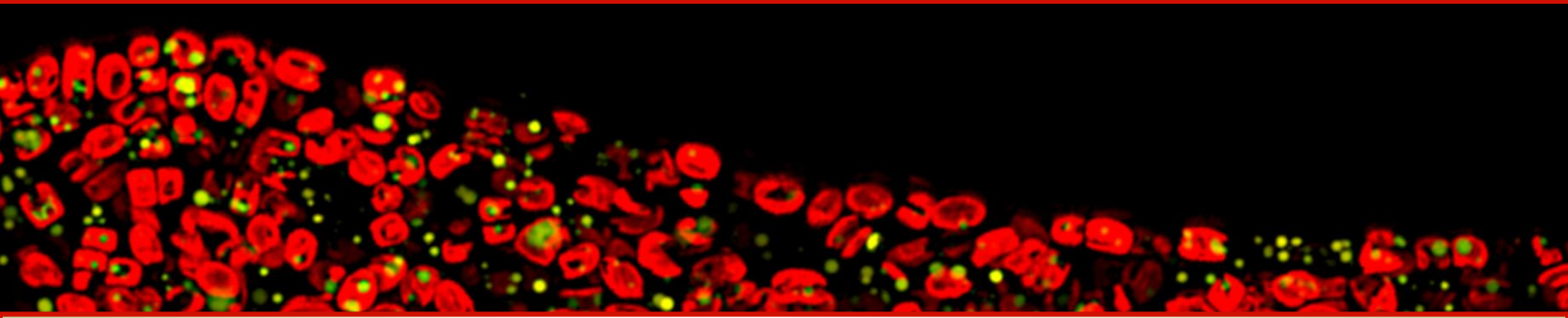
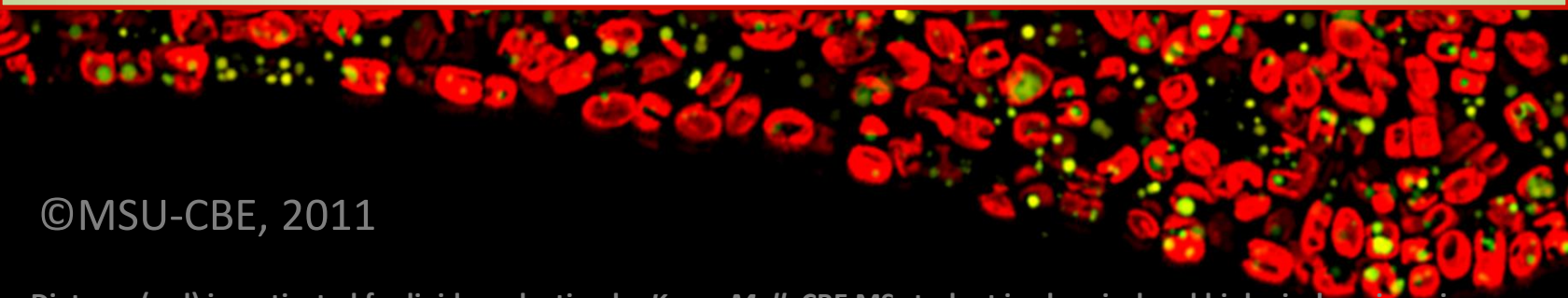


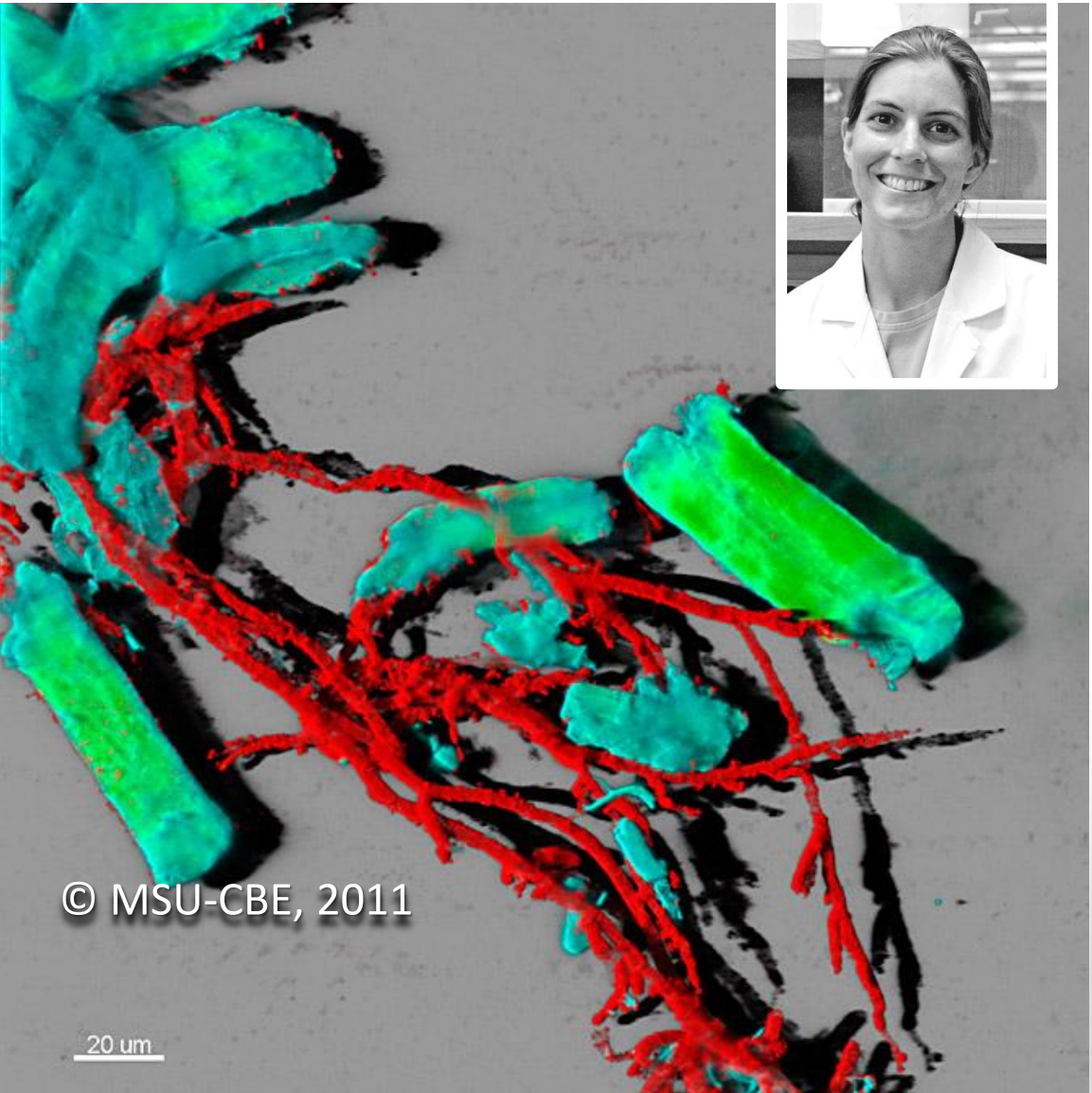
Confocal Scanning Laser Microscopy (CSLM) allows researchers to view living, intact, hydrated biofilms in three dimensions and over time. Cells and matrix material are viewable with the use of reporter genes and stains, or sometimes, auto-fluorescence.

CBE researchers use CSLM to collect quantitative data from biofilms that are important in a number of natural and industrial settings. The scans create compelling images. See more about our facilities at: Montana State University CBE Bioimaging Core Facility.

Several CBE PhD students (noted on slides) were funded by the NSF Integrative Graduate Education and Research Training (IGERT) program.

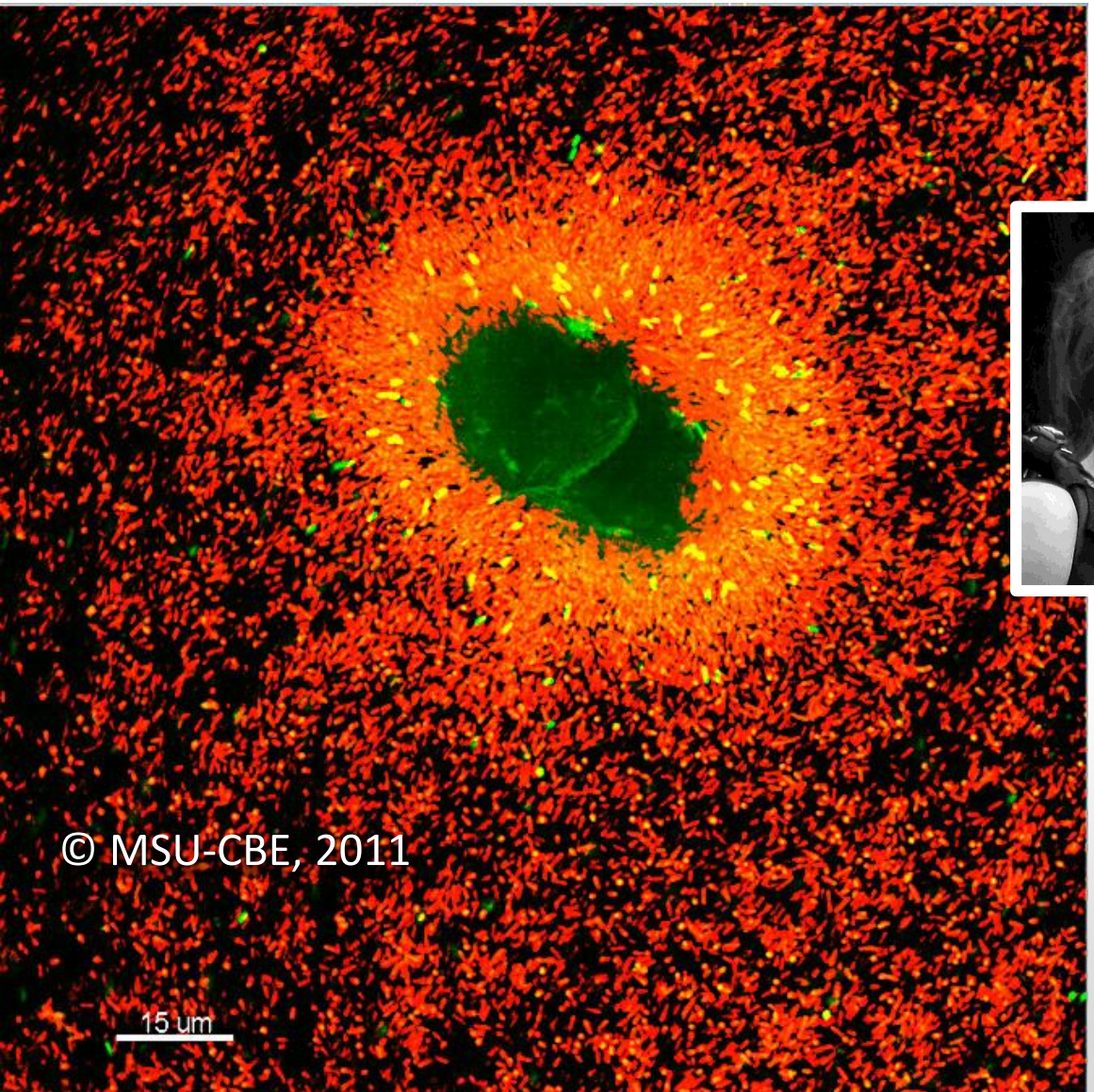


Diatoms (red) investigated for lipid production by *Karen Moll*, CBE MS student in chemical and biological engineering



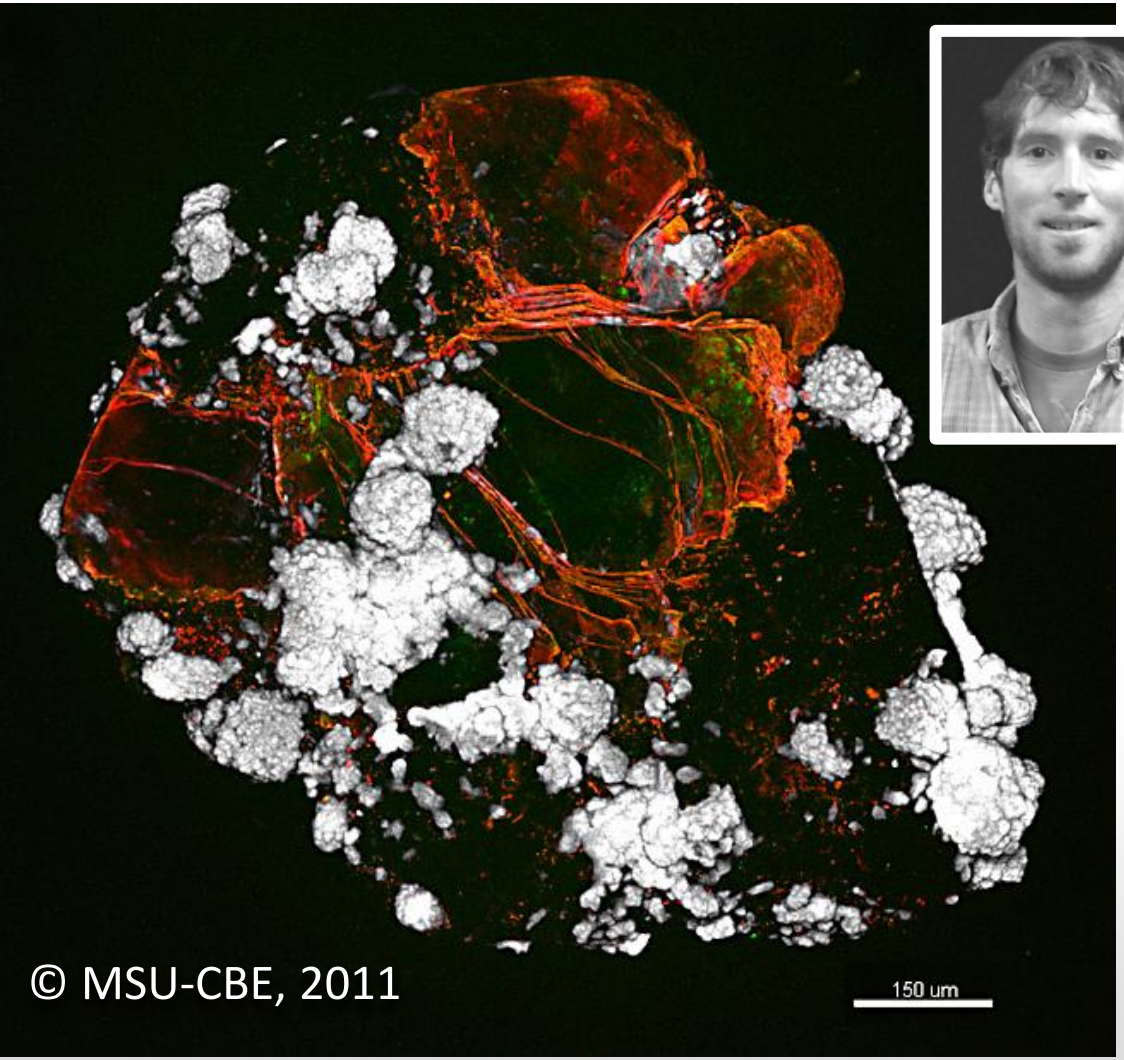
Natasha Mallette, CBE PhD student in chemical and biological engineering, NSF-IGERT awardee

"Ascocoryne sarcoides (red), a filamentous fungi, growing aerobically with cellulose (blue/green) in a submerged liquid culture, was imaged with confocal microscopy. *A. sarcoides* produces myco-diesel, fuel-like compounds."



Carole Nagant, visiting CBE researcher from Université libre de Bruxelles, Belgium

"As part of my PhD research in microbiology, I came to the CBE to use confocal microscopy to collect visual data on the penetration and action of cationic antimicrobial peptides on different *Pseudomonas* biofilms."



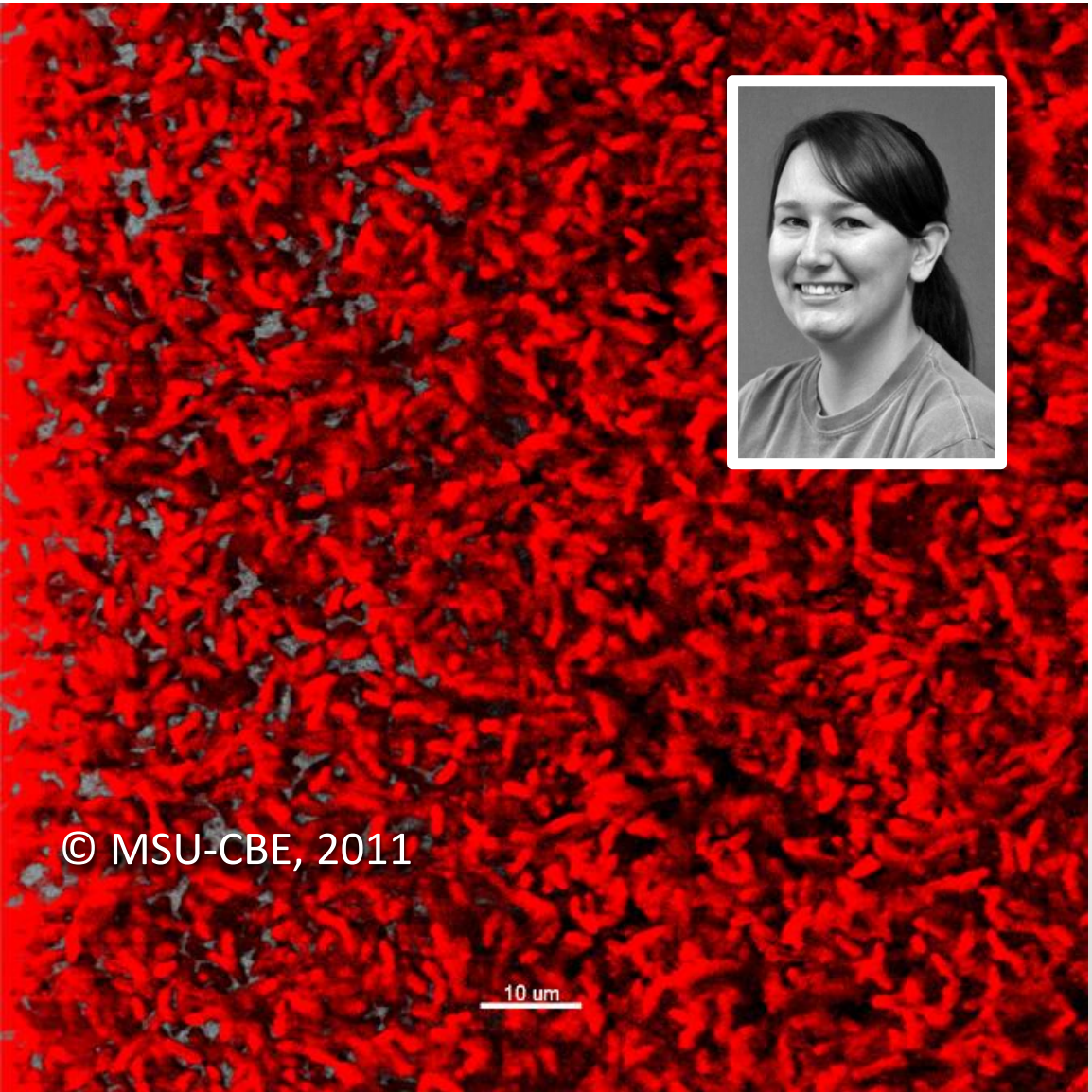
James Connolly, CBE PhD student in environmental engineering, NSF-IGERT awardee

"This image is a CSLM reconstruction of a sand grain colonized by *Sporosarcina pasteurii,* where calcium carbonate (white) has been precipitated. Healthy cells can be seen as green dots. Regions with cells that have compromised membranes or contain extracellular nucleic acids appear as red."

Melis Penic,
CBE undergraduate student in microbiology

"This is a confocal image of a green fluorescent *Pseudomonas aeruginosa* PAO1 biofilm grown in a CDC reactor for 20 hours. The cells are green and the biofilm matrix has been stained red. We are trying to understand more about matrix structure and development through imaging."

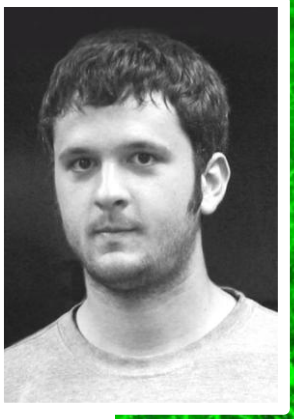
50 um



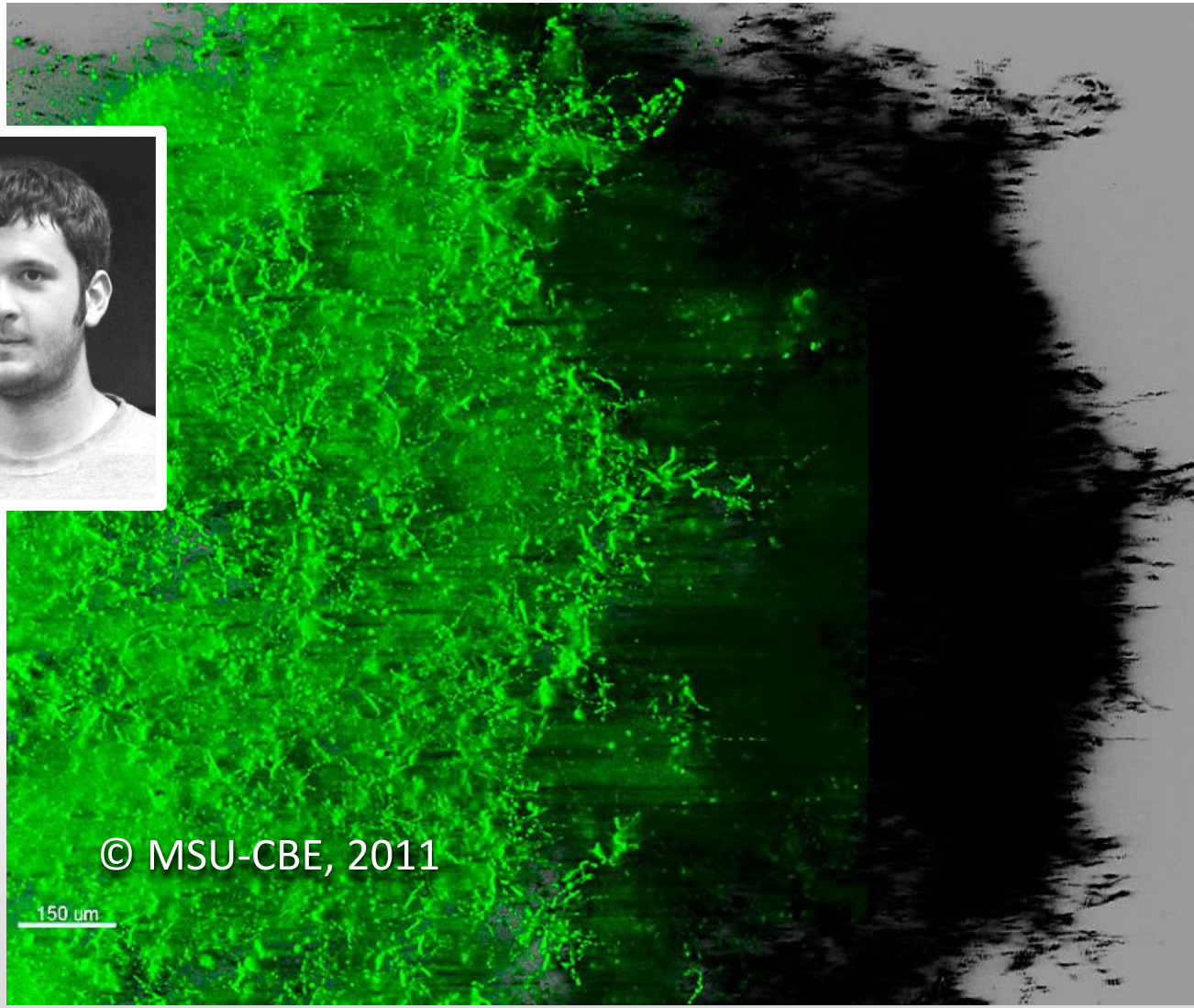
Michelle Tigges,
CBE PhD student in chemistry and biochemistry, NSF-IGERT awardee

"In this confocal image, intrinsic auto-fluorescence produced by a novel non-photosynthetic Antarctic bacterium is intense enough that we can use it to image the bacteria without staining them."

Kristopher Hunt, CBE PhD student in chemical and biological engineering, NSF-IGERT awardee



"This fungal floc (aggregated microorganisms) was stained with a green fluorescent DNA stain. The fungus is an isolate from Thailand."



Hans Bernstein, CBE PhD student in chemical and biological engineering, NSF-IGERT awardee

"This image shows a biofilm containing cyanobacteria (*Synechococcus PCC7002)* in red and *Escherichia coli* in cyan. This interdisciplinary project focuses on engineering and controlling microbial consortial interactions in biofilms."

50 um

Karen Moll, CBE MS student in chemical and biological engineering

"This is a confocal image of a diatom culture, isolated from Yellowstone National Park, which is being screened for algal biofuel production. Generally, we stain for lipids, which show up as yellow, but in this case, we were trying to find a stain that highlighted the diatoms themselves."

Melis Penic, CBE undergraduate student in microbiology

"We are trying to understand more about matrix structure and development through imaging. In this CLSM image, *Pseudomonas aeruginosa* PAO1 are green and the biofilm matrix in which the cells are embedded has been stained red."

15 um

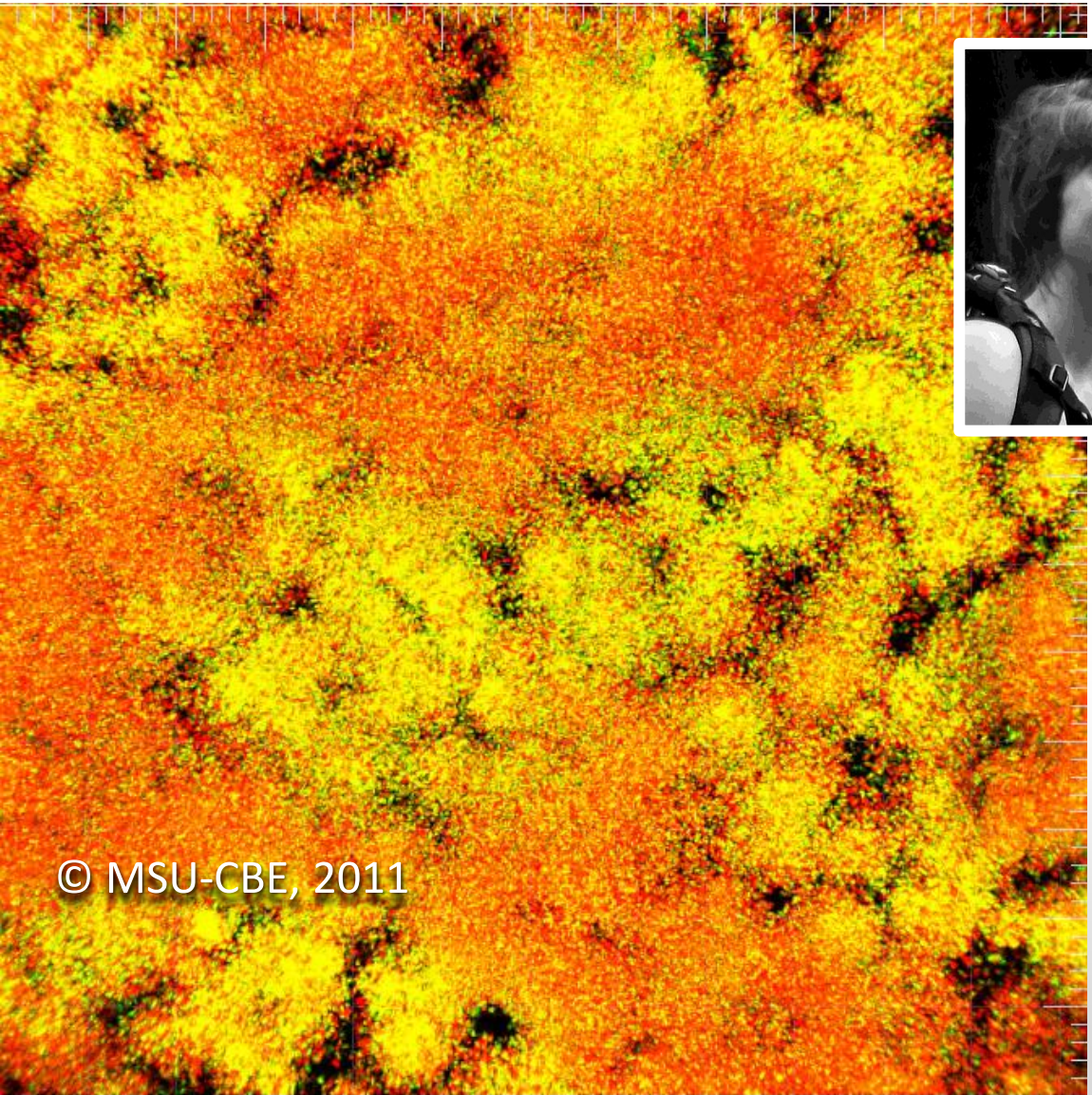


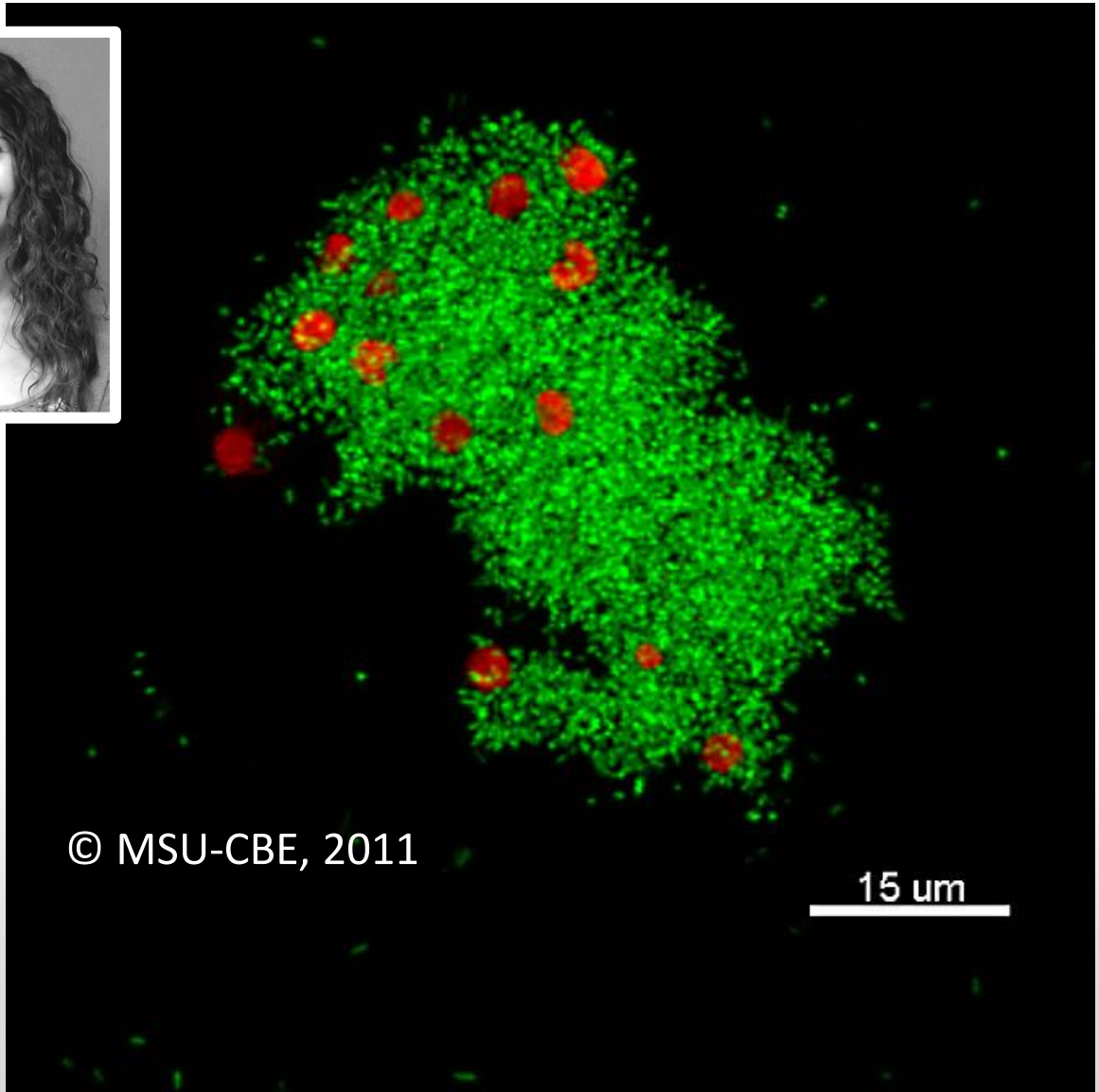
Image by ***Carole Nagant***, visiting CBE researcher Université libre de Bruxelles, Belgium, 2011

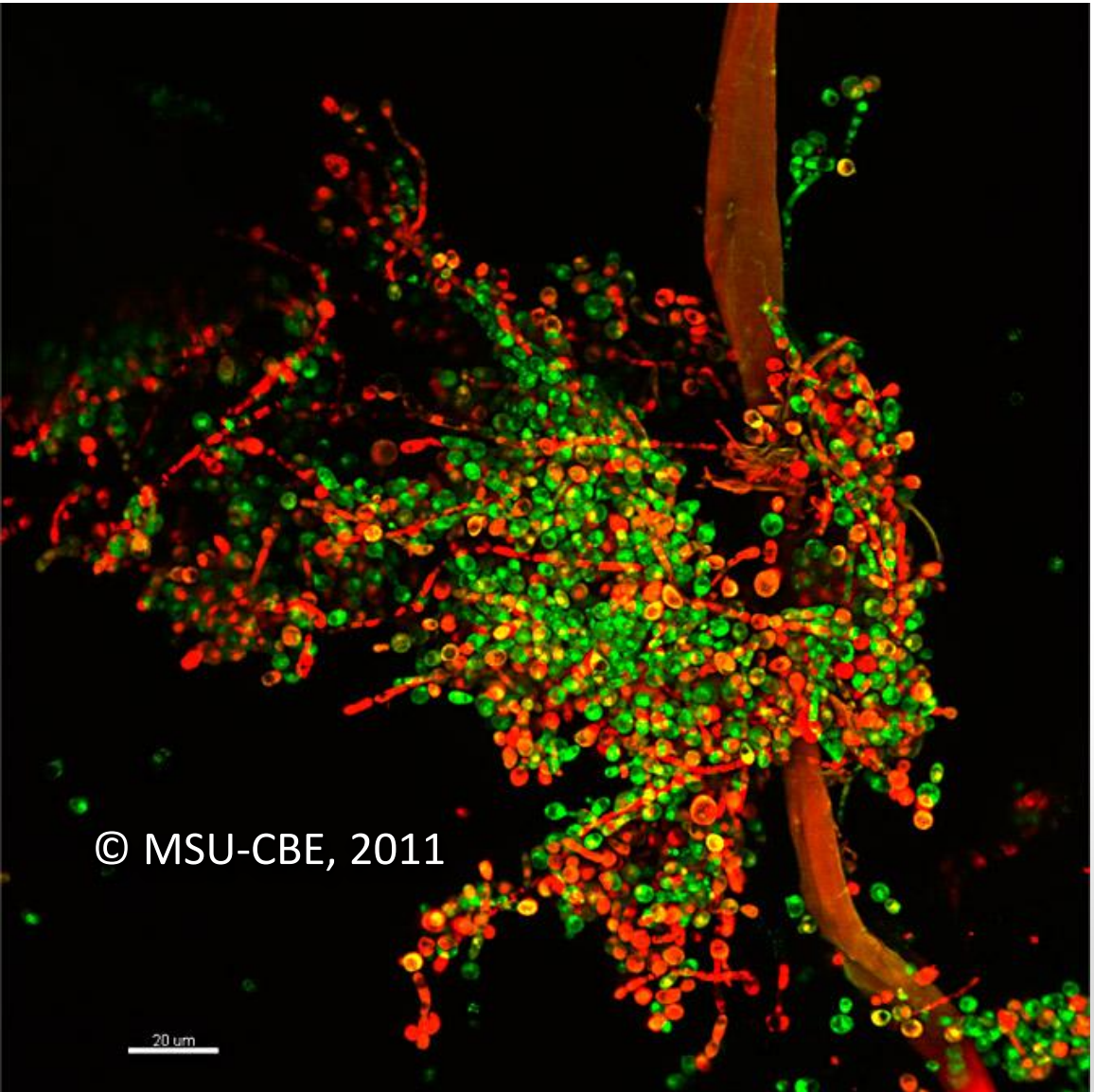
"We don't always get clear answers using stains. With the stains used for this image, live cells should be green, and dead ones red. Yellow or orange color, as shown in much of this image, indicates both live and dead staining, which is not easy to interpret."

Betsey Pitts,
CBE Microscope Facilities Manager

Tisza Bell,
CBE PhD student in microbiology and NSF- IGERT awardee

"This confocal image is of the diatom *Chaetoceros* (red) and associated bacteria (green) which we hypothesize to be a symbiont."





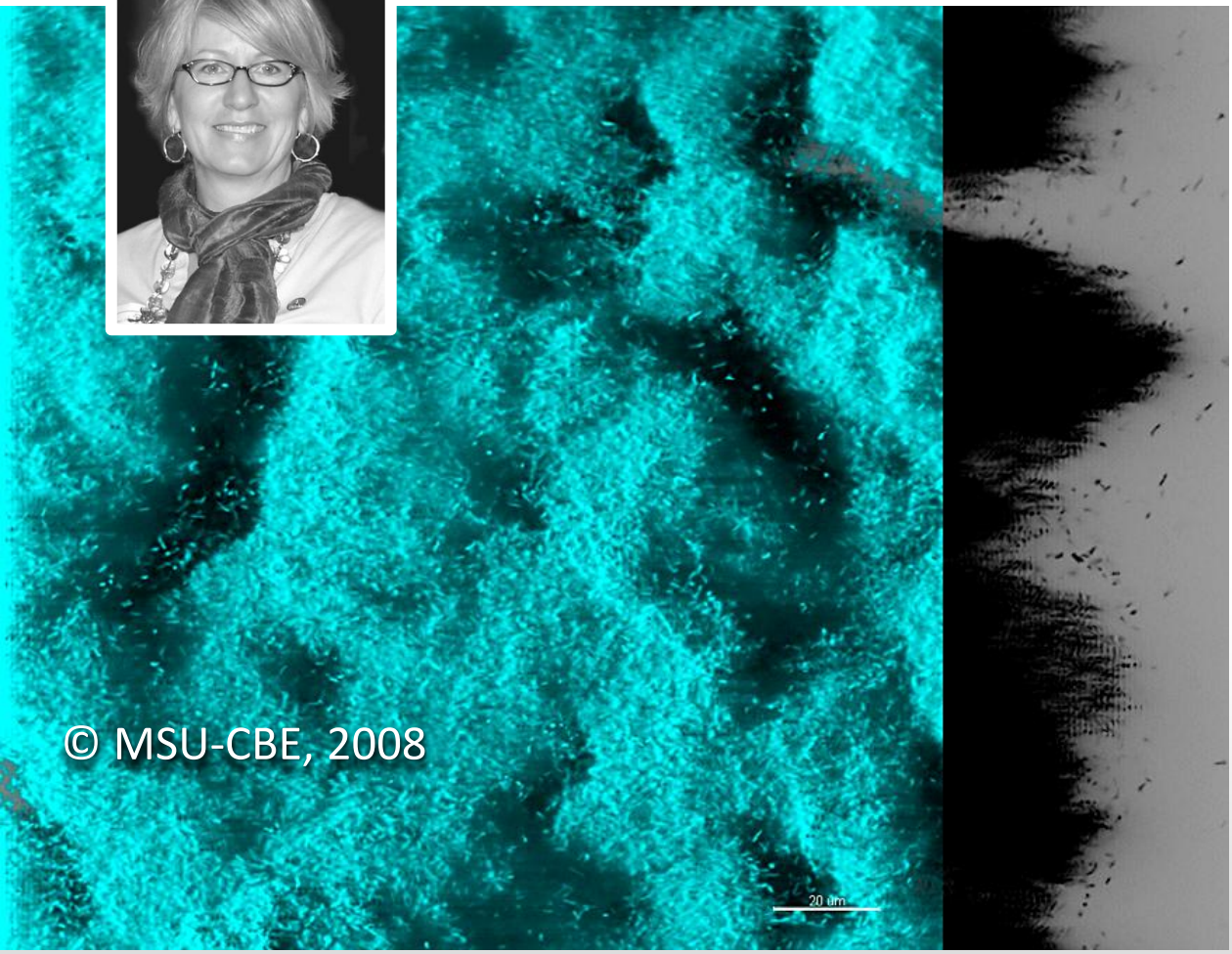
***Alessandra Agostinho**, **DDS**, CBE research scientist*

"This *Candida albicans* biofilm grown on a PMMA coupon is stained to show live cells as green and non-viable cells as red. PMMA—poly (methyl methacrylate)—is used in dental fillings, denture material, and bone cement."

Natasha Mallette, CBE PhD student in chemical and biological engineering, NSF-IGERT awardee

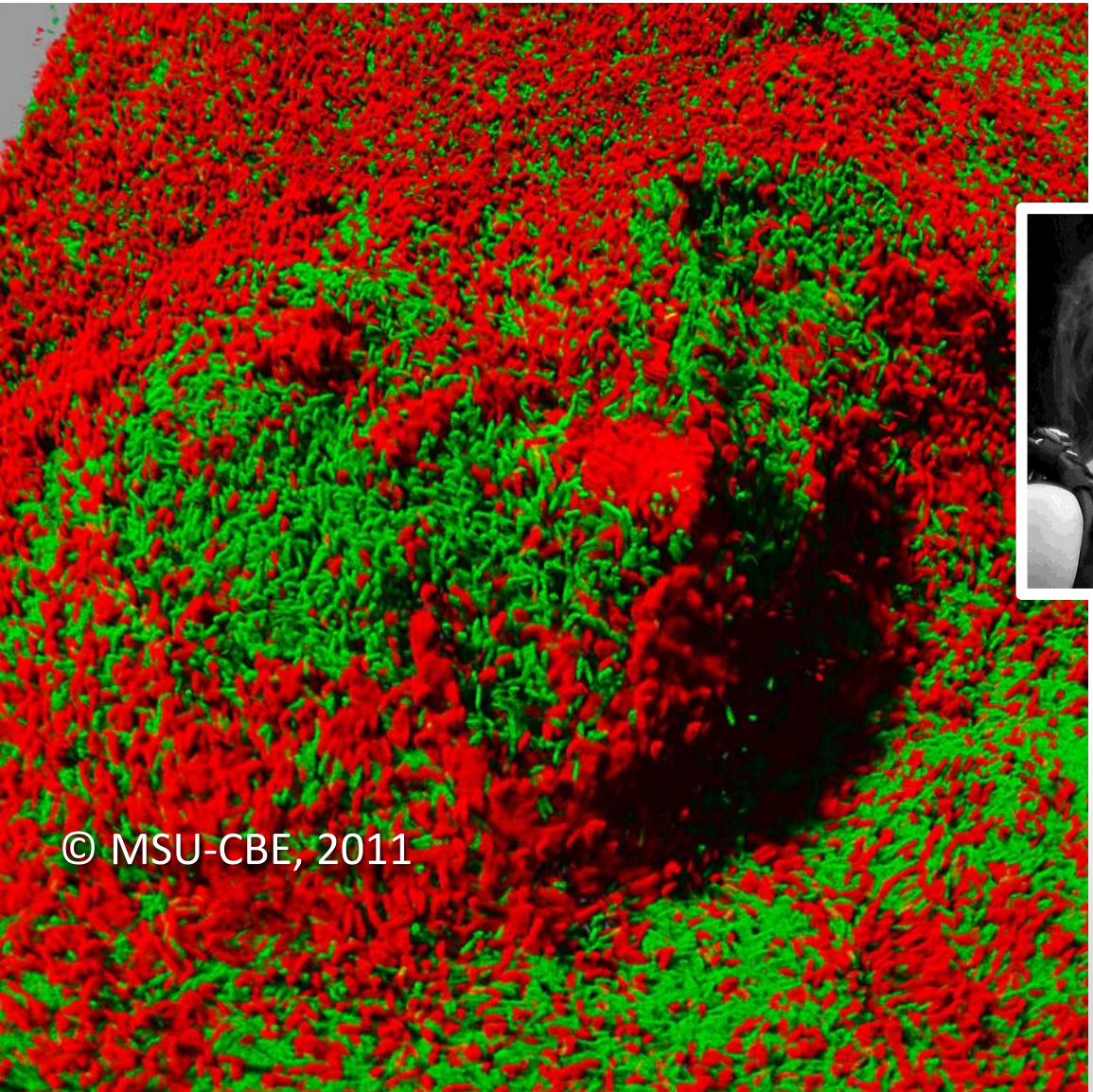
"*Ascocoryne sarcoides* (red), a filamentous fungus, growing aerobically with cellulose (green) in a submerged liquid culture. This image shows *A. sarcoides* entwined with the cellulose, holding the particles together."

20 um



Betsey Pitts,
CBE microscope facilities manager

"This 3-dimensional image of a *Pseudomonas* biofilm was constructed from a large stack of single plane confocal microscope slices through the sample. The biofilm was stained with an amine-reactive aqua fluorescent dye, and is magnified 630 times here."

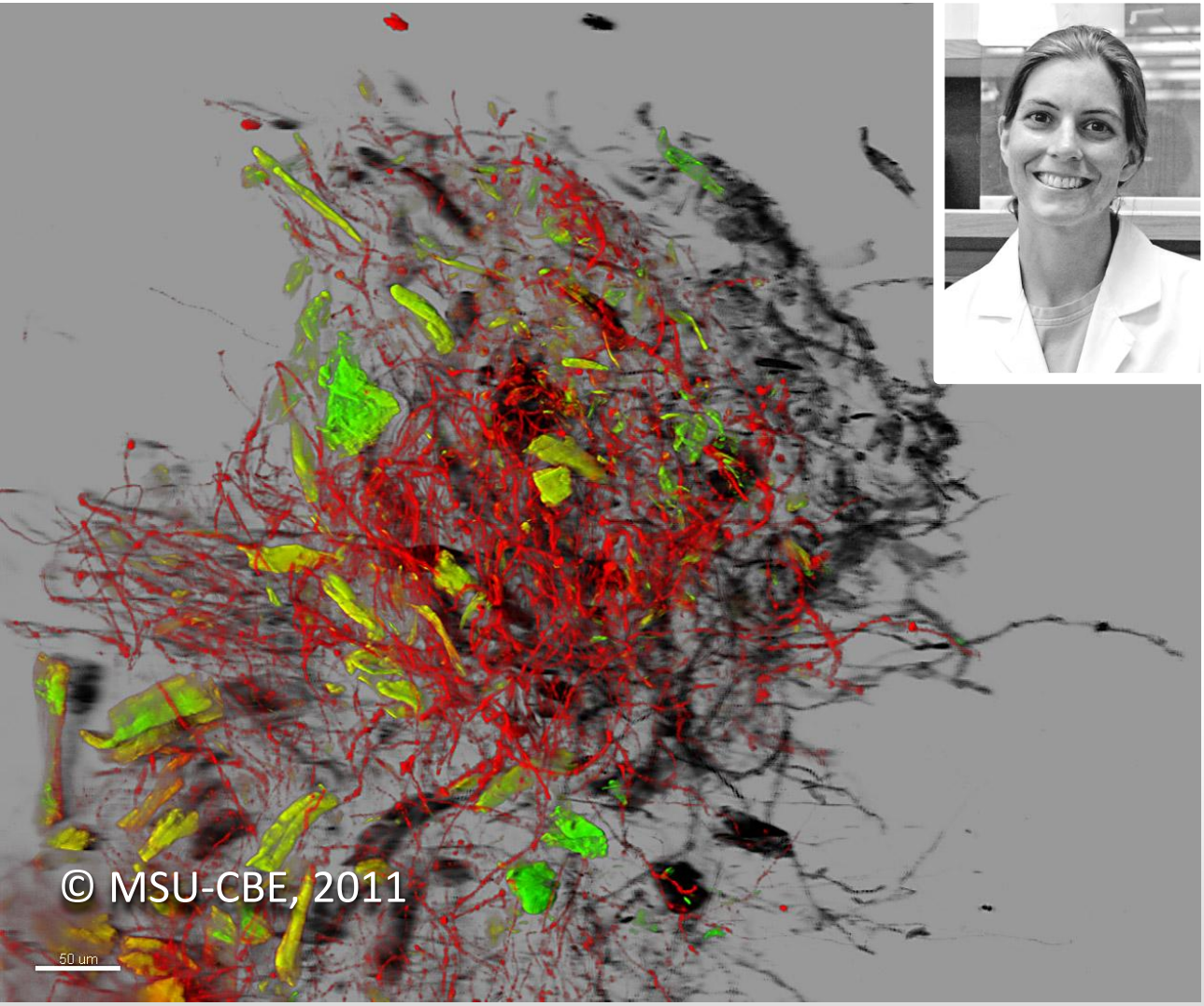


Carole Nagant,
visiting CBE researcher
Université libre de
Bruxelles, Belgium, 2011

"In this microscope image of *Pseudomonas aeruginosa* treated with a peptido-mimetic compound, the live cells appear green and the damaged cells are red."

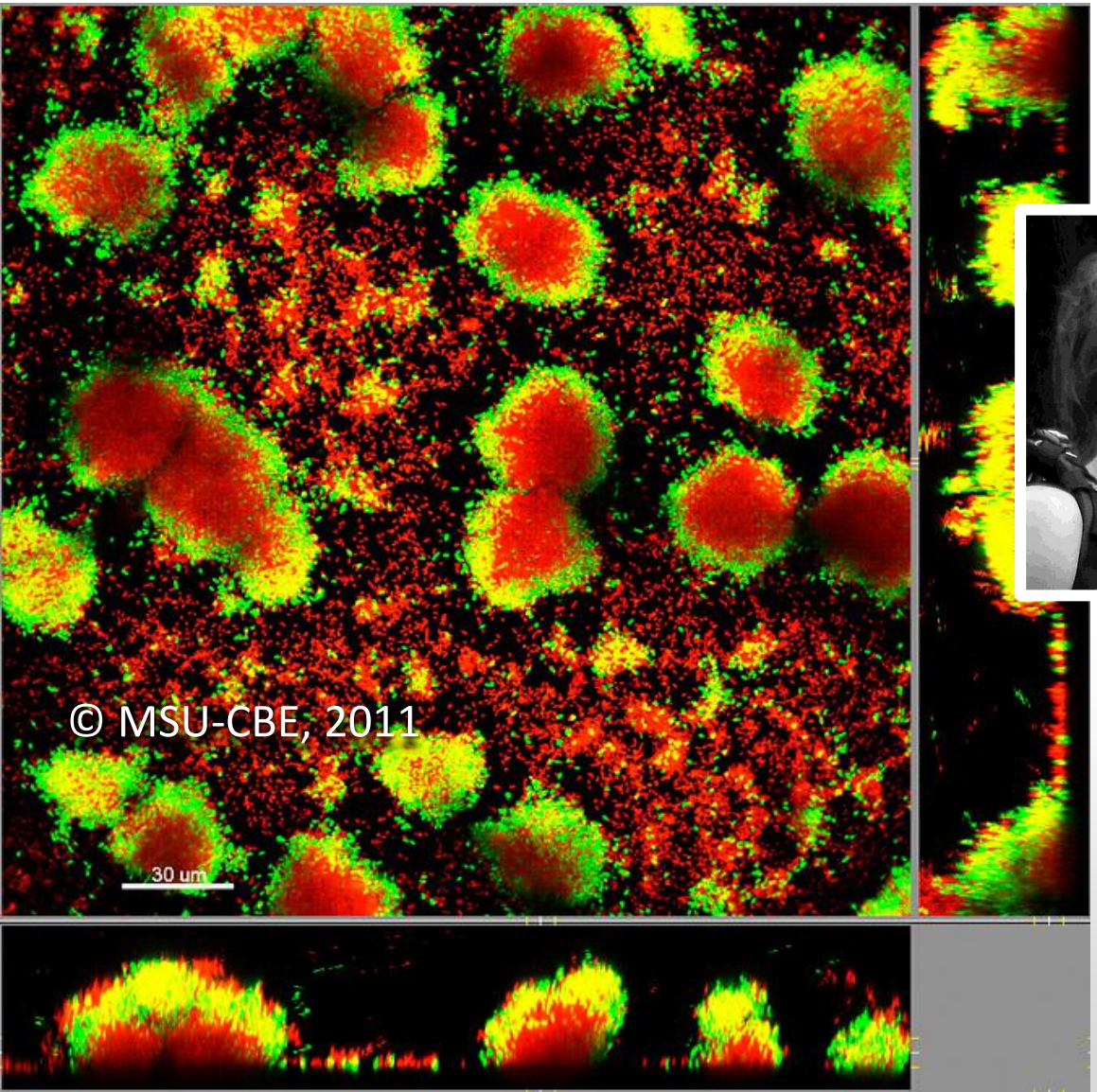
Betsey Pitts,
CBE microscope facilities manager

"Part of my job as facilities manager involves finding new fluorescent stains to use on biofilms. Stains that give information about the physiological state of bacteria in a biofilm are especially valuable. This one is an activity stain (Calcein AM Violet) which indicates that all the *Staphylococcus epidermidis* bacteria visible in this biofilm have intact membranes and are likely alive."



Natasha Mallette, CBE PhD student in chemical and biological engineering, NSF-IGERT awardee

Ascocoryne sarcoides (red), growing aerobically with cellulose (green) in a submerged liquid culture.



Carole Nagant, visiting CBE researcher Université libre de Bruxelles, Belgium, 2011

"This reconstruction shows a cut-away view of *Pseudomonas aeruginosa* ATCC 15442 micro-colonies where we can see the red cells inside are not viable (LIVE/ DEAD® BacLight™ bacterial viability kit).