

---

# AAC Capabilities



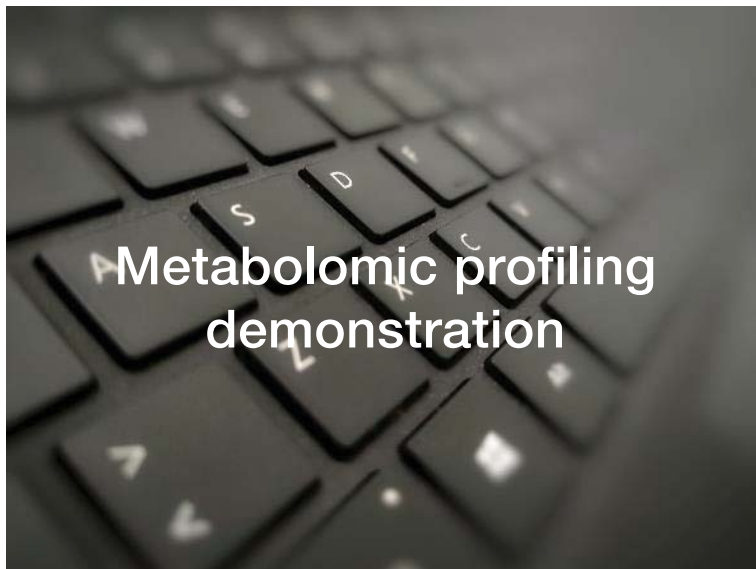
Download info from [microbiome.uoguelph.ca/analytical-services/](https://microbiome.uoguelph.ca/analytical-services/)



**SAMEER AL-ABDUL-WAHID**

---

**NMR**





UNIVERSITY  
of GUELPH

CHANGING LIVES  
IMPROVING LIFE

# Electron Microscopy (EM)

Molecular & Cellular Imaging  
Facility

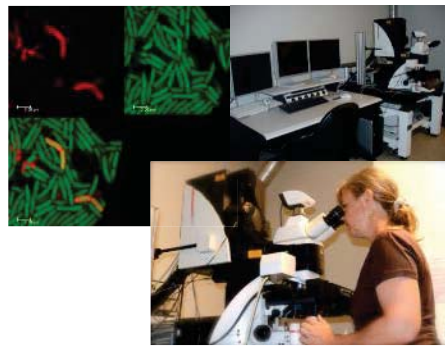
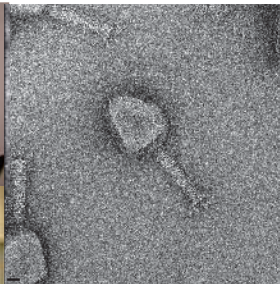
A dvanced  
A nalysis  
C entre

Dr. Elyse Roach  
Electron Microscopy  
May 2023

# Facility Structure



**Electron  
Microscopy  
Unit**

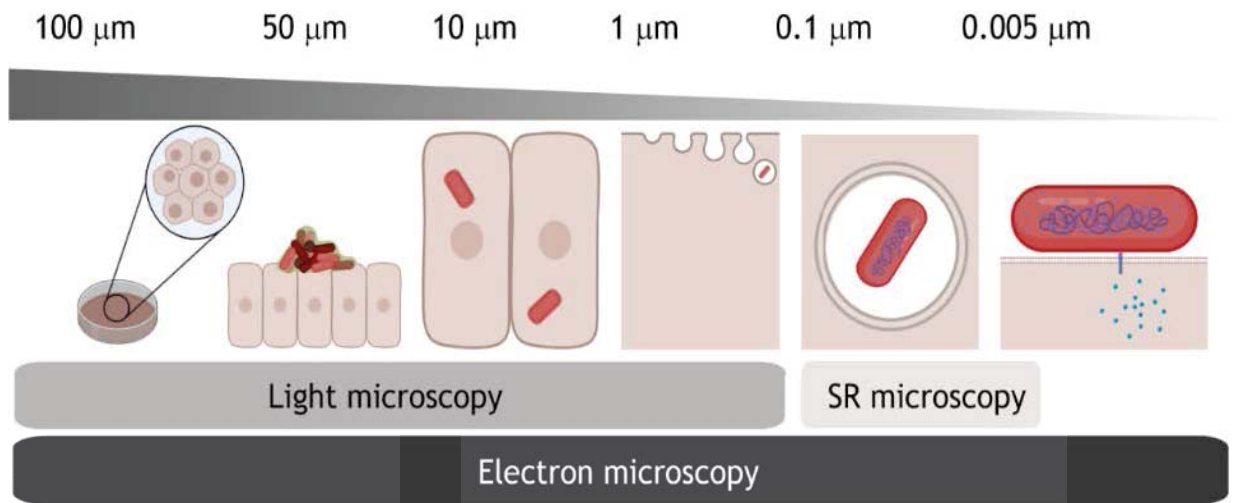


**Advanced Light  
Microscopy  
Unit**

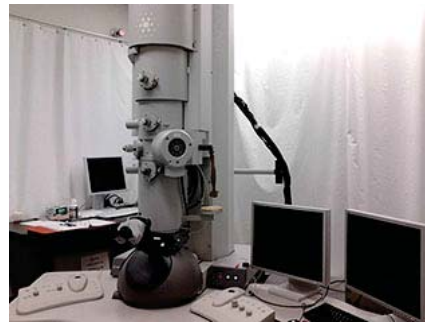
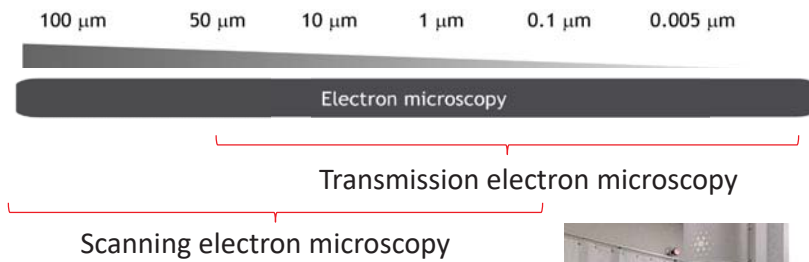


**A**dvanced  
**A**nalysis  
**C**entre





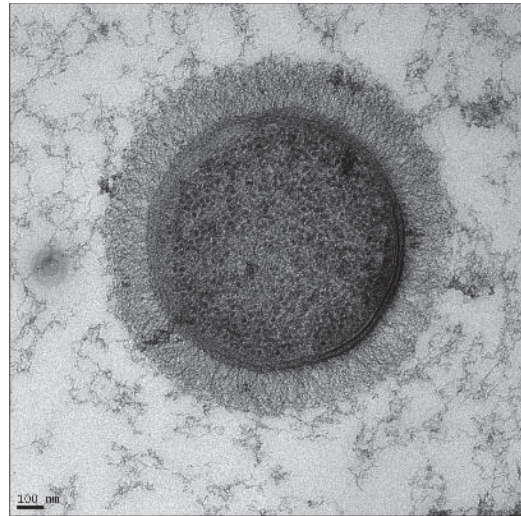
# Electron Microscopy



# Transmission Electron Microscopy (TEM)

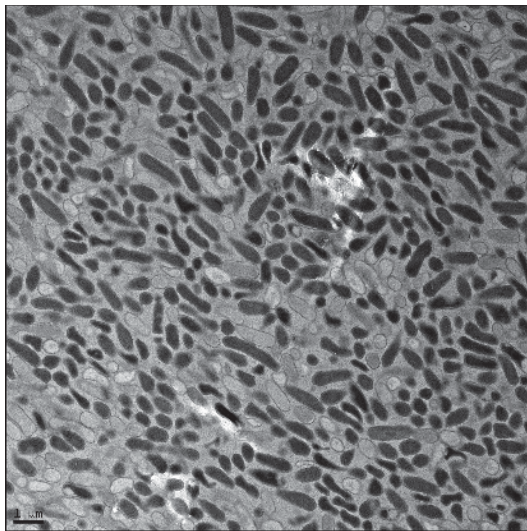


Whole mount, *E. coli*

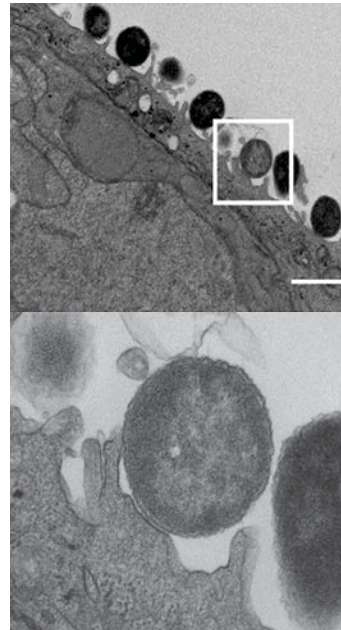


Embedded and sectioned, *A. baumannii*

# Transmission Electron Microscopy (TEM)



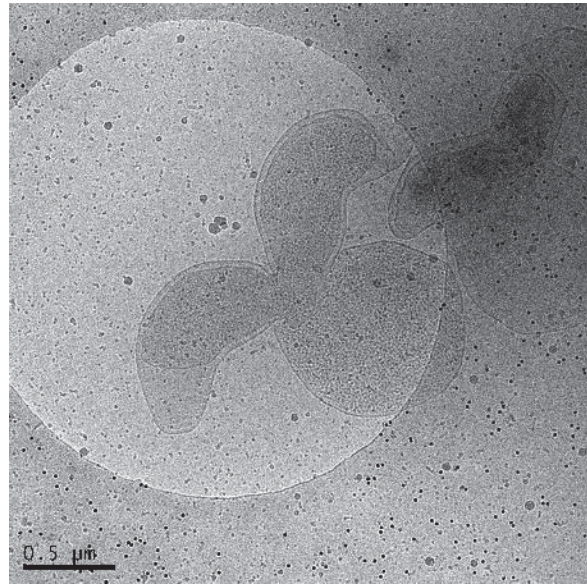
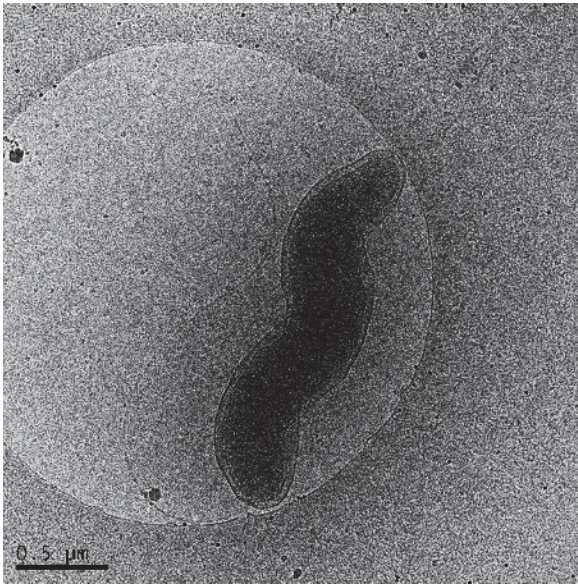
Embedded and sectioned, *P. aeruginosa* biofilm



Embedded and sectioned, *E. coli* infecting Caco-2 cells

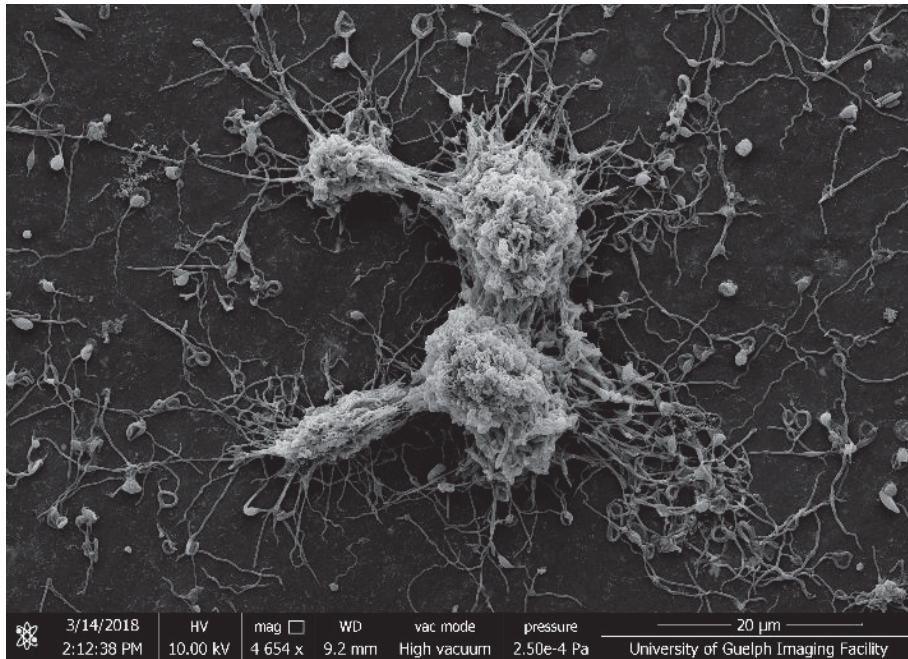


# Cryo-TEM



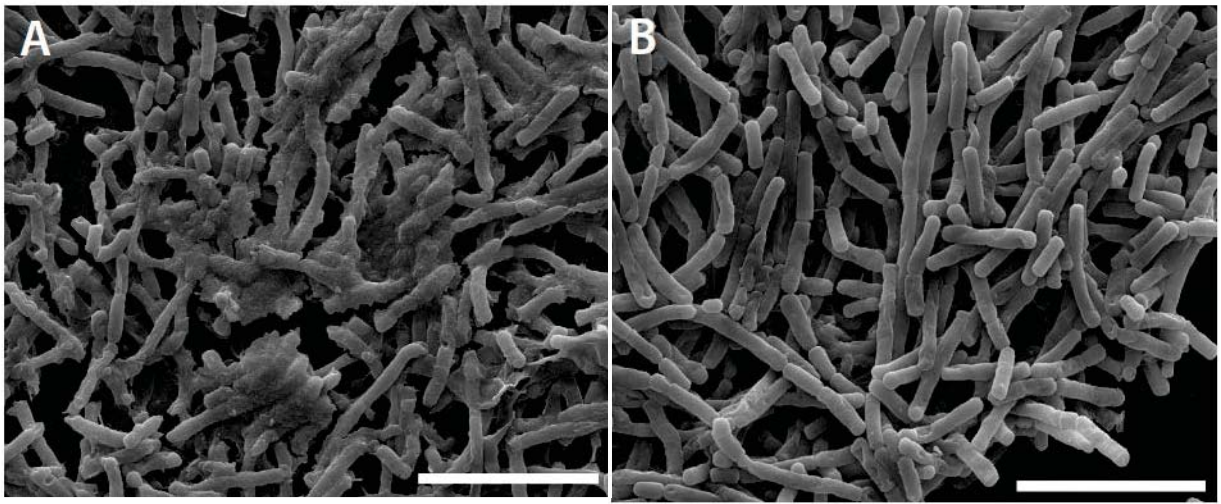
Plunge frozen, *C. jejuni* WT vs mutant

# Scanning Electron Microscopy (SEM)



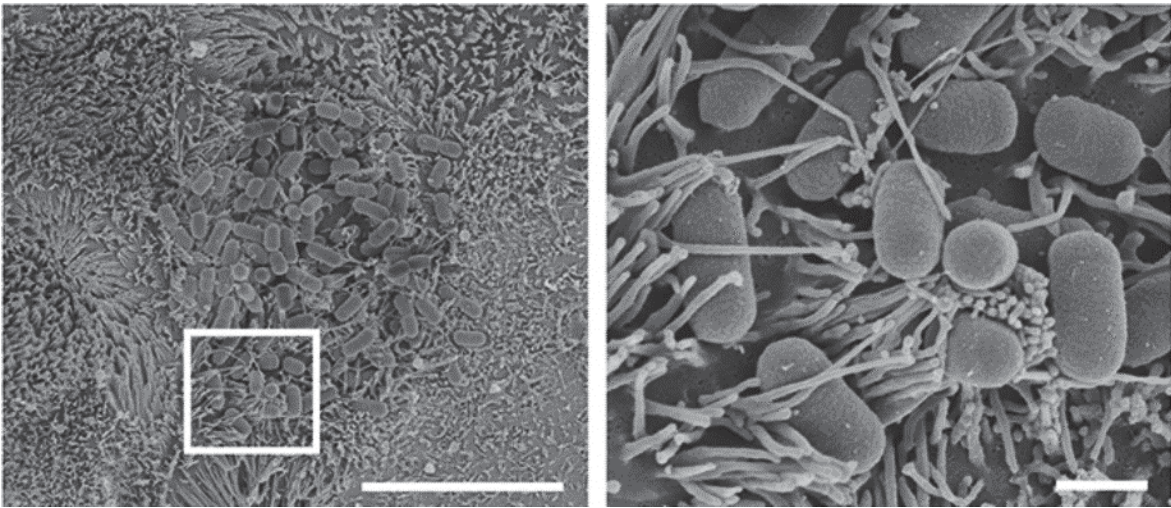
*Borrelia burgdorferi*

# Scanning Electron Microscopy (SEM)



*B. cereus* biofilms, WT vs mutant

## Scanning Electron Microscopy (SEM)



Caco-2 cells infected with enteropathogenic *E. coli*

# Preparatory equipment

## TEM

- Ultra-microtomes
- High pressure freezer
- Freeze substitution machine
- Cryo-plungers
- Cryo ultra-microtome

## SEM

- Critical point drier
- Sputter coater

# EM Services

## Services

### Equipment use

Assisted or independent

### User Training

Mid- to long-term projects

### Full service sample processing

Smaller projects

### Consultation

Sample preparation, experimental setup, etc.

TEM (RT)	\$58.00/hr
TEM (Cryo)	\$68.00/hr
SEM	\$44.00/hr
CPD use	\$17.00/run
Sputter coating	\$37.00/run
Sectioning/staining	\$58.00/hr
Embedding	\$58.00/sample
Technical assistance	\$57.00/hr
SEM training - use	\$202.00/session
SEM training - prep	\$151.50/session
TEM training	\$115.00/hr
Ultramicrotomy by user	\$20.00/hr
HPF/FS	\$175.00/day
TEM grids	\$2.00/each

A scanning electron micrograph (SEM) of a cell surface, showing numerous elongated, finger-like protrusions. The protrusions are color-coded: some are pink, some are light purple, some are blue, and some are green. The background is dark, making the protrusions stand out.

# Molecular & Cellular Imaging Facility

Advanced Analysis Centre  
University of Guelph

[www.uoguelph.ca/aac/mcif](http://www.uoguelph.ca/aac/mcif)

**Dr. Elyse Roach – EM**

[roache@uoguelph.ca](mailto:roache@uoguelph.ca)

Ext. 56409 (office)

**Dr. Michaela Strueder-Kypke – ALM**

[confocal@uoguelph.ca](mailto:confocal@uoguelph.ca)

Ext. 52737 (office)



# Advanced Light Microscopy (ALM)



Molecular & Cellular Imaging  
Facility

**Advanced Analysis Centre**  
**University of Guelph**  
**Guelph, Ontario, Canada**

**A**dvanced  
**A**nalysis  
**C**entre

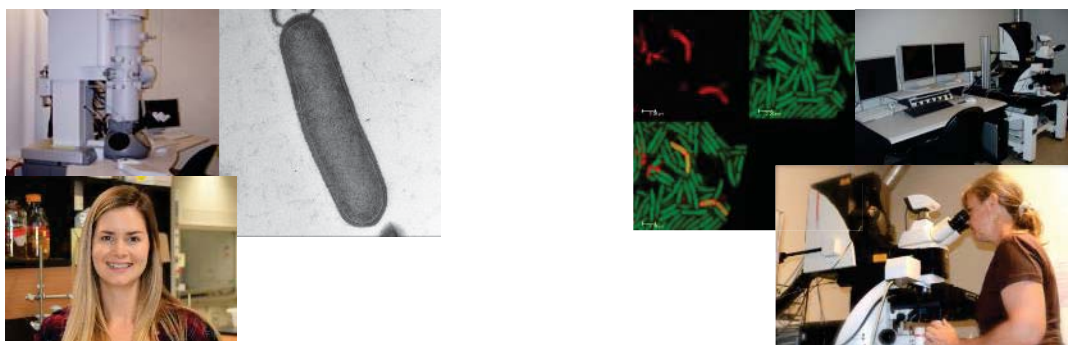
Dr. Michaela Strüder-Kypke  
Advanced Light Microscopy  
May 2023

**A**dvanced  
**A**nalysis  
**C**entre





# Facility Structure



**Electron  
Microscopy  
Unit**

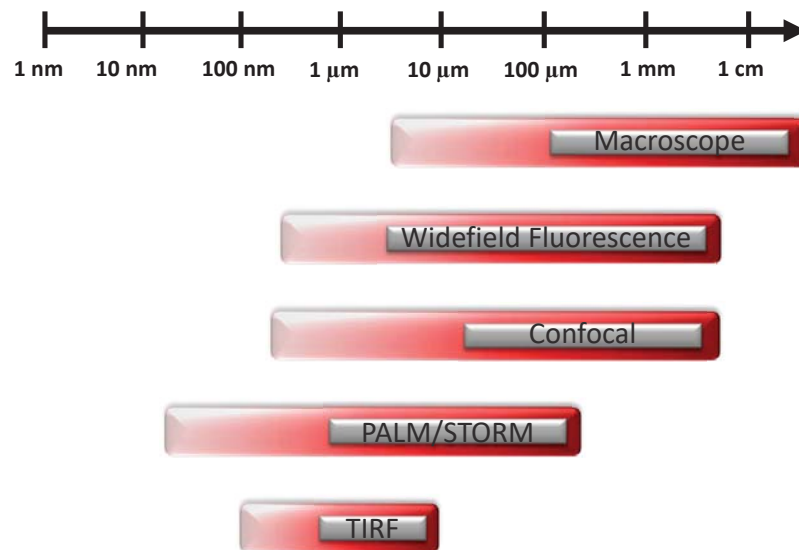


**Advanced Light  
Microscopy  
Unit**

**A**dvanced  
**A**nalysis  
**C**entre



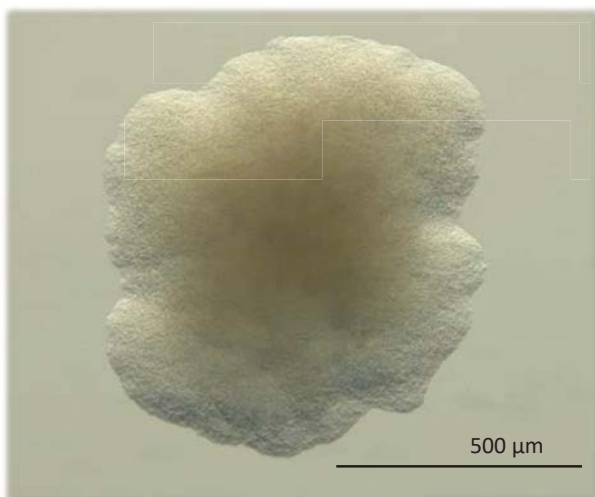
# Optical Microscopy



# Optical Microscopy

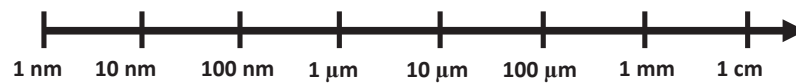


Zeiss Axiozoom V.16



*Mycobacterium* colony on agar  
Sample curtesy of Michael Lapolla, Chemistry

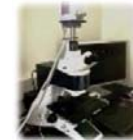
# Optical Microscopy



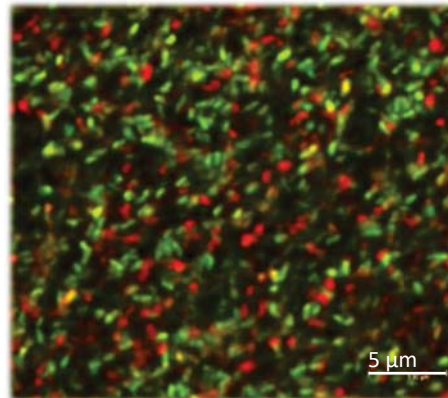
Nikon Eclipse Ti2



Cytation C10

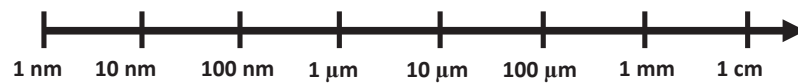


Leica DM 5000B



*Pseudomonas aeruginosa*,  
live-dead stain

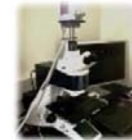
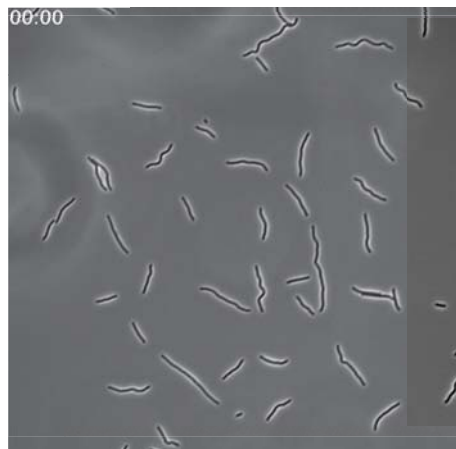
# Optical Microscopy



Nikon Eclipse Ti2



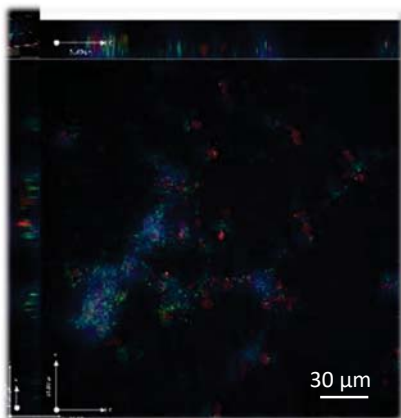
Cytation C10



Leica DM 5000B

*E. coli* 12hr time lapse  
Video courtesy of Dr. Elyse  
Roach, MCIF

# Optical Microscopy



*P. aeruginosa* biofilm  
Image courtesy of Alyssa Banaag, MCB

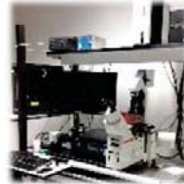
Macroscope

Widefield Fluorescence

Confocal



Leica TCS SP5

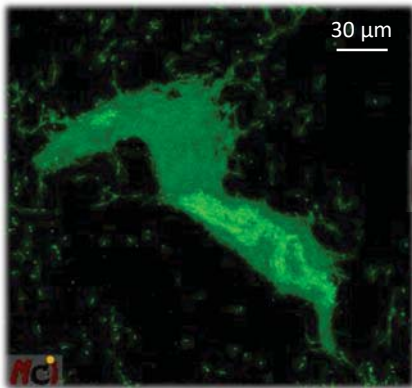


Quorum Discovery



Cytation C10

# Optical Microscopy



Macroscope

Widefield Fluorescence

Confocal



Leica TCS SP5

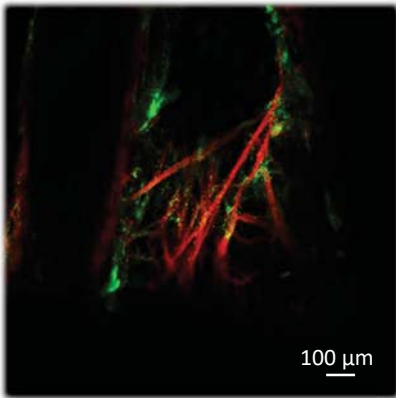


Quorum Discovery



Cytation C10

# Optical Microscopy



Macroscope

Widefield Fluorescence

Confocal



Leica TCS SP5



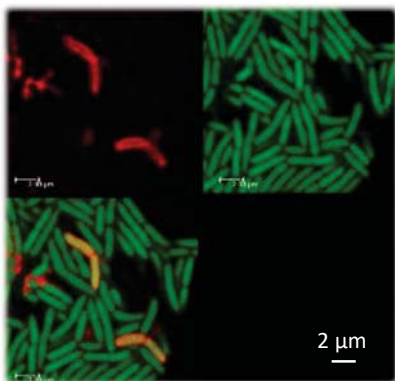
Quorum Discovery



Cytation C10



# Optical Microscopy



*Pseudomonas aeruginosa*,  
membrane stain

Macroscope

Widefield Fluorescence

Confocal



Leica TCS SP5

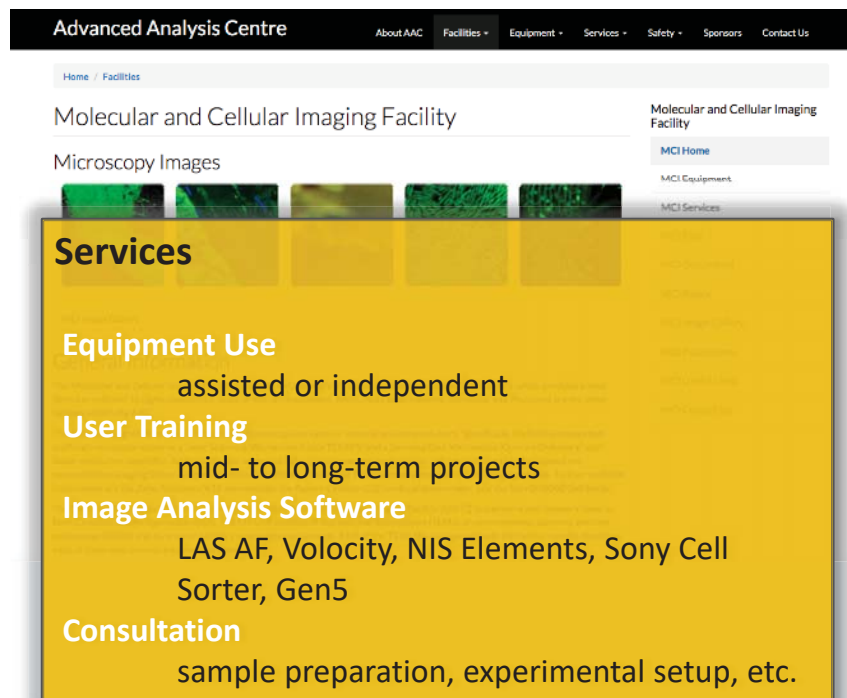


Quorum Discovery



Cytation C10

# ALM Services



The screenshot shows the website for the Advanced Analysis Centre, specifically the Molecular and Cellular Imaging Facility. The navigation bar includes links for About AAC, Facilities, Equipment, Services, Safety, Sponsors, and Contact Us. The main content area is titled "Molecular and Cellular Imaging Facility" and "Microscopy Images". A yellow box highlights the "Services" section, which lists the following offerings:

- Equipment Use**
  - assisted or independent
- User Training**
  - mid- to long-term projects
- Image Analysis Software**
  - LAS AF, Volocity, NIS Elements, Sony Cell Sorter, Gen5
- Consultation**
  - sample preparation, experimental setup, etc.

# Molecular & Cellular Imaging Facility

Advanced Analysis Centre  
University of Guelph

[www.uoguelph.ca/aac/mcif](http://www.uoguelph.ca/aac/mcif)

**Dr. Elyse Roach – EM**

[roache@uoguelph.ca](mailto:roache@uoguelph.ca)

Ext. 56409 (office)

**Dr. Michaela Strueder-Kypke – ALM**

[confocal@uoguelph.ca](mailto:confocal@uoguelph.ca)

Ext. 52737 (office)

**A**dvanced  
**A**nalysis  
**C**entre



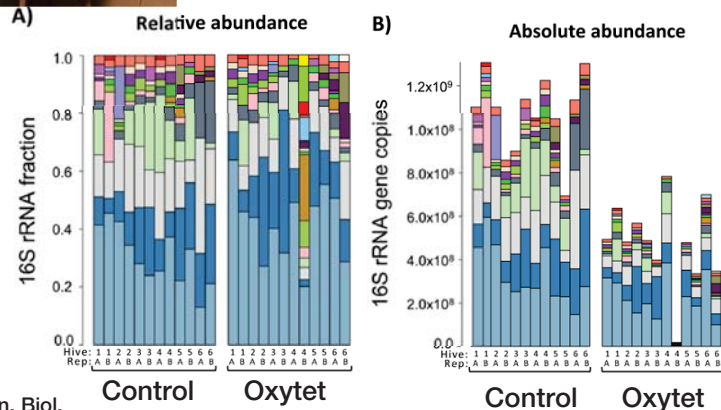
# FACS (FLUORESCENCE-ACTIVATED CELL SORTING)



## Absolute quantification

- Gold-standard for counting microbial cells
- Better than qPCR/ddPCR/CFU-based methods
- Complementary with sequencing-based approaches which are limited by compositionality

Part number	<ul style="list-style-type: none"> <li><input type="checkbox"/> <i>Lactobacillus kunkeei</i></li> <li><input type="checkbox"/> <i>Pseudomonas</i> spp.</li> <li><input type="checkbox"/> <i>Lactococcus</i> spp.</li> <li><input type="checkbox"/> <i>Rosenbergiella</i> spp.</li> <li><input type="checkbox"/> <i>Pediococcus</i> spp.</li> <li><input type="checkbox"/> Remainder</li> <li><input type="checkbox"/> <i>Weissella</i> spp.</li> <li><input type="checkbox"/> <i>Bartonella apis</i></li> <li><input type="checkbox"/> <i>Franconibacter</i> spp.</li> <li><input type="checkbox"/> <i>Bacteroides</i> spp.</li> <li><input type="checkbox"/> <i>Escherichia</i> spp.</li> <li><input type="checkbox"/> <i>Lactobacillus</i> spp.</li> <li><input type="checkbox"/> <i>Enterococcus</i> spp.</li> <li><input type="checkbox"/> <i>Bifidobacterium</i> spp.</li> <li><input type="checkbox"/> <i>Klebsiella</i> spp.</li> <li><input type="checkbox"/> <i>Streptococcus</i> spp.</li> <li><input type="checkbox"/> <i>Commensalibacter</i> spp.</li> <li><input type="checkbox"/> <i>Lactobacillus</i> Firm-4</li> <li><input type="checkbox"/> <i>Frischella perrara</i></li> <li><input type="checkbox"/> <i>Lactobacillus</i> Firm-5</li> <li><input type="checkbox"/> <i>Snodgrassella aliva</i></li> <li><input type="checkbox"/> <i>Gilliamella apicola</i></li> </ul>
Nozzle size	
Cell type examples	



Daisley et al. 2020, Commun. Biol.

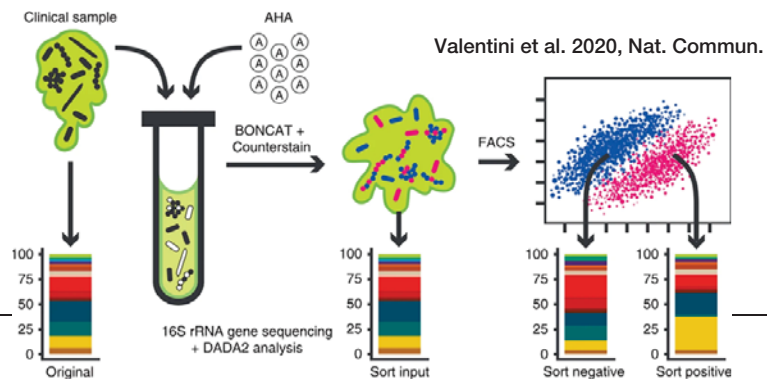
# FACS (FLUORESCENCE-ACTIVATED CELL SORTING)



## Cell sorting and other functionalities

- Sort cells based on phenotype of interest
- Live/dead staining and quantification
- Differentiate active microbes from eDNA and dormant cell populations
- BONCAT-FACS for interrogating translationally active cells in matrix of interest (soil, feces, etc.)

BONAT=Biorthogonal non-canonical amino acid tagging



# EXPERIMENTAL OVERVIEW



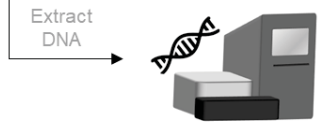
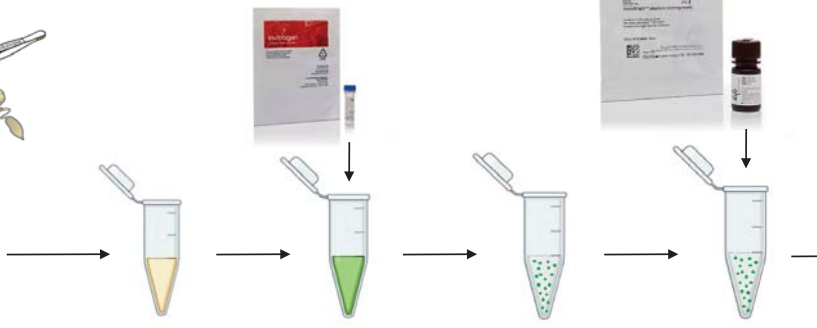
Insects



Feces



Plants and soil

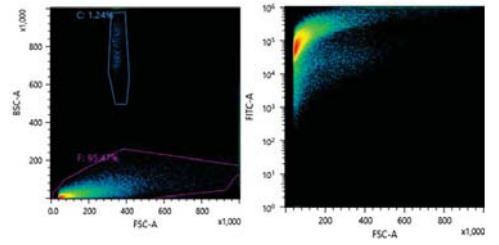


Extract DNA

16S rRNA sequencing on MiSeq



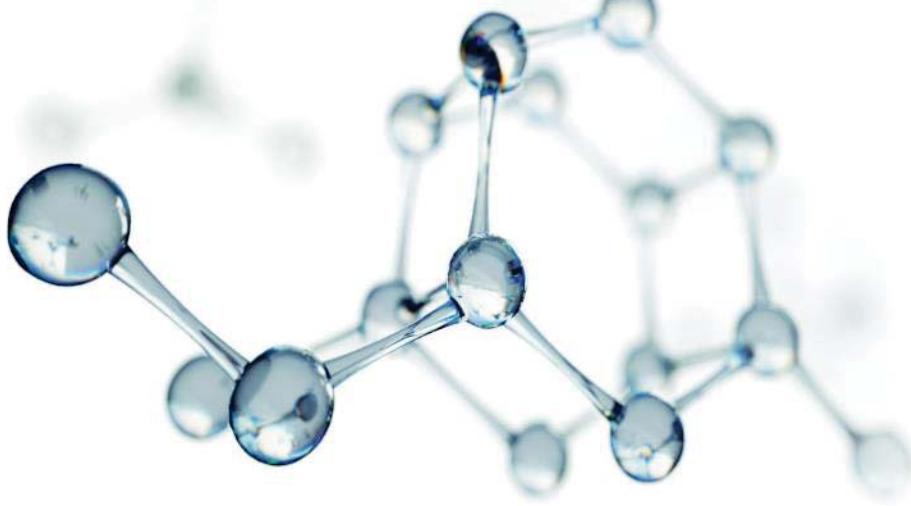
Bioinformatic analysis





# Genomics Facility

**Advanced Analysis Centre  
University of Guelph  
Guelph, Ontario, Canada**



**A**dvanced  
**A**nalysis  
**C**entre



# Genomics Facility Equipment



Nanodrop 8000 Qubit Flex TapeStation 4150

Sample QC



Applied Biosystems  
3730 DNA Analyzer



QuantStudio 7 Pro  
with Orbitor Robot



QX200 ddPCR  
Droplet Reader



Illumina MiSeq



# Workflows



Sanger Sequencing

- ABI 3730
- Sequencing by CE
- Amplicon or Plasmid Sequencing from Isolates
- Taxonomic ID – 16S or ITS



MiSeq

- Sequencing by Synthesis
- Whole Genome or Transcriptome from Isolates
- Taxonomic ID -16S or ITS from community samples
- Low-coverage "shotgun" metagenomics



NovaSeq

- Sequencing by Synthesis
- High-coverage shotgun metagenomics- StrainID, Functional Analysis
- Metatranscriptomics – StrainID, Functional Analysis, Community Gene Expression, RNA Viruses
- Single-Cell Sequencing

# R Study Group

---

---

# Microbiome research and R

(or other statistical tools?)

---

---

---

---

Current state of support

---

---

## Spread across campus

---

---

- R user group
  - Bioinformatics program and associated courses
  - Individual faculty expertise
  - International workshops
- 
-

## Spread across campus

---

---

- R user group
    - Every Friday, 10:30am-12pm
    - SSC3317
    - Slack group - [everybody invited](#)
    - Welcoming, helpful, productive, focus on R
      - (Gwen Freeze participated last week with a practical question, but walked away with feedback on how to structure her folders, R setup, etc etc)
  - Bioinformatics program and associated courses
  - Individual faculty expertise
  - International workshops
- 
-

## Spread across campus

---

- R user group
- Bioinformatics program and associated courses

### Semester Breakdown

Semester	Courses	Total Credits (4.0)
<b>Semester 1</b>	BINF*6210 Software Tools for Biological Data Analysis and Organization	0.50
	BINF*6890 Topics in Bioinformatics	0.50
	Elective (BINF*6410 is highly recommended)	0.50
<b>Semester 2</b>	BINF*6110 Genomic Methods for Bioinformatics	0.50
	BINF*6970 Statistical Bioinformatics	0.50
	Elective ( <a href="#">see list of approved courses</a> )	0.50
<b>Semester 3</b>	BINF*6999 Bioinformatics Master's Project	1.00
<b>Semester 4 (optional)</b>	BINF*6999 Bioinformatics Master's Project – Extension with possible stipend	

- Individual faculty expertise
  - International workshops
-

## Spread across campus

---

- R user group
- Bioinformatics program and associated courses
- Individual faculty expertise

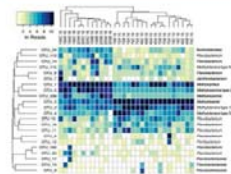
```
53 > ## _ manipulations to use into R -----
54
55 df_otu = x3 |>
56   biom_data() |>
57   as.matrix() |>
58   t() |> # necessary to follow standard statistical conventions
59   as_tibble(rownames = "sample_id") # keep sample id
60 df_otu
61
62 df_sample = x3 |>
63   sample_metadata() |>
64   as_tibble(rownames = "sample_id")
65 df_sample
66
67 df_taxa = x3 |>
68   observation_metadata() |>
69   as_tibble(rownames = "taxa_id")
70 df_taxa
71
72 > ## select columns and filter rows, medium df -----
73
74 df_otu |>
75   select(sample_id, filter(df_taxa, taxonomy1 == "k__Bacteria")$taxa_id) |>
76   filter(df_sample$BODY_SITE == "gut")
77
```

- International workshops
-



## Spread across campus

- R user group
- Bioinformatics program and associated courses
- Individual faculty expertise
- International workshops



### METABARCODING IN MICROBIAL ECOLOGY

#### DATES

6-10 February 2023

To foster international participation, this course will be held online

---

---

Future state of support?

---

---

## Consolidate across campus

---

---

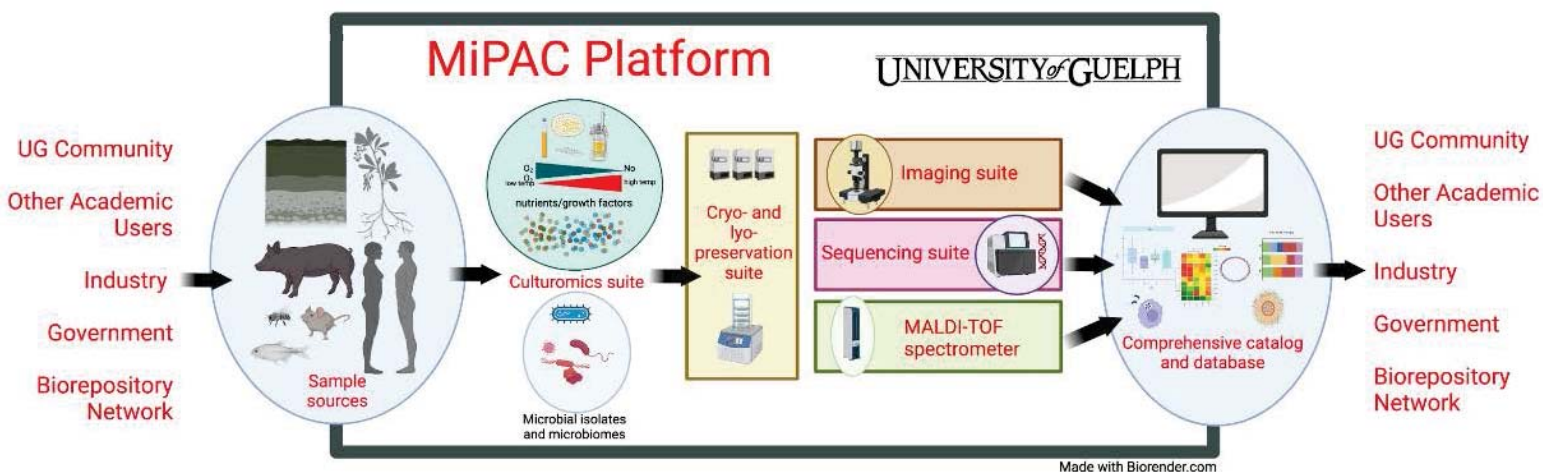
- R user group
  - Need for microbiome-specific resources?
    - R, what packages?
    - QIIME2, Qiita
    - Python, what libraries?
    - Others?
  - Need for a microbiome user group?
  - Questionnaire
    - Your computational needs
    - Where do you get training resources for your researchers
    - What training resources do you provide
  - Report back to the group with a summary and next steps
  - Other suggestions?
- 
-

---

# MiPAC

**MiPAC:**  
**M**icrobiome **P**reservation and  
**A**nalysis **C**entre

*A proposed* resource that will  
be particularly helpful for  
microbiome researchers!



- Total project cost: just over \$11.4M (~\$4.8M from CFI)
- Submitted July 2022 – will hear next month or shortly thereafter if successful or not



---

## CULTUROMICS SUITE: MEDIA PREPARATION, CRYO AND LYOPRESERVATION

- Water purification system
- Plate pourer
- Autoclave
- Bank of 4 ultra-low freezers
- Freeze-dryer (for use with microbial culture)



---

## CULTUROMICS SUITE: MICROBIAL CULTURE INFRASTRUCTURE

- Dual anaerobic workstation
- Single anaerobic workstation
- Hypoxia workstation (microaerophiles)
- Plate-readers (can fit inside chambers for growth curves)
- Bioreactors (2 x 200mLs)
- BioLector - microbioreactor



---

## MASS SPECTROMETRY – MALDI-TOF

- Autoflex maX TOF/TOF MALDI-MS
- A versatile analytic instrument that can be used for a variety of applications
  - E.g., qualitative analysis of high mass “delicate” bio-molecules that cannot be analyzed by other means
- Will include a module for biotyping





---

## SEQUENCING SUITE

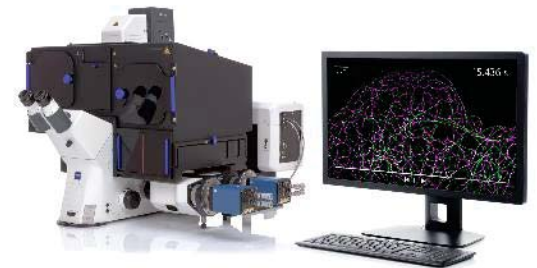
- Illumina NovaSeq 6000 system
  - Super high throughput sequencing
- Biomek i7 hybrid robot
  - Library preparation
- Chromium 10x Genomics system
  - For e.g., Single cell genomics
- Purigen Ionic gDNA extraction instrument



---

## IMAGING

- Zeiss LSM980 confocal microscope with Airyscan
  - Multi-colour experiments with living samples
- Zeiss Elyra7 with Lattice-SIM
  - Live imaging system that allows superresolution
- Plant phenotyping system
  - Multispectral imaging of plants



---

## SERVICE PROVISION MODEL

- Implementation of a first-of-its kind service to isolate, characterize and preserve gut microbes and ecosystems from a variety of sources
    - Provision to researchers without specialist culture facilities as ready-to go inocula for their experiments
    - Vision: service charges will support the MiPAC platform into the future
  - Creation of a networked cross-Canada microbial strain repository
    - Building on, and leveraging security for, strain collections across campus and beyond
  
  - If not successful, we will propose a re-application 😊
-