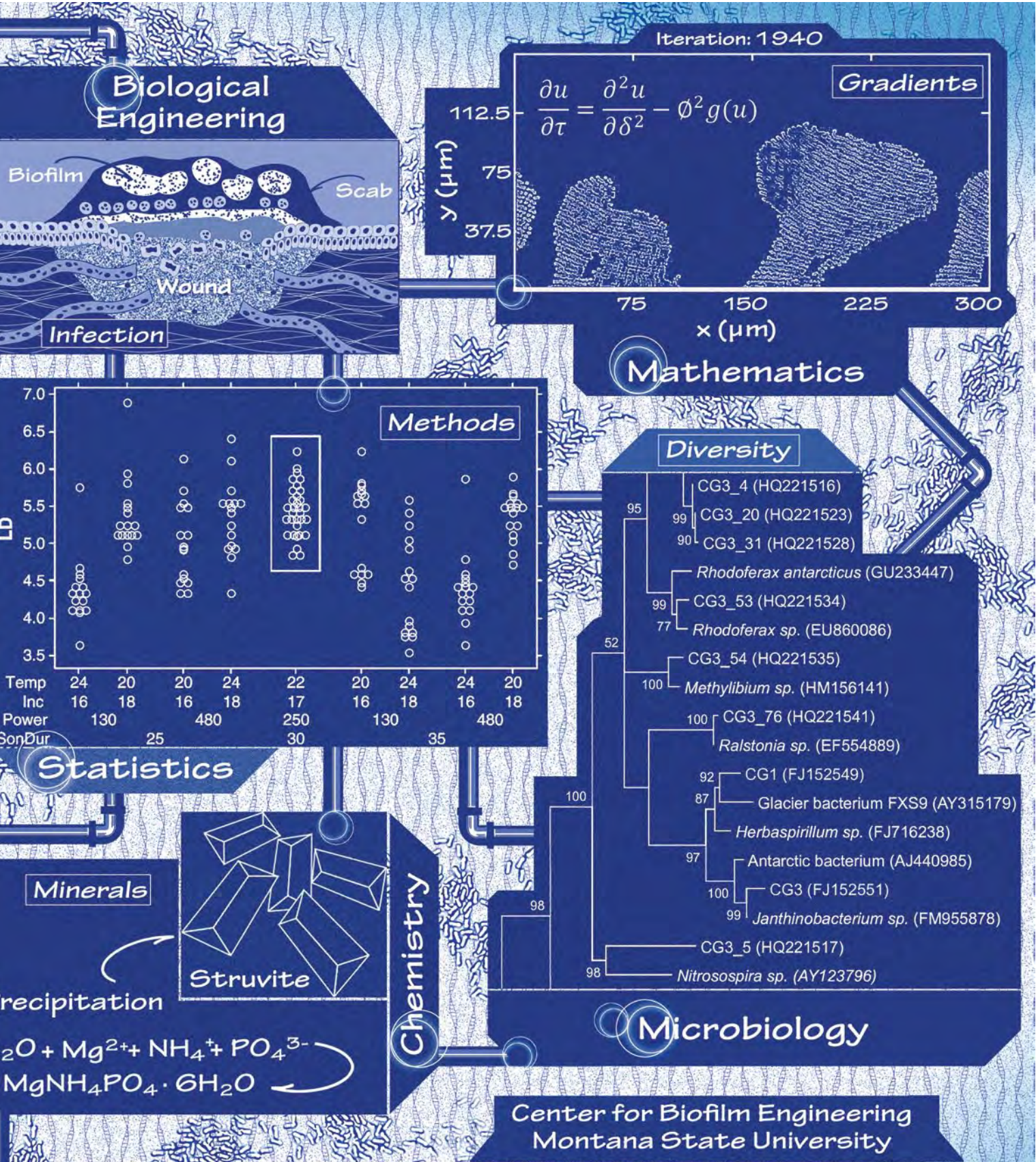


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1990 **Blueprint for Interdisciplinary Education and Research** 2015



Elinor Pulcini, center, of CBE's Medical Biofilm Laboratory, demonstrated reactor sampling to CBE students Amanda Durch and Sarah Mailhot. Photo by K Gorham, MSU News.

Technology TRANSFER



25-YEAR TECH TRANSFER RECAP



Fostering innovation through technology transfer has been a central goal of the CBE from the start. Productive interactions with industry began in the 1980s and 90s with oil and water treatment companies and expanded to consumer products companies, specialty chemical companies, and healthcare companies in the past decade.

With broadening industrial relevance, CBE's sustaining membership has also grown—from 14 member companies in 1990 to 33 in 2014. The range of relevant research has attracted a healthy mix of industry members, including numerous Fortune 500 companies as well as innovative start-ups.

These members and additional companies have funded hundreds of sponsored research and testing projects in Center labs. A relatively recent area of funding growth, these projects have brought in over \$5 million in the past 5 years.

CBE has aided companies in such diverse efforts as: solving biofilm water line contamination for a manufacturing plant, documenting the efficacy of novel surface disinfectants against biofilms, collecting data to be included in 510(k) submissions to FDA, optimizing the formulation of novel anti-biofilm chemistries for a surgical lavage, and developing technologies for increasing lipid production for biofuels.

CBE has consistently engaged with industry through regular visits, workshops, and conferences. Our Montana Biofilm Meeting is a premiere biofilm technology event, with participants from member companies, academia, government agencies, and invited speakers from around the globe.

Full-day biofilm methods and microscopy workshops began as occasional offerings, but have become regular add-ons to the biannual meetings. Enrollments are limited to enable hands-on practice by participants. Recent topics have included epifluorescence microscopy for assessing surface biofilm accumulation and removal efficacy, instruction in use of the CBE-developed Single Tube Method and Treatment Flow Cell, and basic biofilm methods.

We enter our next 25 years with renewed efforts to broker interaction and dialogue with FDA and EPA by holding meetings in the Washington, D.C. area.

Comments from our members

“The Standard Biofilm Methods team has helped to move the foundation of biofilm claims and registration forward, enabling us to deliver better performing products that address microbial control in a real world manner.” — **Bret Glembocki, Research Scientist, SealedAir**

“Through various collaborations at the CBE we have been fortunate to expand the fundamental learning about deposition of actives on surfaces, to understand the properties of agents and their relationship to penetration in oral biofilms, to study mode of action of the actives, to produce videos demonstrating the working of CP products against oral biofilms, and to publish this work. The CP-CBE relationship has matured over the years and the mutual respect and appreciation of each party has grown.” — **Harsh Trivedi, Manager of Technology, Colgate-Palmolive**

<p><i>Healthcare/Medical</i></p> <p>3M</p> <p><i>9 years</i></p>	<p><i>Testing Lab</i></p> <p>ATS Labs</p> <p><i>2 years</i></p>	<p><i>Healthcare/Medical</i></p> <p>Bard Access Systems</p> <p><i>3 years</i></p>	<p><i>Specialty Chemicals</i></p> <p>BASF</p> <p><i>6 years</i></p>
<p><i>Healthcare/Medical</i></p> <p>Baxter Healthcare</p> <p><i>6 years</i></p>	<p><i>Specialty Chemicals</i></p> <p>BCG Solutions</p> <p><i>3 years</i></p>	<p><i>Healthcare/Medical</i></p> <p>Bend Research</p> <p><i>4 years</i></p>	<p><i>Energy</i></p> <p>BP</p> <p><i>(15 +) 2 years</i></p>
<p><i>Consumer Products</i></p> <p>Church & Dwight</p> <p><i>12 years</i></p>	<p><i>Consumer Products</i></p> <p>Colgate-Palmolive</p> <p><i>15 years</i></p>	<p><i>Healthcare/Medical</i></p> <p>Curza</p> <p><i>1 year</i></p>	<p><i>Specialty Chemicals</i></p> <p>Dow Corning</p> <p><i>3 years</i></p>
<p><i>Specialty Chemicals</i></p> <p>Dow Microbial Control</p> <p><i>9 years</i></p>	<p><i>Specialty Chemicals</i></p> <p>Ecolab</p> <p><i>6 years</i></p>	<p><i>Energy</i></p> <p>ExxonMobil</p> <p><i>(10 +) 5 years</i></p>	<p><i>Healthcare/Medical</i></p> <p>ICU Medical</p> <p><i>7 years</i></p>

Montana Biofilm Research Consortium

The CBE Industrial membership program includes this group of small Montana companies, which has a combined single vote in the CBE industrial membership. In recognition of support from the State of Montana and Montana State University, the MBRC members pay no subscription fee. The mission of the MBRC program is to facilitate statewide economic development of small biofilm technology related companies in Montana.

**BioScience Laboratories | BioSurface Technologies
Center for Innovation | Lupine Medical | Microbion**

U.S. Government

NASA

10 years

Consumer Products

Johnson & Johnson

5 years

Healthcare/Medical

Kane

6 years

Healthcare/Medical

KCI

4 years

Consumer Products

Masco

10 years

Specialty Chemicals

NCH

4 years

Healthcare/Medical

Next Science

2 years

Specialty Chemicals

Novozymes

9 years

Consumer Products

Procter & Gamble

17 years

Specialty Chemicals

Sani-Marc

2 years

Specialty Chemicals

Sealed Air

2 years

Specialty Chemicals

Sterilex

1 year

Healthcare/Medical

STERIS

4 years

Consumer Products

The Clorox Company

10 years

Consumer Products

The Sherwin-Williams Company

6 years

Healthcare/Medical

W.L. Gore & Associates

18 years

Healthcare/Medical

Zimmer

1 year

CBE and FDA co-sponsor first public biofilm workshop

Montana State University's Center for Biofilm Engineering was pleased to co-sponsor a public workshop on biofilms in partnership with the U.S. Food and Drug Administration on February 20, 2014, at the FDA campus in Silver Spring, Maryland. This public workshop sought to share scientific information between the academic and healthcare communities, U.S. government scientists, and companies interested in developing products to address biofilm contamination.

The workshop generated an extraordinary response. Within a few days of the opening of registration, all 160 seats on site had been claimed. At the peak of the day, an additional 285 people watched the live webcast. Forty FDA employees participated in person; 53 different companies were represented.

Speaker highlights

Keynote lecturer **Javad Parvizi, MD**, of Thomas Jefferson University, an orthopaedic surgeon, examined the role of infection in the **failure of hip and knee replacements**. Infections associated with these devices cause trauma for the patient and pose an economic burden estimated to exceed \$1 billion annually. Of particular concern: prosthetic joint infections continue to appear even a decade after implantation.

Weaving together evidence from the literature as well as case histories from his personal experience, **Todd Heniford, MD**, Professor of Surgery in the Carolinas HealthCare System, spoke about **biofilm infections associated with meshes used in hernia repair**. Dr. Heniford presented data showing that only 13% of infected meshes could be salvaged. The cost of hernia repair with complications due to mesh infection was 3.7 times the cost of an uncomplicated surgery.

Rod Donlan, director of the biofilm laboratory at the Centers for Disease Control and Prevention in Atlanta, Georgia, discussed the evidence for **biofilm infection of devices such as catheters, needle-free connectors, and ventilator tubes**. An analysis of healthcare associated infections (HAI) in U.S. hospitals in 2002 tallied over 1.7 million. More than half of these (61%) were cases of catheter-associated urinary tract infection, central line-associated bloodstream infection, or ventilator associated pneumonia.

In an update from CBE, **Garth James**, director of the Medical Biofilms Laboratory, examined the evidence of antibacterial efficacy of a first-generation antimicrobial central venous catheter containing chlorhexidine and silver sulfadiazine. This device demonstrated antimicrobial efficacy in an *in vitro* biofilm model, in three studies in rats, and in a meta-analysis of 13 clinical studies. This interesting example of concordant results from different types of experiments demonstrated the **potential for *in vitro* and animal models to correlate with clinical outcomes**.

Paul Sturman, industrial coordinator at CBE, described several biofilm methods that have been certified as ASTM standard methods and explained the **desired features of a standardized method**. He also highlighted the difference between biofilm disinfection and biofilm

removal and emphasized the unmet need for standardized approaches to measuring biofilm prevention and biofilm removal.

Angela Krueger, Regulatory Advisor/Product Jurisdiction Officer in the Office of Device Evaluation, addressed **regulatory challenges faced by FDA in evaluating biofilm claims for medical devices**. These challenges include the variety of possible indications or claims related to anti-biofilm technologies, the lack of standardized testing, the lack of relevant internal standards or guidance documents within the Center for Devices and Radiological Health, and the complexity of considering combination (device plus drug) products in many cases.

Steve Tomasino, a Senior Scientist in the Biological and Economic Analysis Division of EPA, announced EPA's intention to define a basis for the **approval of a public health claim for liquid surface disinfectants against biofilms** later this year. He provided specific, if preliminary, details of what to anticipate in this method. The approach will use the single tube disinfection method ASTM E2871-12.

Innovative anti-biofilm technologies appeared throughout the day. **Matthew Libera**, Professor of Materials Science at the Stevens Institute of Technology, Hoboken, NJ, made the important point that **next generation biomaterials or device coatings will ideally induce differential effects on cells**: they will repel or kill microorganisms while integrating with and promoting healing responses of host cells and tissues.

At the end of the afternoon a **panel of experts tackled questions about the future research needed to enable development of safe and effective anti-biofilm products**. Panel members recognized the **need for new methods for detecting biofilms on medical devices and for diagnosing biofilm infections**. Clinicians, attuned to the difficulty of resolving a biofilm infection, voiced the need for **medical devices that incorporate anti-biofilm technologies designed to prevent device-related infections from establishing in the first place**. They also recognized that it may be appropriate to target the use of anti-biofilm devices to patients whose co-morbidities put them at higher risk of infection. The need to **consider the issue of antimicrobial resistance in the development of new anti-biofilm technologies** was discussed.

Another focus of panel comments was the identification and refinement of appropriate *in vitro* and *in vivo* animal model methods for evaluating anti-biofilm technologies. Strategies that could be considered in this regard include **pursuing method standardization through standard setting organizations**, having a **task force make consensus recommendations on methods**, and taking **lessons from the experience at EPA**.

The workshop concluded with a call to maintain this platform of integration and interdisciplinary interaction with a resolve to continue this event. Many of the presentations and slides shown at the workshop can be viewed through an FDA website at: <http://www.fda.gov/MedicalDevices/NewsEvents/WorkshopsConferences/ucm381336.htm>

In response to positive feedback from industry members, CBE planned a second workshop:

"Anti-Biofilm Technologies: Pathways to Product Development," Wednesday, February 11, 2015, in College Park, Maryland.

CBE and Industry: Intentional interaction for productivity

"CBE stays connected with industrial members and keeps up-to-date with current needs for anti-biofilm strategies and technologies. This is reflected in the relevant, quality research conducted at the Center and in the choice of presentations and invited speakers at the biannual meetings. CBE has maintained itself as a Center of Excellence, with state-of-the-art microscopy and analytical capabilities. Access to these resources by the industrial members is invaluable. Finally, connections that CBE has made with the CDC and NIH, as well as regulatory agencies such as FDA and EPA, help provide unbiased education on the impact of biofilms with serious clinical and environmental outcomes. Industrial members benefit from these relationships by having an opportunity to communicate with the experts in a non-competitive and collaborative setting."

Ruth Cutright, Corporate Microbiologist, W.L. Gore & Associates

Full-day methods and imaging workshops

Biannual meetings

Regulatory agency interactions

Networking

Research and testing projects

Faculty visits and member consulting

"The P&G collaboration with the CBE at MSU has allowed us to develop technical models and methods that enable optimal design and delivery of consumer product technologies. In particular, we have uncovered key insights about the performance of these technologies through the state-of-the-art visualization capabilities at the CBE. These microscopic images bring to life the traditional product efficacy results that are generated with our industry standard methods."

Chuck Pettigrew, Principal Scientist, Procter & Gamble

hands-on biofilm workshops



K GORHAM, MSU NEWS

"Participation in the CBE's Industrial Associates program continues to pay dividends to our organization. A key benefit is the enrichment of our employees' basic knowledge of biofilms, as well as a broad understanding of applied biofilm control technologies. Participation in the Montana Biofilm Meetings not only provides a way to communicate scientific advances and regulatory progress, but has also provided an important opportunity for the development of collaborative industrial partnerships."

Tony Rook, R&D Manager, The Sherwin-Williams Company

"One of the issues that we had in our recent biological water processor test was sloughing of biofilms in recycle lines. The presentation Jim Wilking gave at the Montana Biofilm Meeting about techniques to better understand the physical and mechanical characteristics of biofilms helped us understand characteristics of biofilms in our system(s), enabling us to develop strategies to mitigate biofilm formation and sloughing."

Letty Vega, Scientist, Life Support Systems, NASA

"The CBE conference forum and industrial partnership program has brought us together with another member company with common goals in the healthcare field. Together, we have developed a viable product that has shown great results in biofilm reduction through *in vitro* testing at the CBE and should be able to be introduced to the market within the next year."

Chris Samuel, VP Business Development, Next Science

networking and project development



Montana Biofilm S&T Meetings for members



Biofilm Education OUTREACH

25-YEAR OUTREACH RECAP



As the world's oldest, largest and best known research center for microbial biofilms, CBE regularly attracts students, staff, faculty, industry representatives, and health clinicians from around the world to visit Bozeman with the aim of incorporating CBE biofilm expertise into their applied areas. Nearly 270 visiting researchers from over 30 countries, plus 38 U.S. states, have spent from several weeks to a year or more studying biofilms in CBE labs.

This global collegiality fosters interdisciplinary collaboration, which has resulted in numerous publications and grants. It also broadens exposure to new ideas and cultural differences, for visitors as well as for MSU-CBE students, staff and faculty. These working visits often create long-lasting friendships and on-going collaborations.

Another branch of outreach includes numerous workshops and retreats planned and hosted by CBE through the years on topics including: chronic wounds, biofilm methods, microscopy techniques, subsurface biotechnology and bioremediation, the manufacture and application of microsensors, biofilm research methods in dentistry, biomineralization, and biofilm mechanics, among others. CBE leadership was also instrumental in introducing the ASM Biofilm conference series, beginning in 1996 with the Snowbird, Utah, meeting.

CBE personnel have played host and tour guide to countless groups, large and small, of visitors young and old—providing many people their first glimpse of the amazing complexity of microbial communities.

Meanwhile, the world has continual access to the Center through CBE's web site, which has provided basic information about biofilms, abstracts for CBE publications and theses, people, and events since 1996. Perennial favorites, the Center's iconic biofilm conceptual graphics have been downloaded for use in conference presentations, classrooms, and dissertations by requesters from all 50 U.S. states and 58 countries, from Afghanistan to Venezuela.





Visiting graduate student Fernanda Godoy Santos, left, from Universidade Federal de Viçosa, Brazil, learned confocal imaging with microscope facilities manager Betsey Pitts, right. She recently spent a year investigating *Staphylococcus* biofilms at the CBE. Photo by K Gorham, MSU News.

Recent visiting researchers & projects

Cristina Cattò, PhD student, University of Milan, Italy. Project: Innovative biomaterials incorporated into medical devices. Host: Garth James.

Greg Characklis, faculty, and **Adam Hise**, PhD student, University of North Carolina. Collaborative project: Development of sustainable algal biorefineries. Hosts: Robin Gerlach, Brent Peyton and Matthew Fields.

Merle de Kreuk, research faculty, Delft University of Technology, The Netherlands. Project: Anaerobic biofilm techniques and analytical methods. Host: Matthew Fields.

Rosa Dutra de Oliveira, Universidade Estadual Julio de Mesquita, Sao Paulo, Brazil. Project: Biofilms in a drip flow reactor. Host: Garth James.

Erika Espinosa-Ortiz, UNESCO-IHE, Delft, The Netherlands. Project: Mycogenic production of elemental selenium nanoparticles. Hosts: Robin Gerlach and Brent Peyton.

Josh Felicia, undergraduate, Little Bighorn College, Montana. Project: Use of nitrogen-fixing cyanobacteria as a plant biofertilizer. Host: Rich Macur.

Raquel Ferrer, PhD student, Universidad De Navarra, Pamplona, Spain. Project: Combinations of antimicrobial peptides and antibiotics against *P. aeruginosa* biofilms grown in the CDC biofilm reactor. Host: Phil Stewart.

Michael Gilmore, faculty, Harvard Medical School, Boston, Massachusetts. Project: Spent sabbatical time working on antibiotic resistance of enterococci, staphylococci, and streptococci. Host: Phil Stewart.

Fernanda Godoy Santos, PhD student, Universidade Federal de Viçosa, Brazil. Project: *Staphylococcus* biofilm studies. Host: Phil Stewart.

Pia Herrling, graduate student, Karlsruhe Institute of Technology, Germany. Project: Diffusion in biofilms grown on wastewater treatment carriers using NMR. Hosts: Sarah Codd and Joe Seymour.

Johannes Hommel, PhD student, University of Stuttgart, Germany. Project: Returned to CBE for collaborative work with the bioremediation group. Host: Al Cunningham.

Andrew Keenan, undergraduate, Boise State University, Idaho. Project: Microfluidics. Host: Connie Chang.

Kirsten Küsel, faculty, Friedrich Schiller University, Jena, Germany. Project: Solid matrix biofilm and molecular ecology techniques. Host: Matthew Fields.

Shinya Matsumoto, postdoctoral researcher, Nagoya University, Japan. Project: Two-year study of antimicrobial tolerance. Host: Phil Stewart.

Jennifer McIntosh, faculty, University of Arizona, Tucson. Project: Coal-bed methane research. Hosts: Matthew Fields and Al Cunningham.

Federica Villa, postdoctoral researcher, University of Milan, Italy. Project: Biofilm deterioration of stone artwork. Host: Phil Stewart.

Yeni Yung, PhD student, University of Illinois at Chicago. Project: Basic biofilm culturing methods for spatially resolved mass spectrometry analysis to identify biofilm proteins and metabolites. Host: Ross Carlson.



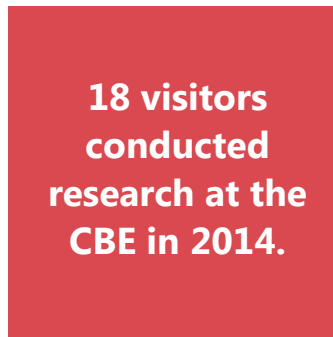
Yung



Villa



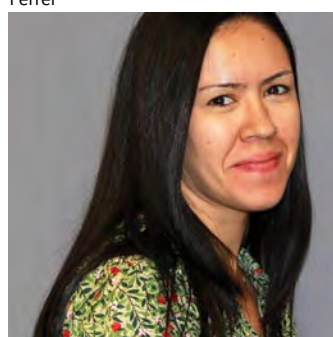
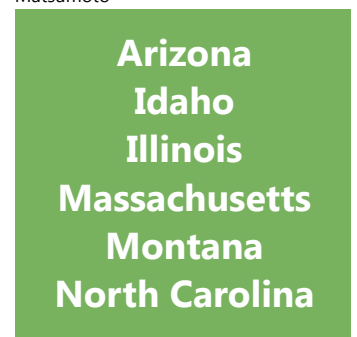
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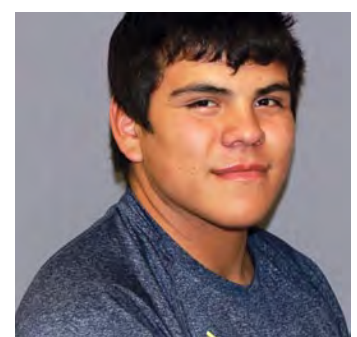
Matsumoto



Ferrer



Dutra de Oliveira



Felicia



**Biofilm breaks rock:
Visitor links microbial
decay and stone art**

High above the Hudson River, on the property of the Vanderbilt Mansion National Historic Site in Hyde Park, New York, Federica Villa (pictured top, right, page 22) worked with Judith Jacob, conservator of the National Park Service, to non-destructively sample the surface of a 15th Century baptismal font. Several days later, back at the CBE in Bozeman, Villa examined her miniscule specimen with confocal microscopy and the images confirmed her suspicions.

The font, like much of the world's most precious stone artwork, is being threatened by irreversible deterioration due to biological attack. Villa is on a mission to demonstrate the role of microbial biofilms in the erosion of the world's stone artwork.

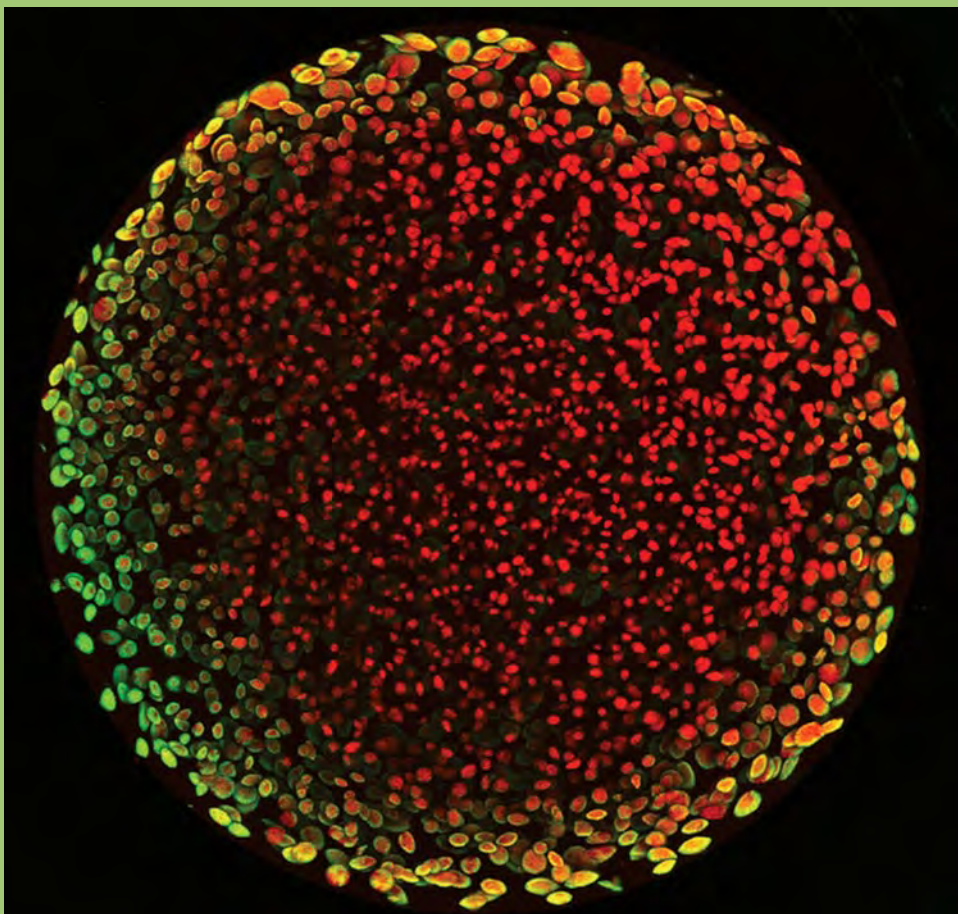
Funded by the European Union under the FP7 Marie Curie People program IOF (International Outgoing Fellowship), Villa left her home institution, the University of Milan, Italy, to spend two years at the CBE on her project: "ESENCYA- Environmental SENSory perception in CYAnobacterial biofilms: Understanding biodeterioration of outdoor stone materials in a changing environment."

As she studies the formation of phototrophic biofilms at the stone/air interface, Villa is particularly interested in the ability of microbes to sense and respond to environmental cues, because such microbial community changes may influence the biodecay of stone-built treasures. She hopes her research will contribute to improving restoration and conservation strategies.

Other U.S. sites she has received permission to study include the Federal Hall National Memorial in New York City and the Lincoln Memorial in Washington, D.C.



A Renaissance baptismal font (top) now in the garden of the Vanderbilt Mansion National Historic Site, Hyde Park, NY. Federica Villa worked with National Park Service conservator Judith Jacob to non-destructively obtain a surface sample for testing and imaging. (Bottom) 3D projection of the sub-aerial biofilm that colonized the dusty marble surface of the font (F Villa and B Pitts).



Confocal microscopy of agarose gel beads seeded with *Staphylococcus aureus*. The bacteria were tagged with GFP and fluoresced green under low oxygen conditions. The beads were counter-stained red with propidium iodide. B Pabst and B Pitts.

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An additional special thank-you to the many CBE staff, faculty, students and visitors who contributed photography, imaging, information and proofreading—and made this a collaborative project. Concept, editing, and design by Peg Dirckx. Executive editor, Phil Stewart.

It has been a privilege to work with so many bright, creative, and dedicated people through the years. Thank you all. *pd*

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