

Beyond genomic

Microbiome Kickstart workshop – 2024

Penn State

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Current World Population

7,814,251,039

<https://www.worldometers.info>



Stick figure $8 \cdot 10^9 / \text{planet}$

Star $100 \cdot 10^9 / \text{Milky way}$



Circle $1 \cdot 10^9 / \text{g soil}$

$1 \cdot 10^{30} / \text{planet}$

A $1 \cdot 10^{31} / \text{planet}$

microbiome



Yana Bromberg

George Peabody Library, Baltimore, U.S.A



DNA extraction

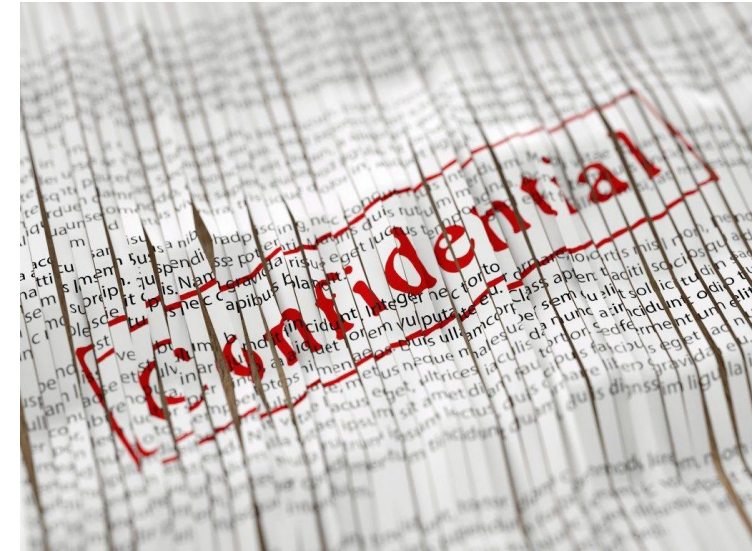
Metagenome reads



Metagenome reads



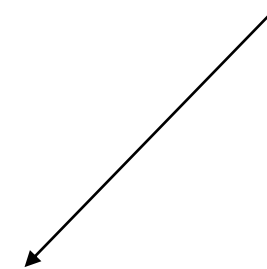
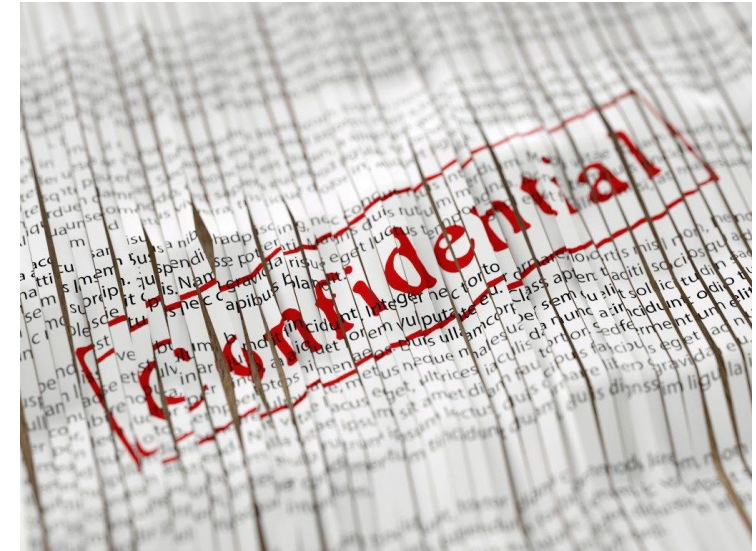
bioinformatic



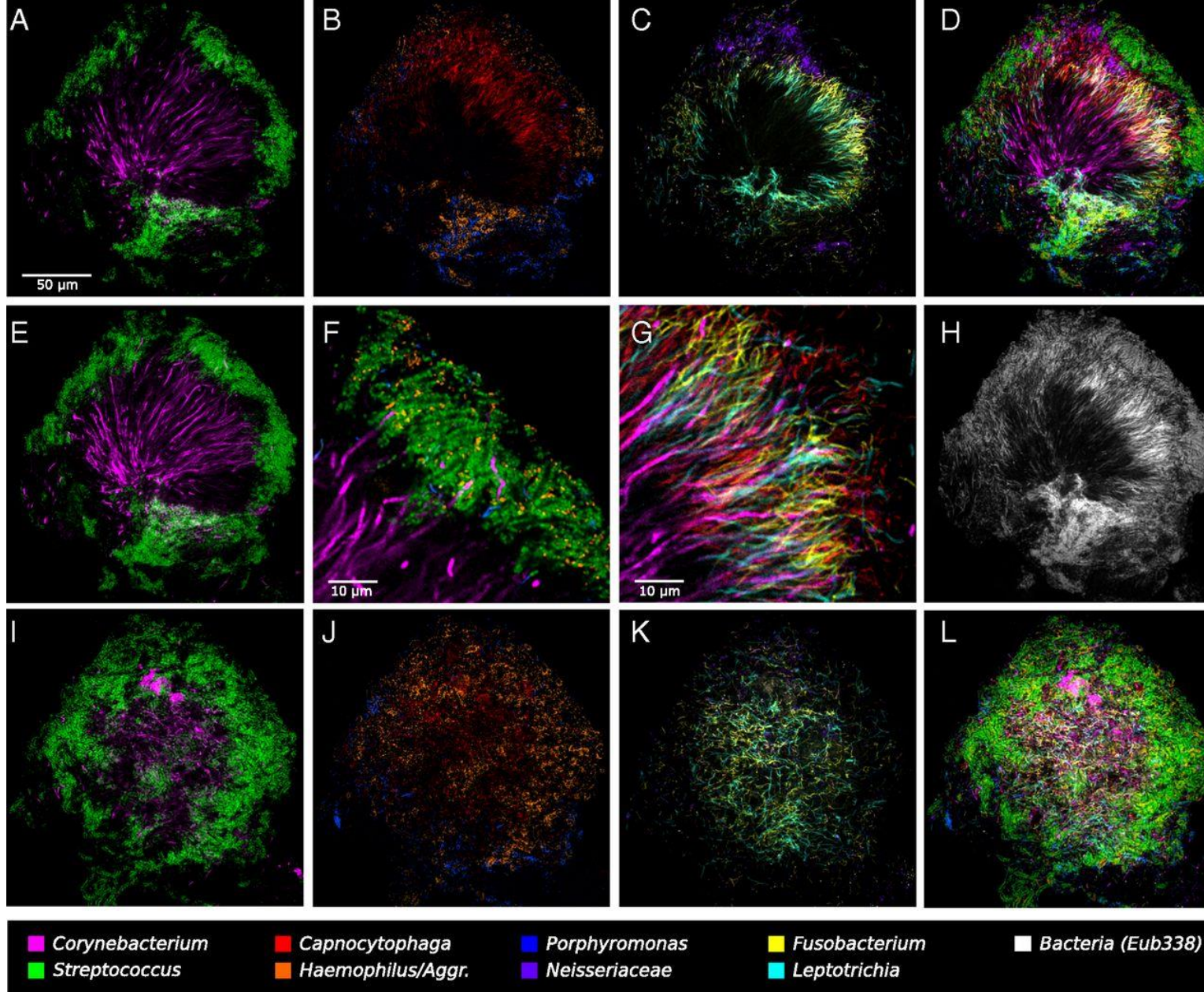
Metagenome reads



bioinformatic



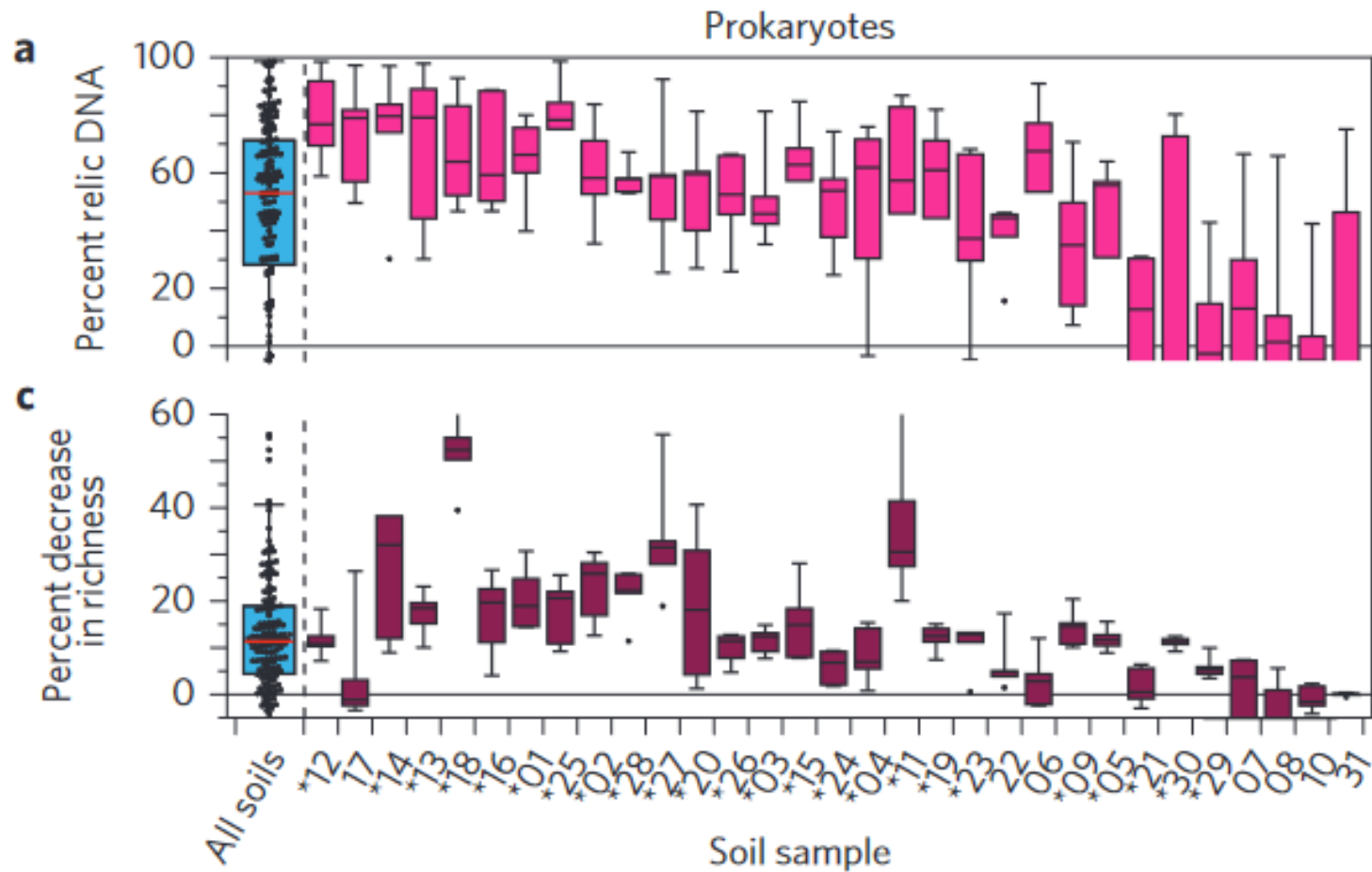
metagenome assembled genomes
MAG



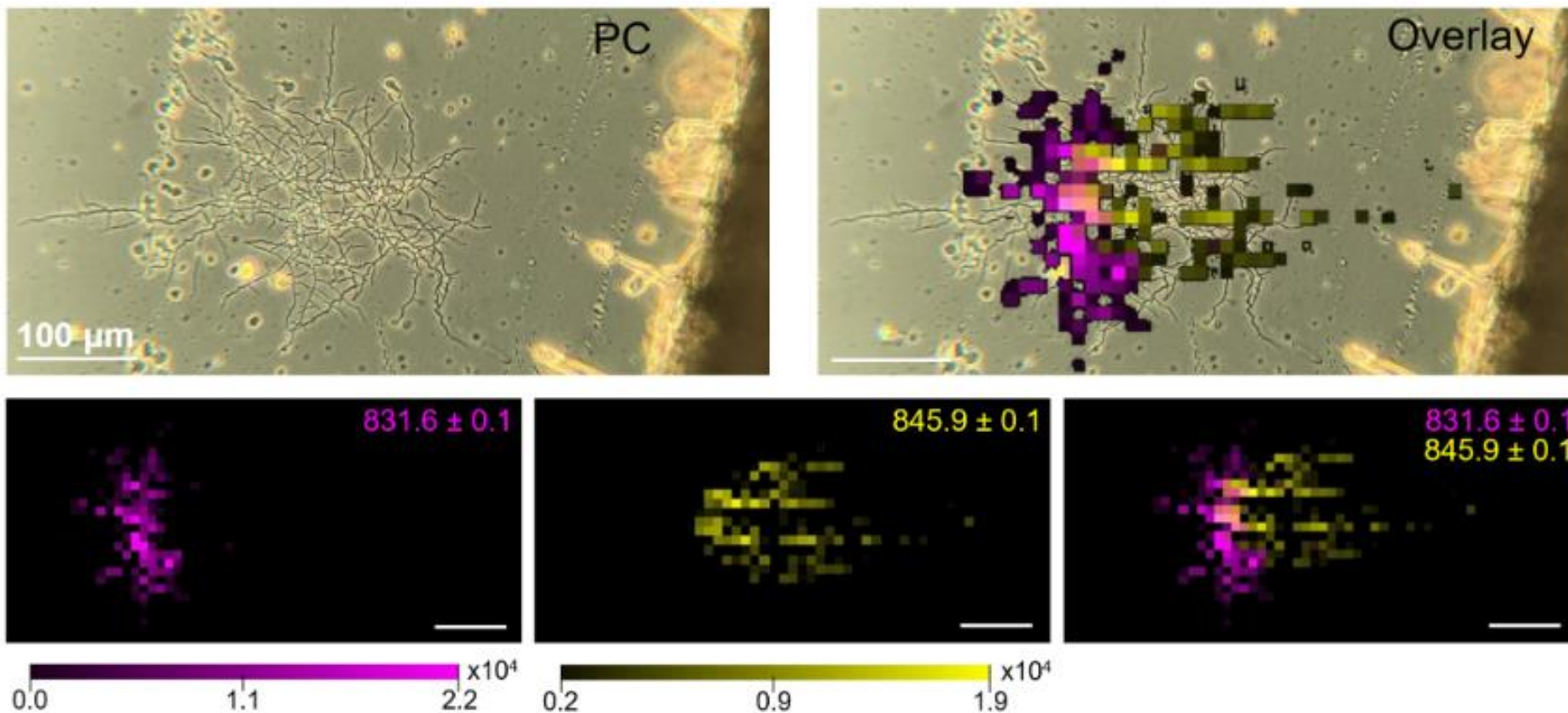
Biogeography of a microbiome at micron scale

Jessica L. Mark Welch, Blair J. Rossetti, Christopher W. Rieken, Floyd E. Dewhirst, Gary G. Borisy

PNAS Feb 2016, 113 (6) E791-E800; DOI: 10.1073/pnas.1522149113



Carini, P., Marsden, P., Leff, J. *et al.* Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. *Nat Microbiol* **2**, 16242 (2017).
<https://doi.org/10.1038/nmicrobiol.2016.242>

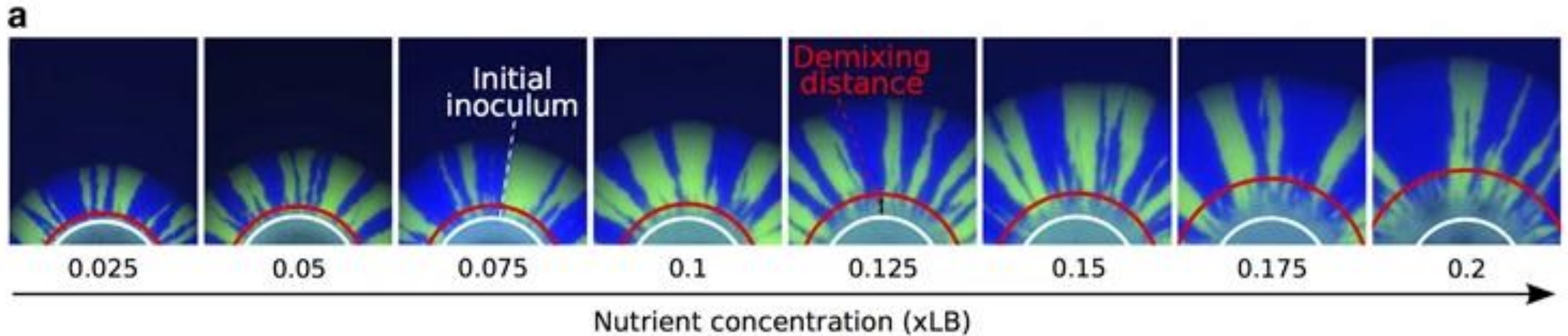


High Spatial Resolution Imaging Mass Spectrometry Reveals Chemical Heterogeneity Across Bacterial Microcolonies

Rita de Cassia Pessotti, Bridget L. Hansen, Vineetha M. Zacharia, Daniel Polyakov, and Matthew F. Traxler

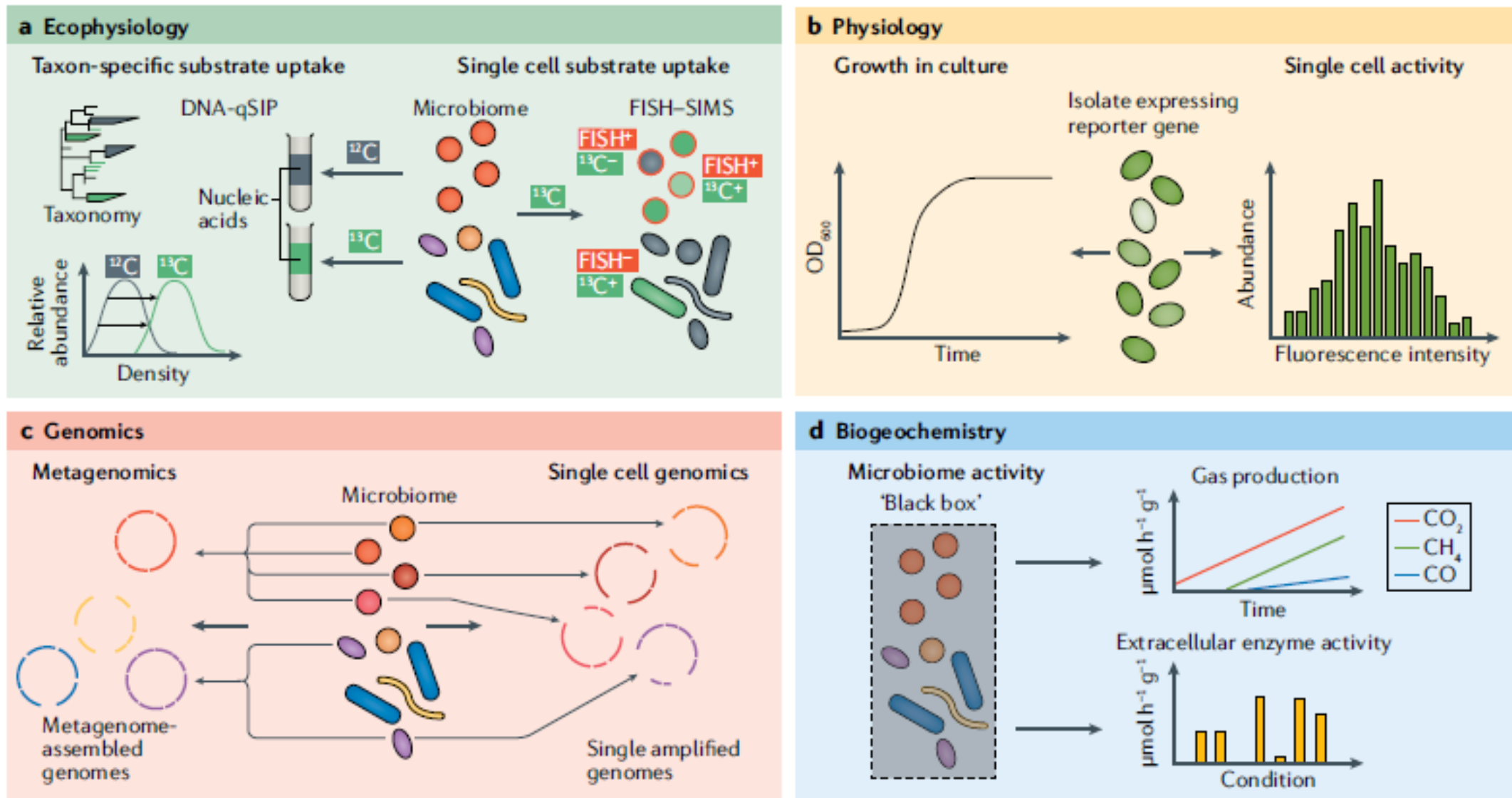
Analytical Chemistry **2019** 91 (23), 14818-14823

DOI: 10.1021/acs.analchem.9b03909

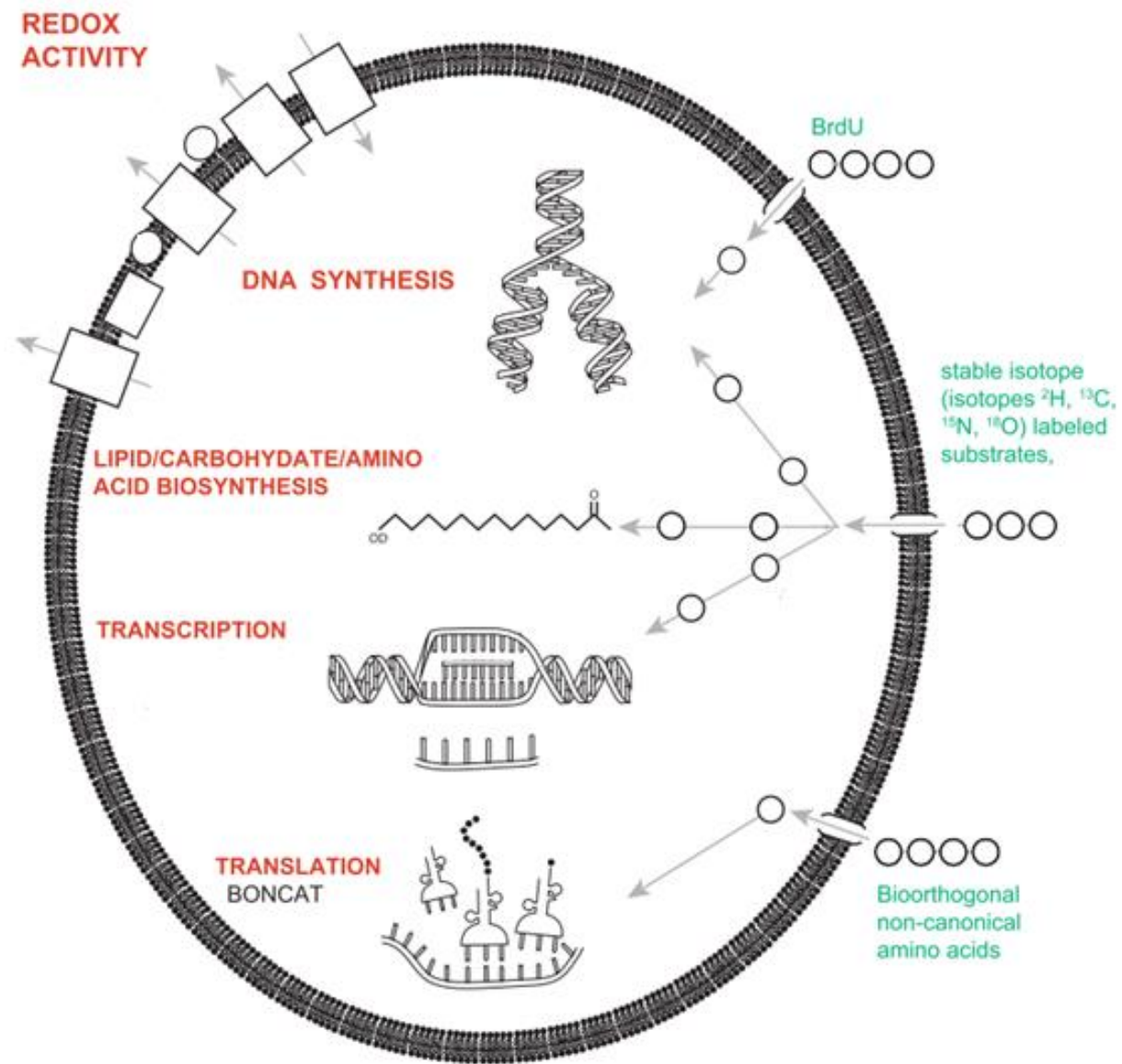


P. aeruginosa colonies grown from a 1:1 mixture of YFP- and CFP-labeled cells.

Mitri, S., Clarke, E. & Foster, K. Resource limitation drives spatial organization in microbial groups. *ISME J* **10**, 1471–1482 (2016). <https://doi.org/10.1038/ismej.2015.208>

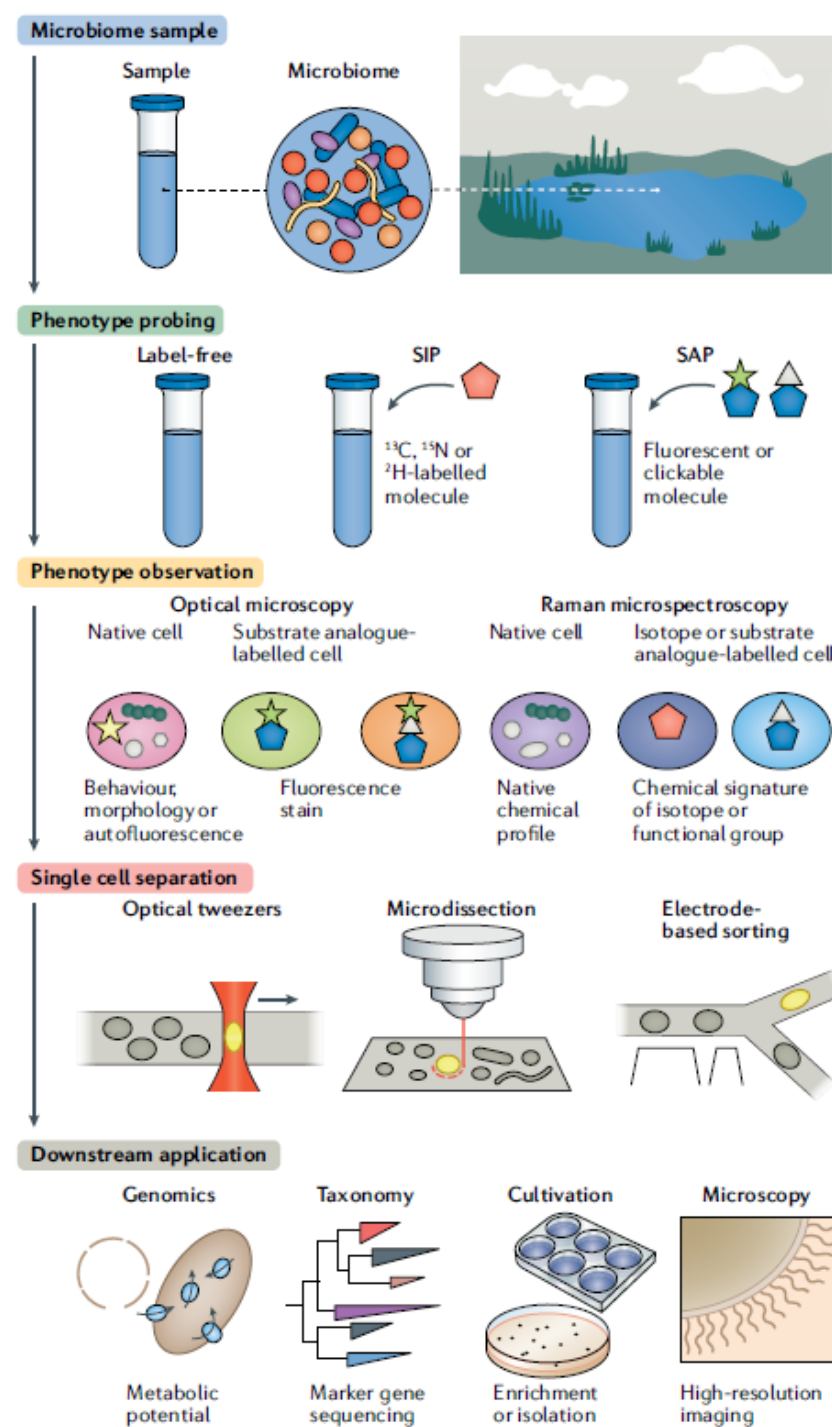


Hatzenpichler, R., Krukenberg, V., Spietz, R.L. *et al.* Next-generation physiology approaches to study microbiome function at single cell level. *Nat Rev Microbiol* **18**, 241–256 (2020). <https://doi.org/10.1038/s41579-020-0323-1>



modified from Singer et al. 2016

Next-generation physiology



Hatzenpichler, R., Krukenberg, V., Spietz, R.L. *et al.* Next-generation physiology approaches to study microbiome function at single cell level. *Nat Rev Microbiol* **18**, 241–256 (2020).
<https://doi.org/10.1038/s41579-020-0323-1>

One Health Microbiome Center Resources

<https://www.huck.psu.edu/institutes-and-centers/microbiome-center/member-resources/microbiome-research-resources>

-Research Collaboratory (Bisanz lab)

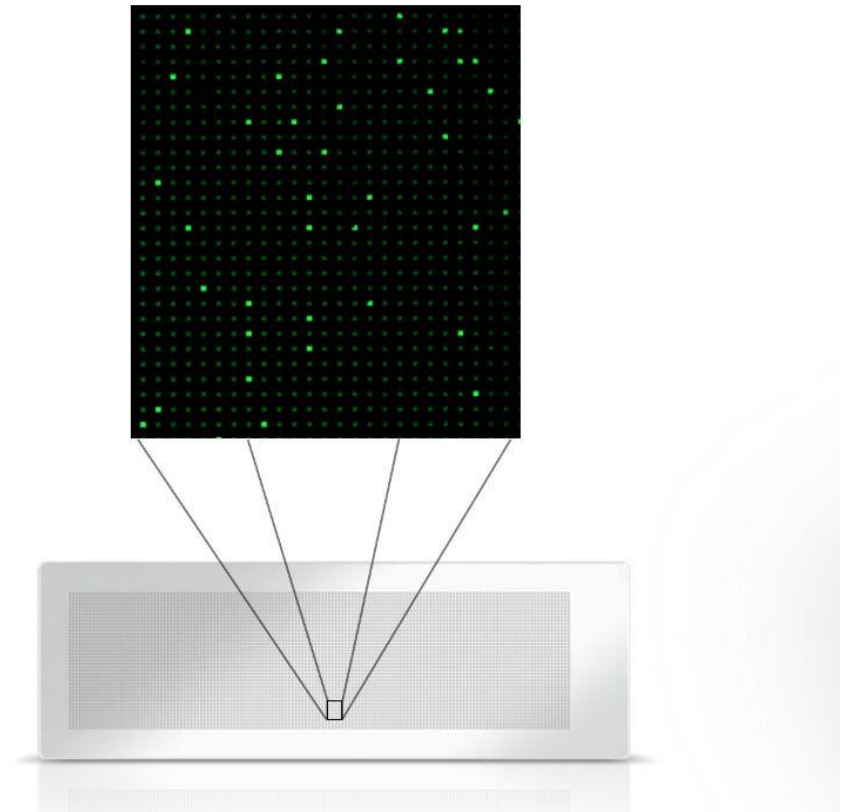
-Echo microscope



Galt – Prospector (Buckout lab)

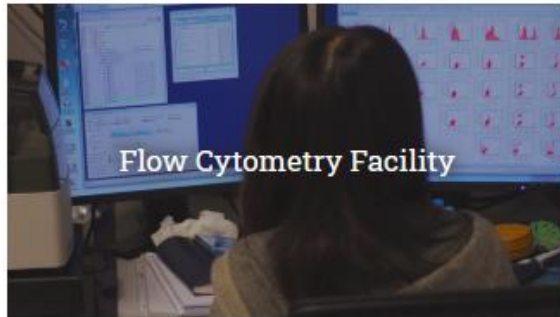


Prospector system; General Automation Lab Technologies Inc.



High-throughput microbial cultures

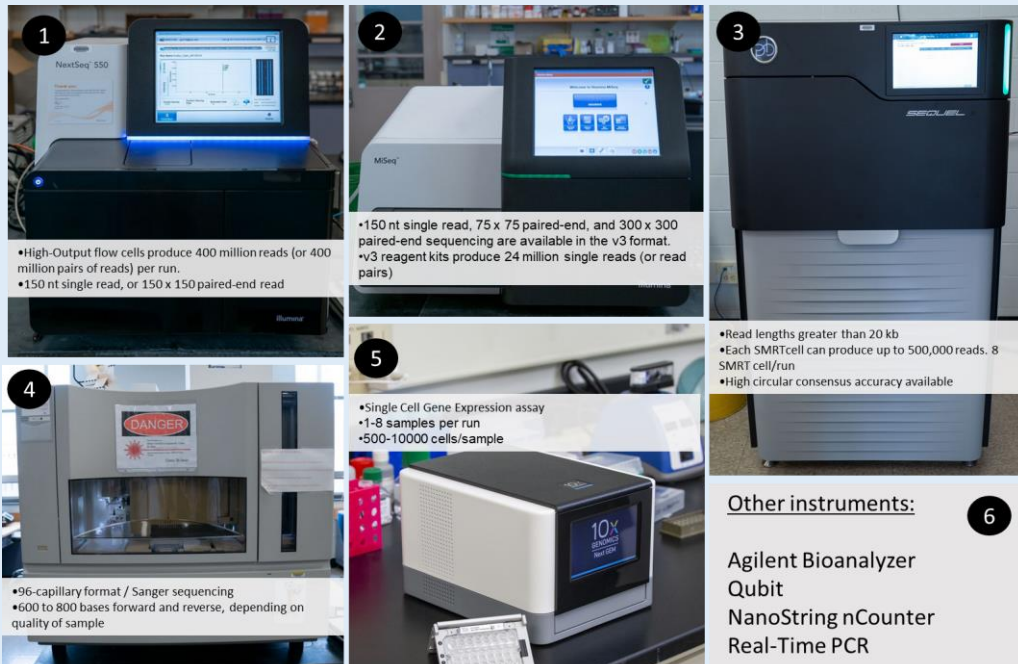
Huck Core Facilities



Genomics & Transcriptomics

Location: 412 Chandlee Lab

INSTRUMENTS



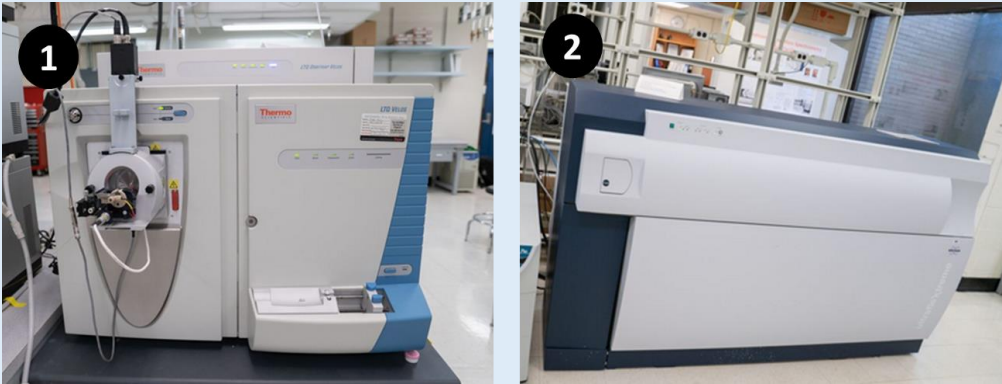
APPLICATIONS

- Full genome sequencing
- Metagenomic + genome binning
- Metatranscriptomics (under varied conditions) + reads mapping
- 16S rRNA gene, ITS and other amplicons sequencing
- pure product sequencing (single colony PCR or clone)

Proteomics

Location: 3 Althouse lab

INSTRUMENTS



(1) Thermo LTQ Orbitrap – nano-flow 2D LC System, (2) Bruker ultrafleXtreme MALDI-TOF/TOF

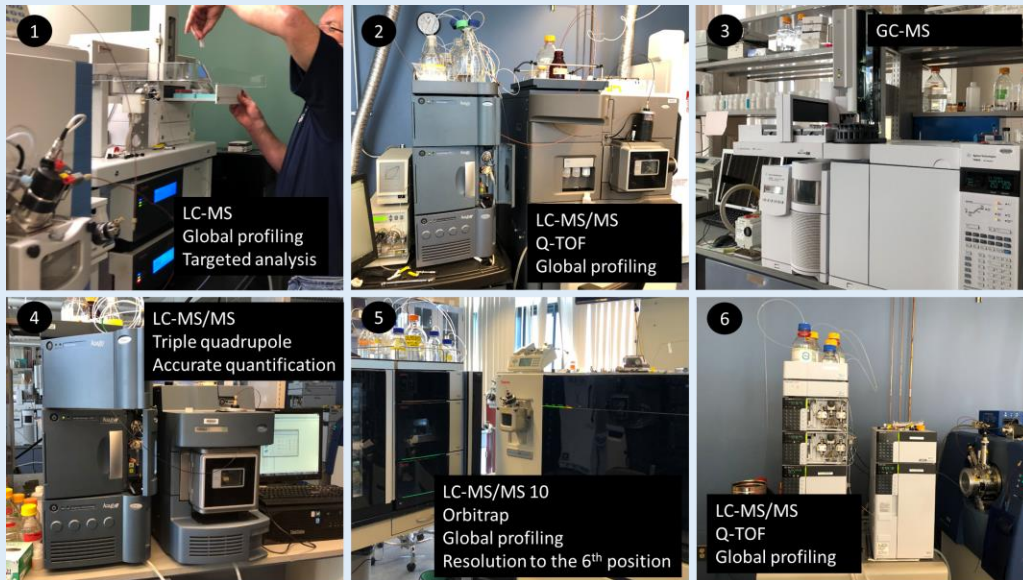
APPLICATIONS

- Microbial identification – biotyper
- Exploring microbiome proteomes under varied condition / link diversity to enzymatic functions
- Identifying new proteins – bioactive peptides

Metabolomics

Location: Huck Life Sciences Building, first floor

INSTRUMENTS



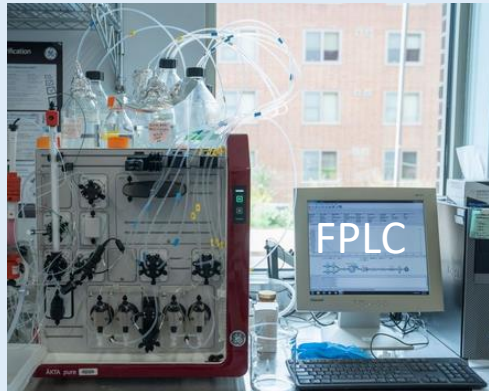
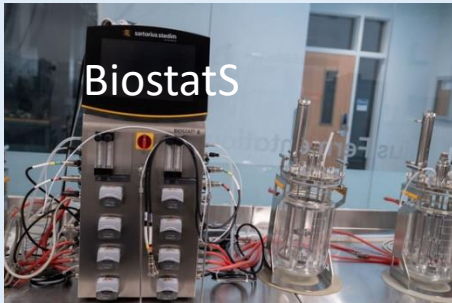
APPLICATIONS

- Microbial rapid identification
- Probing microbe-microbe interactions through the exchange of metabolites
- Synthesizing defined culture media
- Surveying microbial population under varied condition
- Identifying/quantifying new natural product of interest
- Tracking the fate of a known compound

CLS Behring Fermentation

Location: 114 Agricultural Engineering Building

INSTRUMENTS



Freeze drier
Tube centrifuge...

APPLICATIONS

- Produce large quantity of microbial inoculant for environmental restoration
- Produce and purify proteins/products of interest – such as natural products

Microscopy

Location: N048 Millennium Science Complex

INSTRUMENTS

Type	#	Name	Type	Application
Dissecting	1	Olympus SZ-PT	Dissecting	sample dissecting, trimming
	2	Olympus SZ-ST	Dissecting	sample dissecting, trimming
	3	Zeiss SteREO	Dissecting	sample dissecting, trimming
Fluo Stereo	4	Zeiss AxioZoom V16	Fluorescence stereozoom	Fluorescence imaging, confocal like-optical slicing
Wide-field	5	Olympus BX50	Wide-field, upright	BF; Slide scanning
	6	Olympus BX51	Wide-field, upright	BF/FL/Pola,
	7	Olympus BX60	Wide-field, upright	BF/DIC, slide scanning
	8	Olympus BX61	Wide-field, upright	BF/FL/DIC/Pola/Reflection/motorized
	9	Keyence	Wide-field, inverted	BF/FL/phase, motorized, live-cell. All-in-one
Laser Capture	10	Zeiss Axio Observer	Wide-field, inverted	BF/FL/phase
Confocal	11	Olympus FV1000	Confocal, inverted	BF/FL/DIC; 40 water, 60x, 100x
	12	Zeiss LSM88 Airyscan	Confocal, inverted	BF/FL/DIC; 40 W, 40 O, 63 O, 100 O,
	13	Olympus FV10i	Confocal, inverted	BF/FL/Phase; live-cell, motorized, 10x 60x water
Multiphoton	14	Leica DIVE	Multiphoton, upright	MP lens, motorized lens
Super Res	15	Nikon N-SIM/STORM	Super Resolution	Resolution 110 nm (SIM) and 20 nm (STORM)
	16	BioVision VT-iSIM	Super Resolution	Resolution 120 nm, fast imaging (live cell)
EM	17	FEI Spirit G2	TEM	Thin section, cryo, tomo
	18	Zeiss Sigma	VP-FESEM	low vacuum, EDS, serial block-face imaging, cryoSEM, low T control

APPLICATIONS

- Imaging of live cells, cell counting
- Imaging of fluorescent cells / or fluorescently labelled cells
- FISH fluorescent in situ hybridization and variation inc. *in situ* transcriptomics
- Use of various fluorophore inc. live/dead, ratiometric dyes to measure intracellular pH or Ca concentration for instance
- Imaging cells environment, inc. mineral and organic associated to the cell and map elements
- Sectioning and visualizing intracellular organization of microbes, biofilms and colonies

Flow Cytometry

Location: W124A Millennium Science Complex

INSTRUMENTS



Flow cytometry (1 & 5), cell sorting - FACS (2 & 4), flow imaging (3)

APPLICATIONS

- Evaluation of fluorescence using live-dead staining
- Sorting microbial populations of interest (based on size, fluorescence etc..)
- Sorting single cells (for single cell genomics) sorting cell populations (for mini-metagenomes or metagenomes)
- Sorting cells + their associated viruses
- Absolute count of cells in a sample (cell per ul of sample)

and everything else....

EESL CORE FACILITIES

- Center for quantitative imaging
- Deployable equipment
- Organics laboratory
- Radiocarbon laboratory
- Solar Energy laboratory
- Water quality laboratory

<https://iee.psu.edu/labs>

Runs the **EESL Green Student Seed Grant**
Competition (Proposal due in March)

MCL CORE FACILITIES

- Atomic force microscopy (AFM)
- Focused Ion Beam
- Infrared spectroscopy
- Raman spectroscopy
- Particle size analysis
- Zeta potential
- TEM/SEM and more....

<https://www.mri.psu.edu/materials-characterization-lab/mcl-characterization-techniques>