Beyond genomic

Microbiome Kickstart workshop - 2021

estelle@psu.edu

Current World Population

7,814,251,039

https://www.worldometers.info

8.10^9/planet

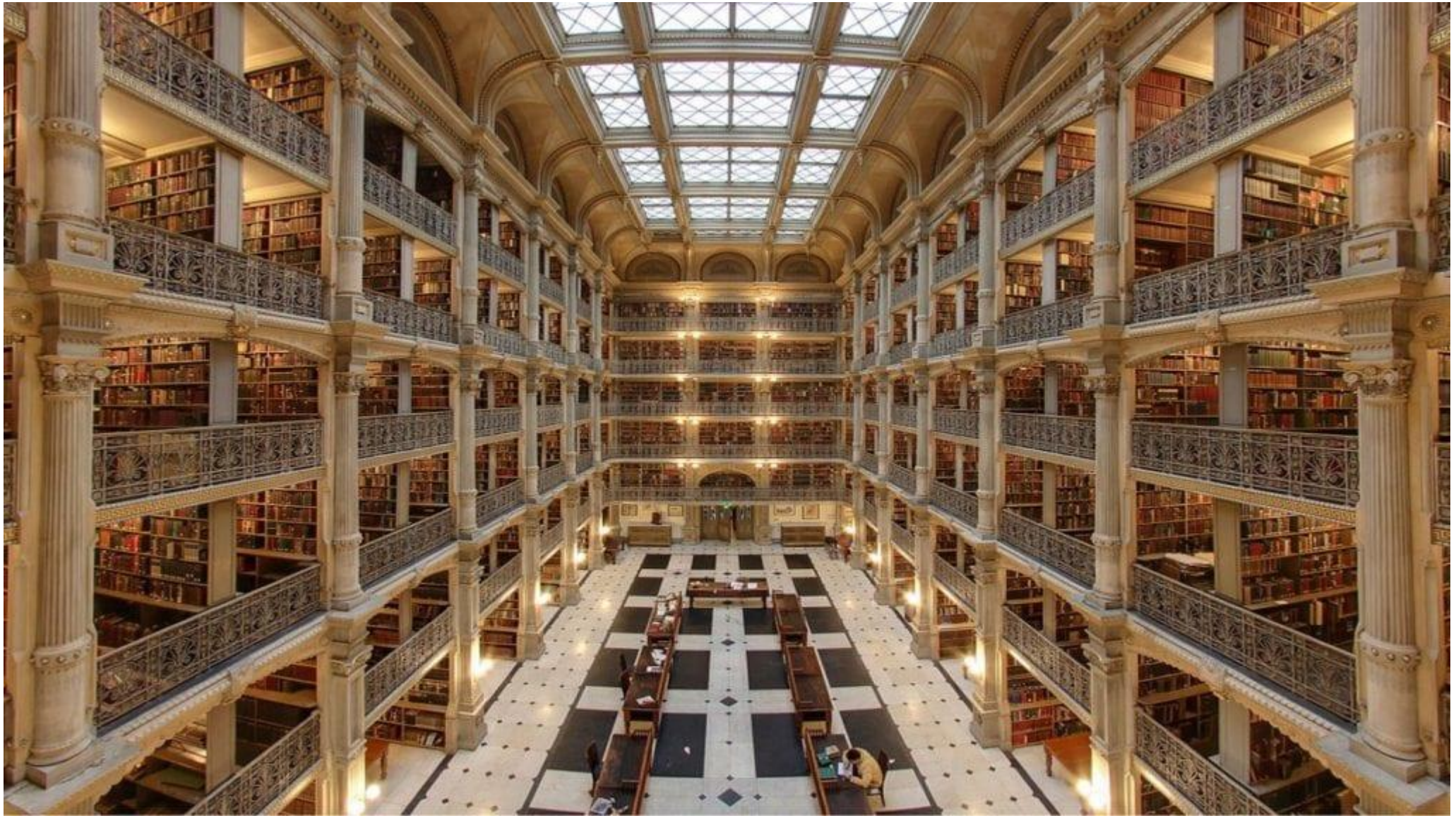
100.10^9/Milky way

1.10^9/g soil

1.10^{30}/planet

1.10^{31}/planet

microbiome



Yana Bromberg

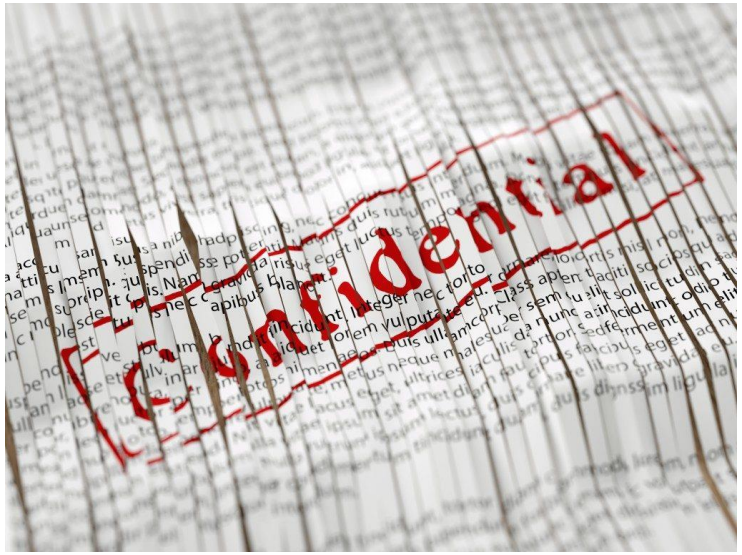
George Peabody Library, Baltimore, U.S.A


DNA extraction

Metagenome reads

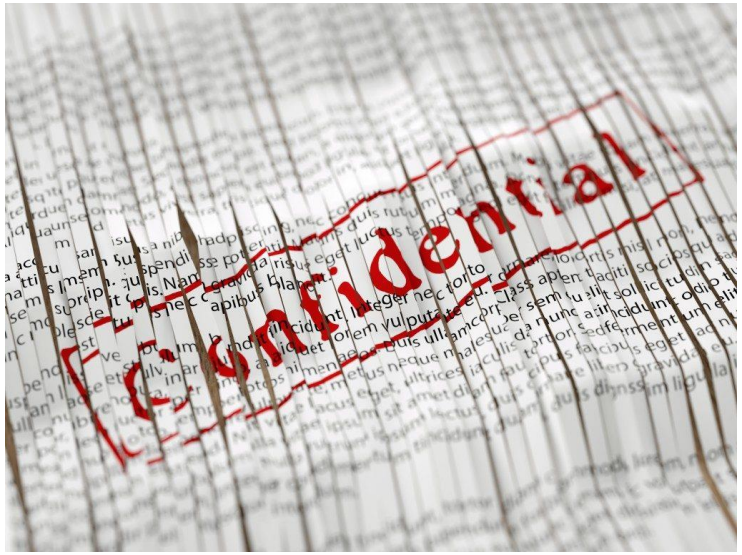
Metagenome reads

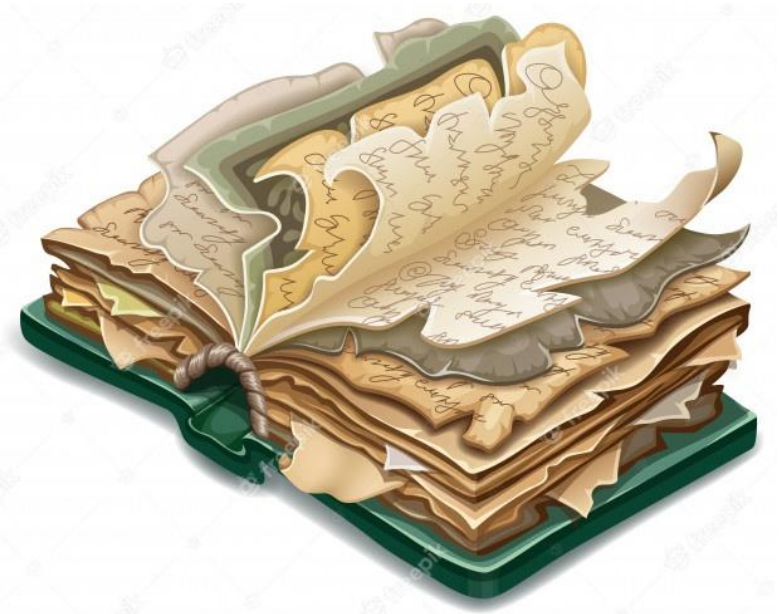
bioinformatic



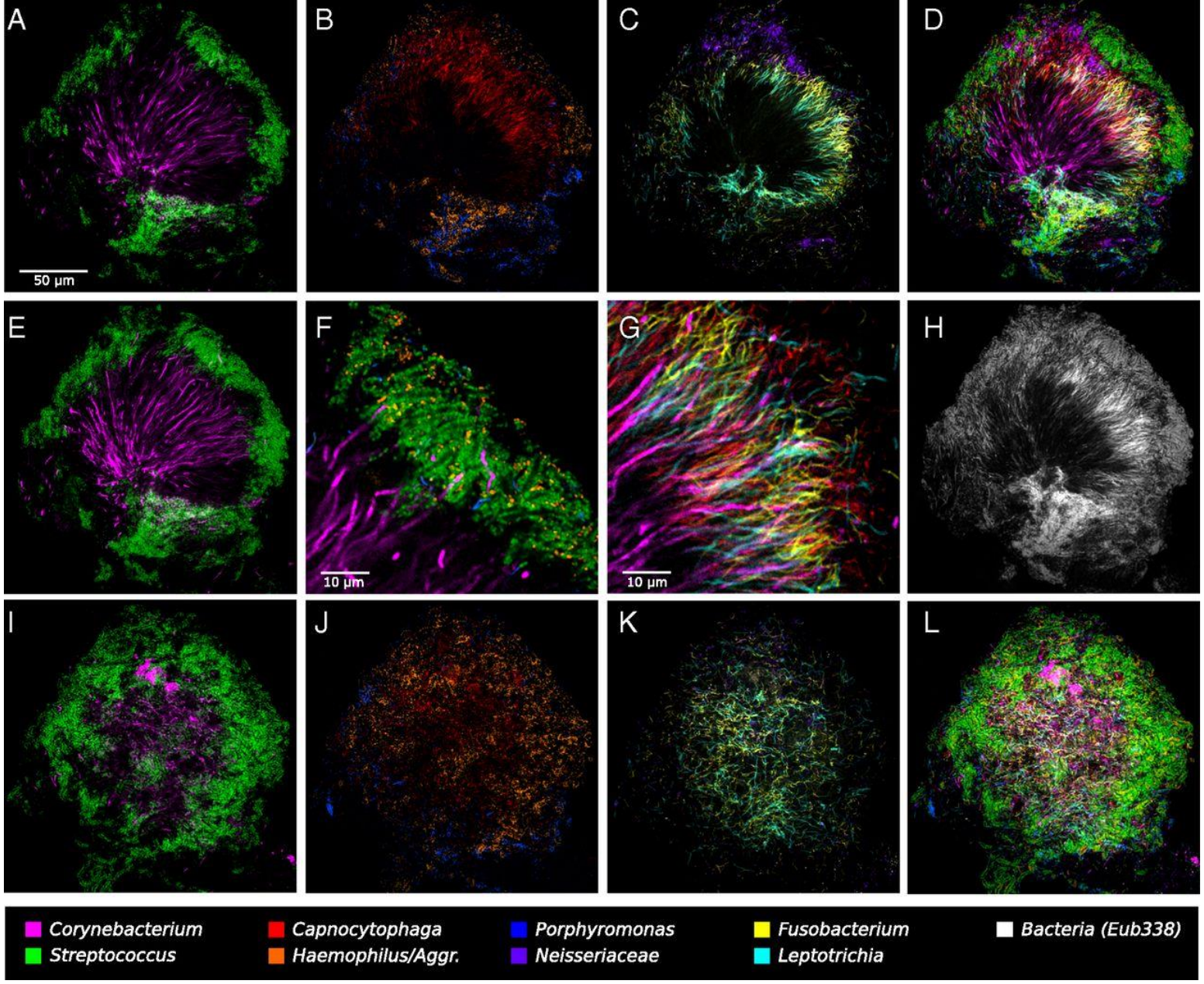
Metagenome reads

bioinformatic

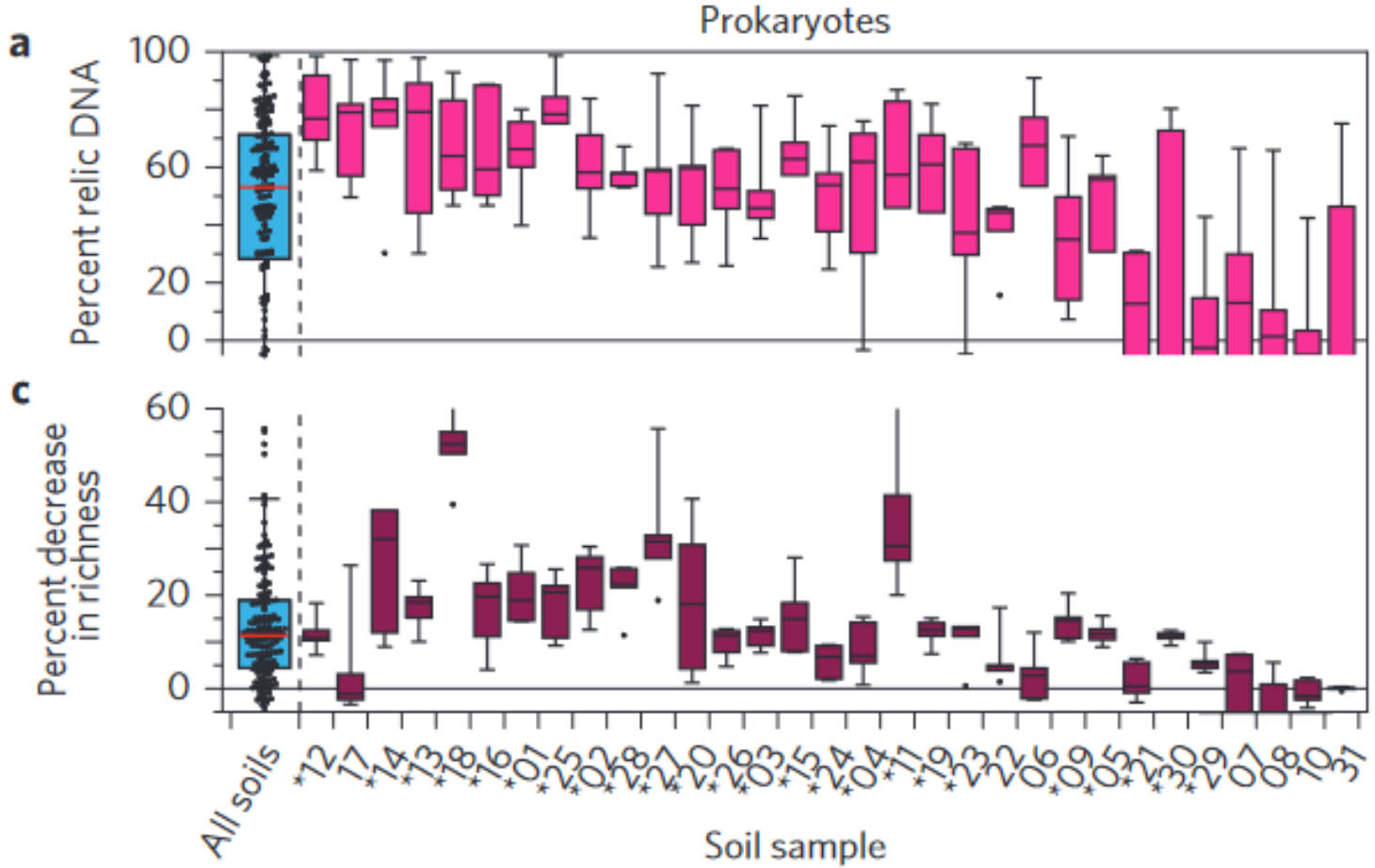




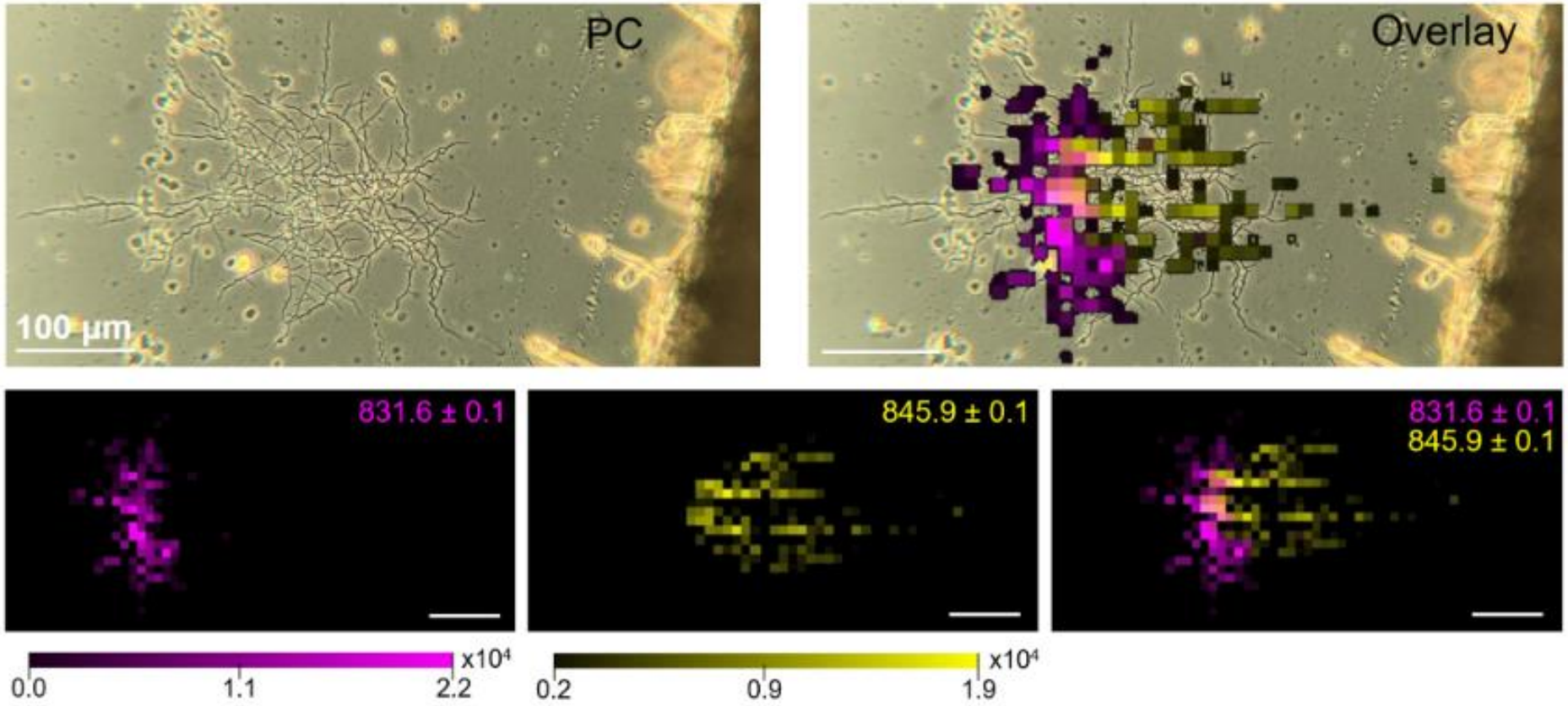
metagenome assembled genomes
MAG



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



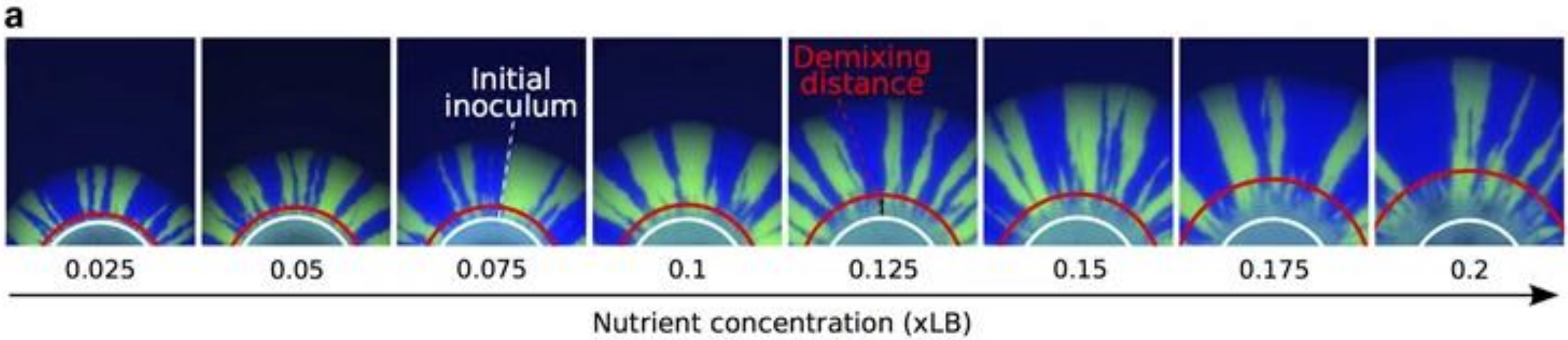
Carini, P., Marsden, P., Leff, J. *et al.* Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. *Nat Microbiol* **2,** 16242 (2017). https://doi.org/10.1038/nmicrobiol.2016.242



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

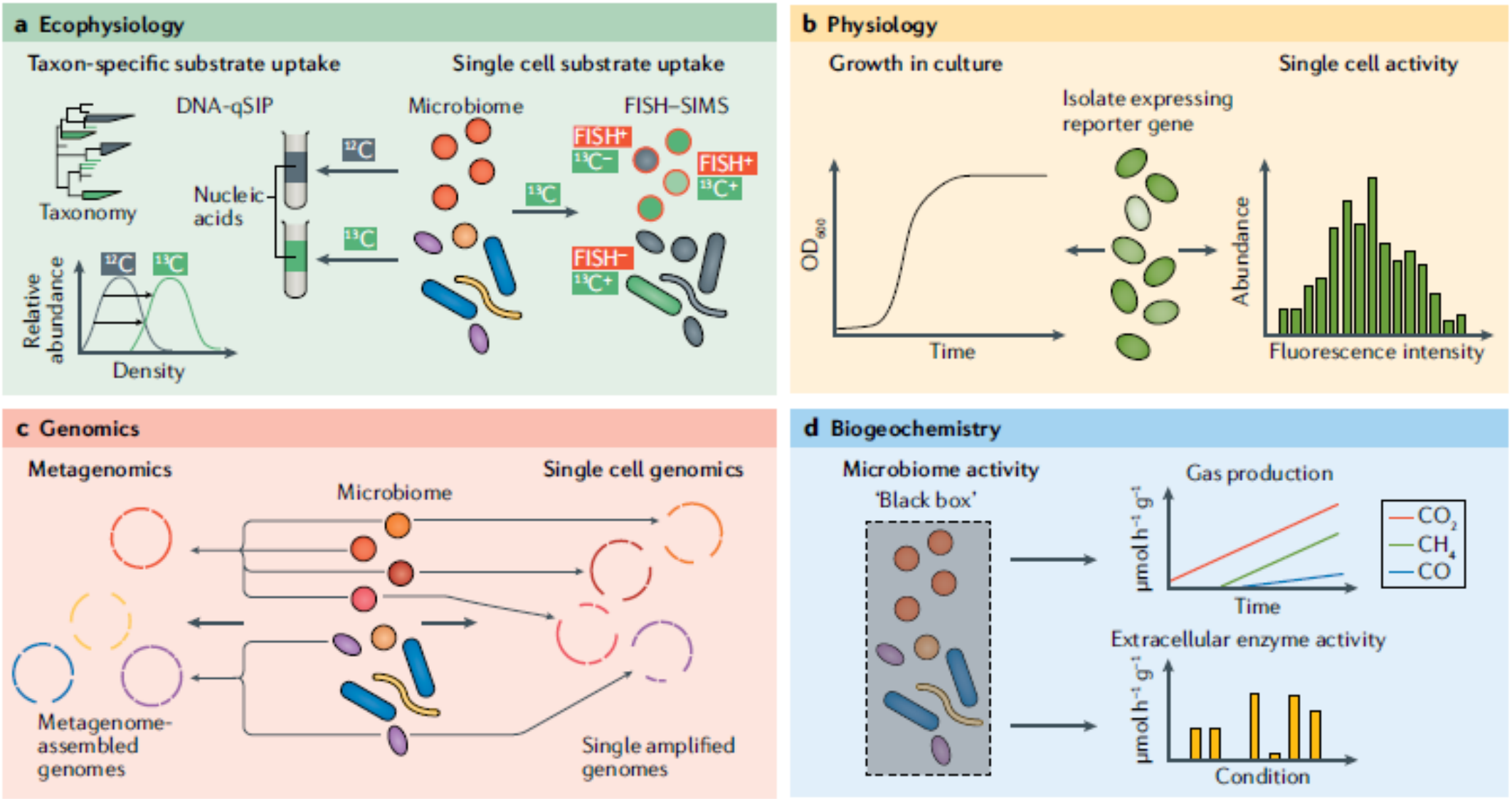
Rita de Cassia Pessotti, Bridget L. Hansen, Vineetha M. Zacharia, Daniel Polyakov, and Matthew F. Traxler

Analytical Chemistry **2019** *91* (23), 14818-14823
DOI: 10.1021/acs.analchem.9b03909

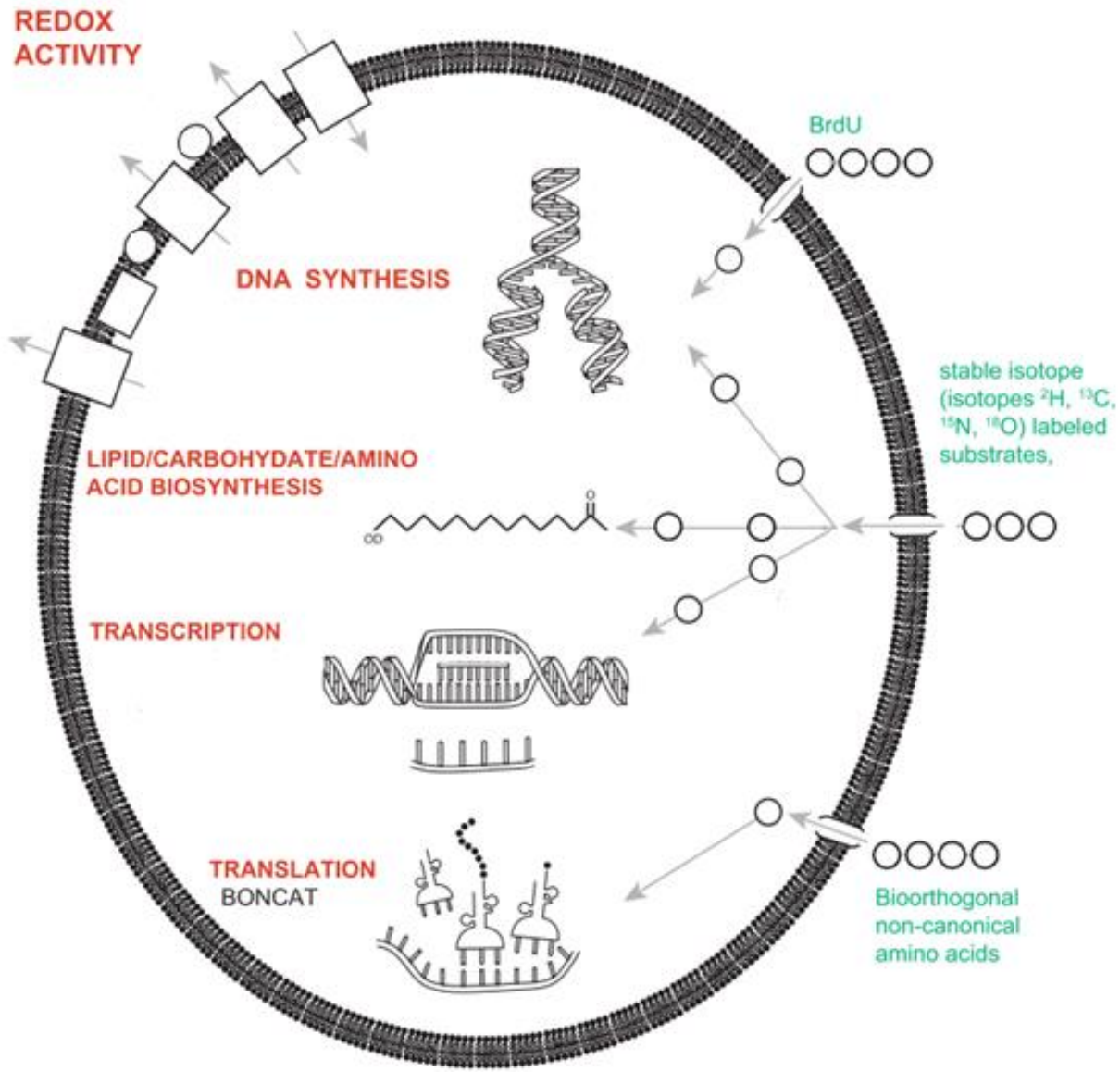


P. aeruginosa colonies grown from a 1:1 mixture of YFP- and CFP-labeled cells.

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

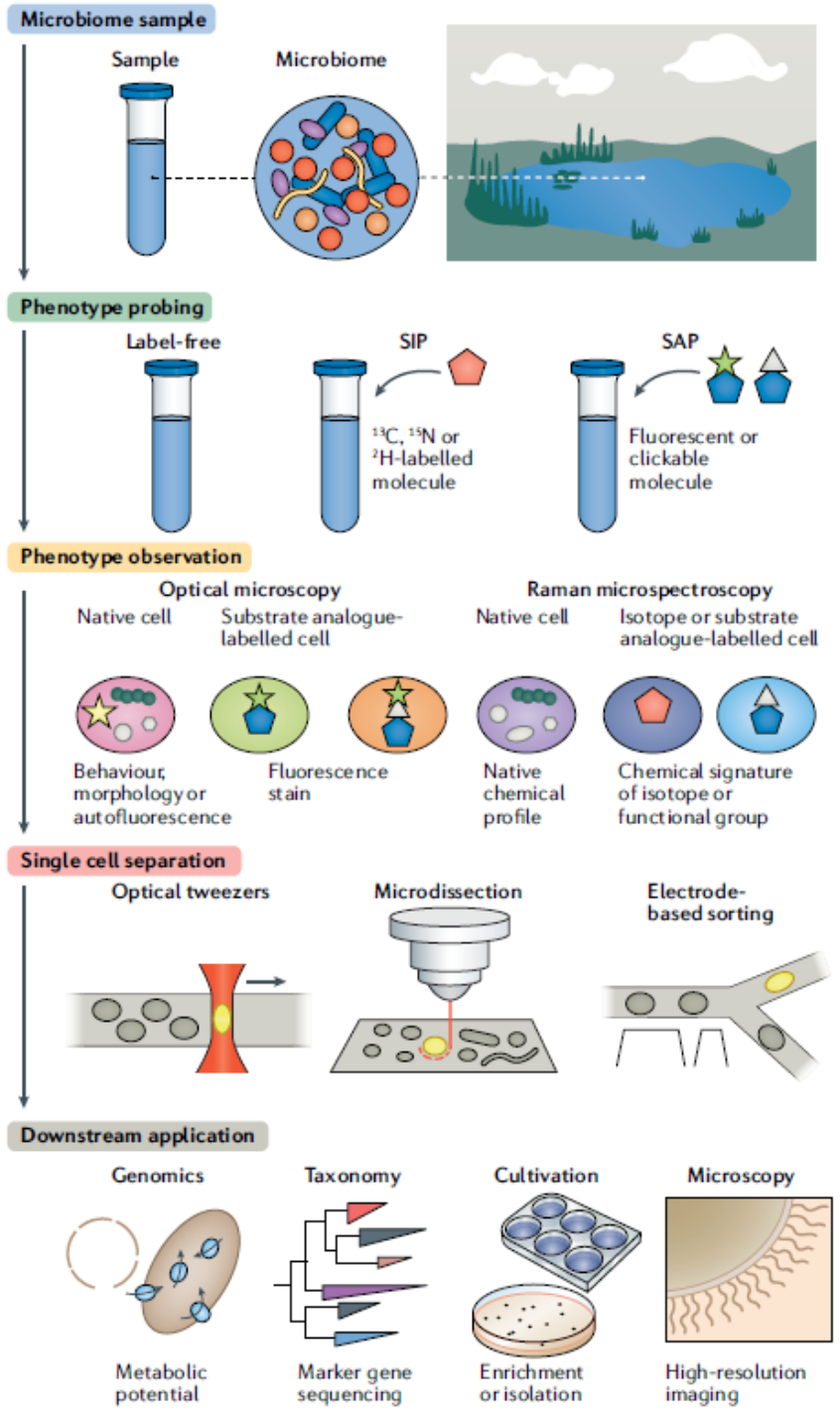


Hatzenpichler, R., Krukenberg, V., Spietz, R.L. *et al.* Next-generation physiology approaches to study microbiome function at single cell level. *Nat Rev Microbiol* **18,** 241–256 (2020). https://doi.org/10.1038/s41579-020-0323-1



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

Huck Core Facilities

Genomics & Transcriptomics

Contact: Craig Praul, cap142@psu.edu

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

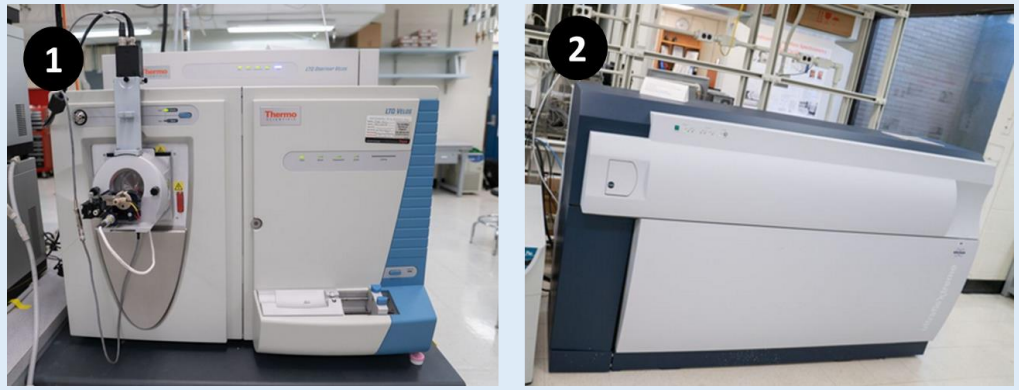
-pure product sequencing (single colony PCR or clone)

Proteomics

Contact: Tatiana Laremore, tnl1@psu.edu , Ganesh Anand, gsa5089@psu.edu

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

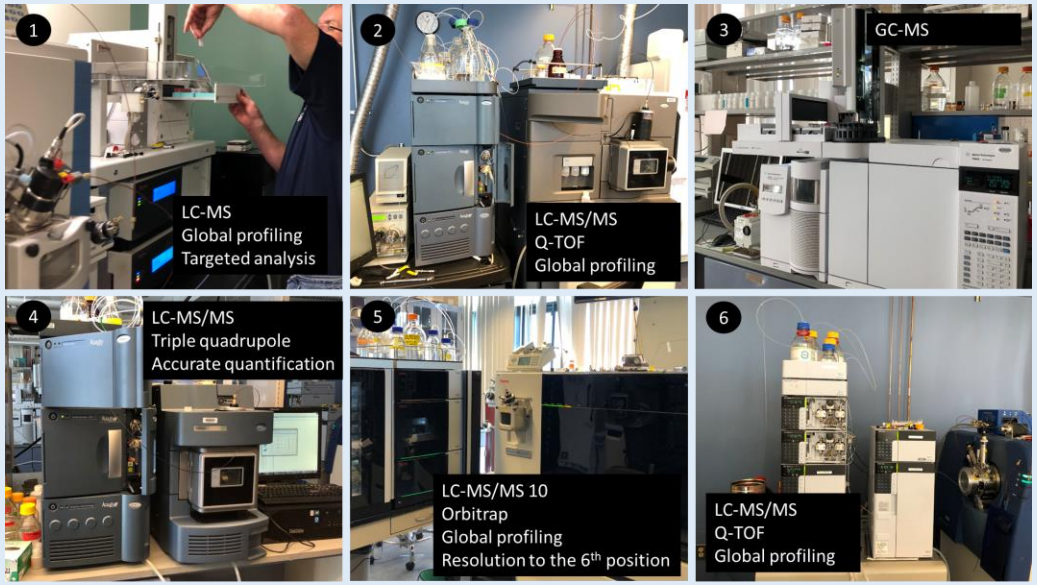
-Identifying new proteins – bioactive peptides

Metabolomics

Contact: Phil Smith, pbs13@psu.edu

Location: Huck Life Sciences Building, first floor

INSTRUMENTS



APPLICATIONS

-Microbial rapid identification

-Probing microbe-microbe interactions through the exchange of metabolites

-Synthetizing defined culture media

-Surveying microbial population under varied condition

-Identifying/quantifying new natural product of interest

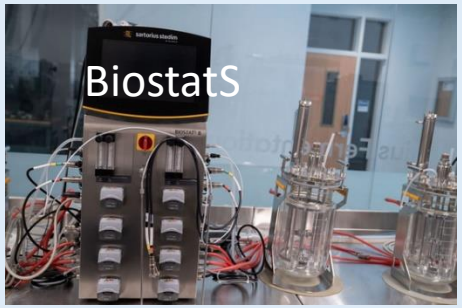
-Tracking the fate of a known compound

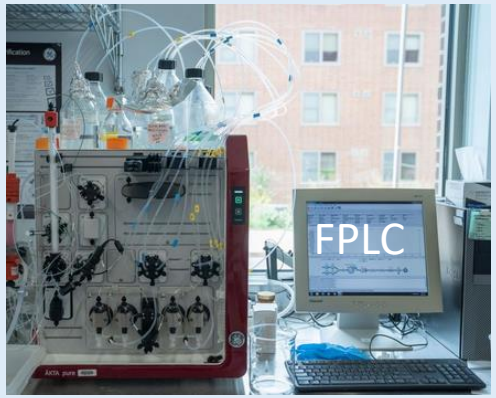
CLS Behring Fermentation

Contact: Mark Signs, mws6@psu.edu, Ali Demirci, axd29@psu.edu

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

Contact: Greg Ning, gxn7@psu.edu

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 slicin
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/moterized
	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

Contact: Sarah Neering, sjn16@psu.edu

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