BioLoop: Embedded Electrochemical Impedance Spectroscopy for Autonomous Microfluidic Biofilm Experiments

Presented by Hannah Hobbs[1]

Principal Investigator: Samuel M.D. Oliveira[2]

Additional Authors: Tolulope Oyeniran[2], Jianna Evans[2], Harnish Shah[2]

[1]Department of Biomedical Engineering, Clemson University, 301 Rhodes Research Center, Clemson, SC 29634, USA [2]Joint School of Nanoscience and Nanoengineering (JSNN), North Carolina Agricultural and Technical State University (NCAT), Greensboro, 27401-NC, USA

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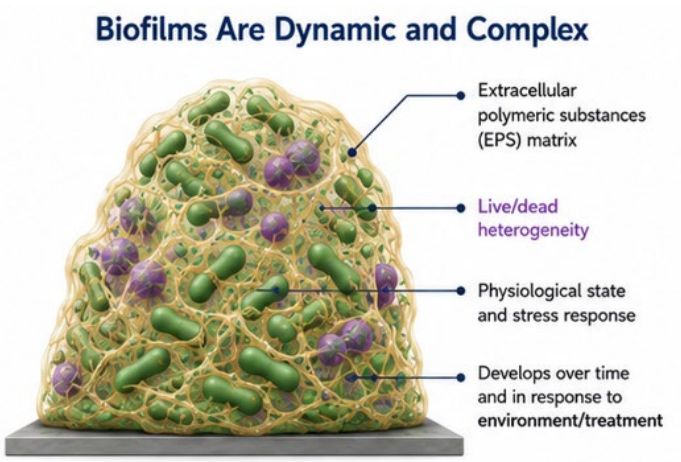
The Microbial Biofilm Challenge in Health Care



Healthcare-associated infections

- **687,000** HAIs annually in U.S. acute care hospitals [1]
- **~72,000** associated in-hospital deaths
- **1 in 31** hospitalized patients has an HAI on any given day

Earlier detection and improved characterization of healthcare-associated infections could support more timely and effective treatment decisions.

Medical device infections

For **cardiac implantable electronic device (CIED)** infections:

- **64%** of patients underwent device extraction after infection [2]
- **25%** died within one year of infection
- Medicare expenditures commonly ranged from **$50,000–$77,000** per patient, depending on management strategy

Once mature biofilms develop on implanted devices, treatment frequently requires device removal because antimicrobial therapy alone is often insufficient.

Chronic & persistent infections

- Chronic wounds affect approximately **10.5 million** Medicare beneficiaries in the United States and impact the quality of life of nearly **2.5%** of the U.S. population [3]
- **Require continuous monitoring for adaptive treatments to eliminate infection: No validated, non-invasive clinical diagnostic scale currently exists for identifying biofilm-associated infection in chronic wounds.**

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How can microbial biofilm characterization and treatment evaluation be improved?

CURRENT APPROACHES

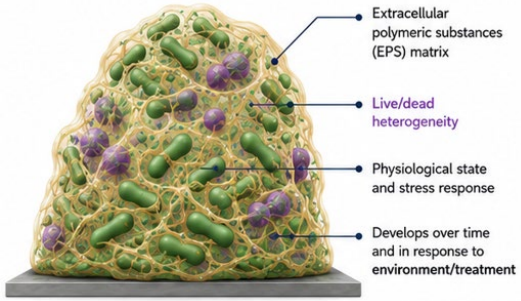
Microscopy (time-lapse, confocal)
- Gold standard for structure and visualization
- Labor-intensive, complex workflows

Culture-Based Assays
- Viability and CFU counts
- Slow (hours–days), not real-time

Biochemical Stains (Crystal Violet, etc.)
- Quantify biomass
- Multiple steps, endpoint measurements

Molecular Analysis (qPCR, sequencing)
- Identify species and genes
- Expensive, specialized equipment

Biofilms Are Dynamic and Complex



Information We Need

- Total biomass and structure
- Physiological state
- Antimicrobial efficacy
- Temporal trends

THE IMPROVED FUTURE

Real-Time, Label-Free Monitoring
Continuously track biofilm development and response without disrupting the system.

Quantitative, Multi-Parameter Insight
Simultaneously assess biomass, physiological state, and treatment efficacy.

Continuous Treatment Evaluation
Detect treatment effects earlier and adapt strategies in real time.

Point-of-Care Applicable
Portable, automated, and easy to use in clinical and field settings.

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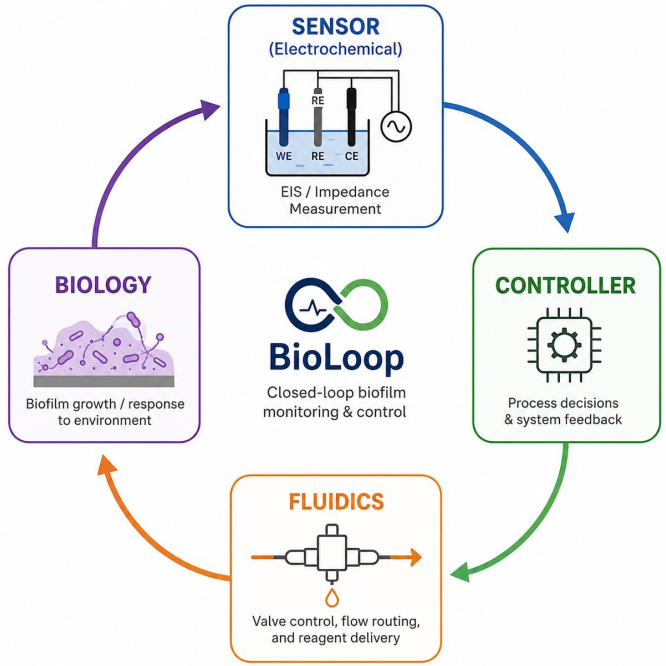
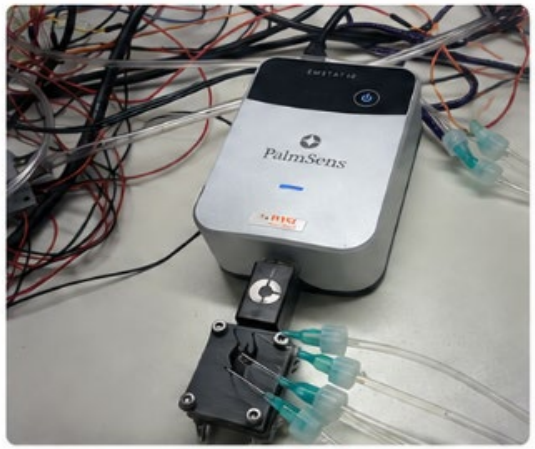
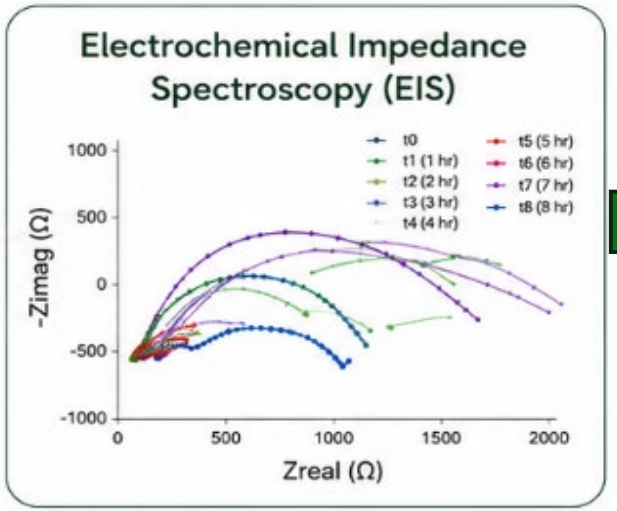
Proposed Solution

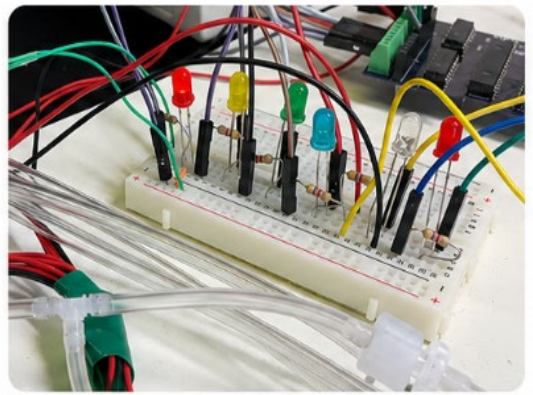
BioLoop integrates label-free monitoring with automated fluidics and system feedback to deliver continuous insight into biofilms.





Microfluidic Biofilm Chamber
with Integrated SPE Working Electrode





Real-Time Analysis
& System Feedback

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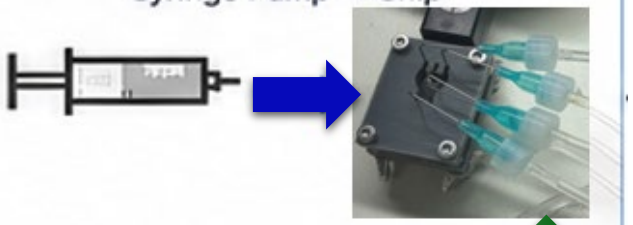
Bioloop Platform: Hardware

1. STEADY FLOW (INDEPENDENT)

Syringe Pump → Chip

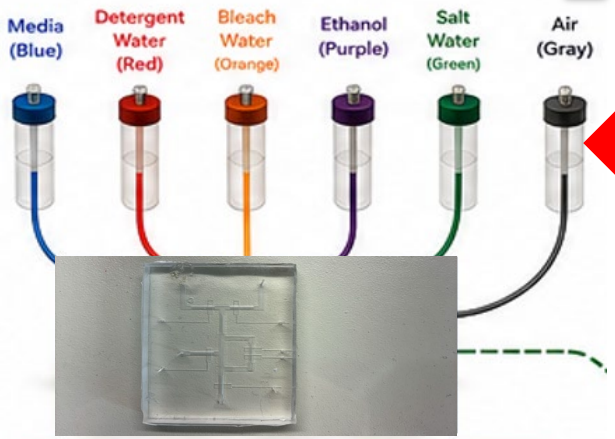


Constant media flow for biofilm growth and seeding

4. ELECTROCHEMICAL MEASUREMENT

2. PERTURBATION ROUTING SYSTEM

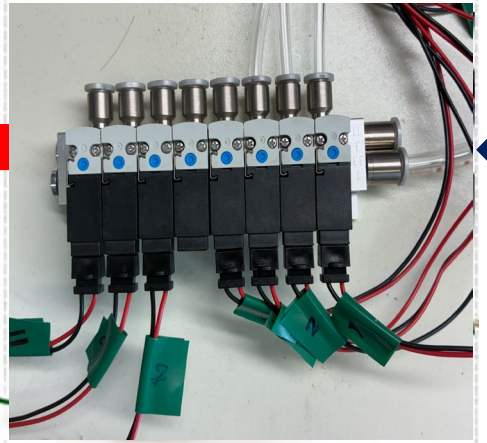
Independent Valve Manifold → Chip Inlet



- Routes perturbation fluids for experiments and recirculation
- Completely separate from steady flow path
- Enables precise environmental perturbation

3. AIR ACTUATION

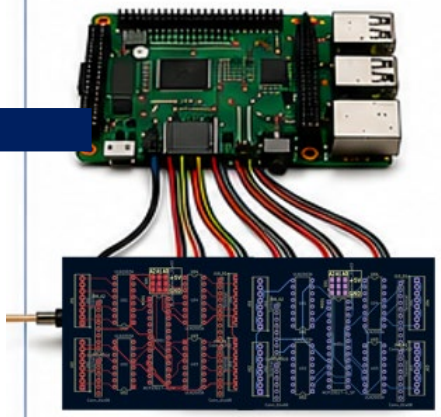
Solenoid Valves + Air Pump



- Air pressure actuates quaker valves on chip for reliable switching

5. CENTRAL CONTROL

Raspberry Pi + Driver Board



- Controls pumps, valves, and acquires EIS data
- Automated scheduling, logging, and analysis

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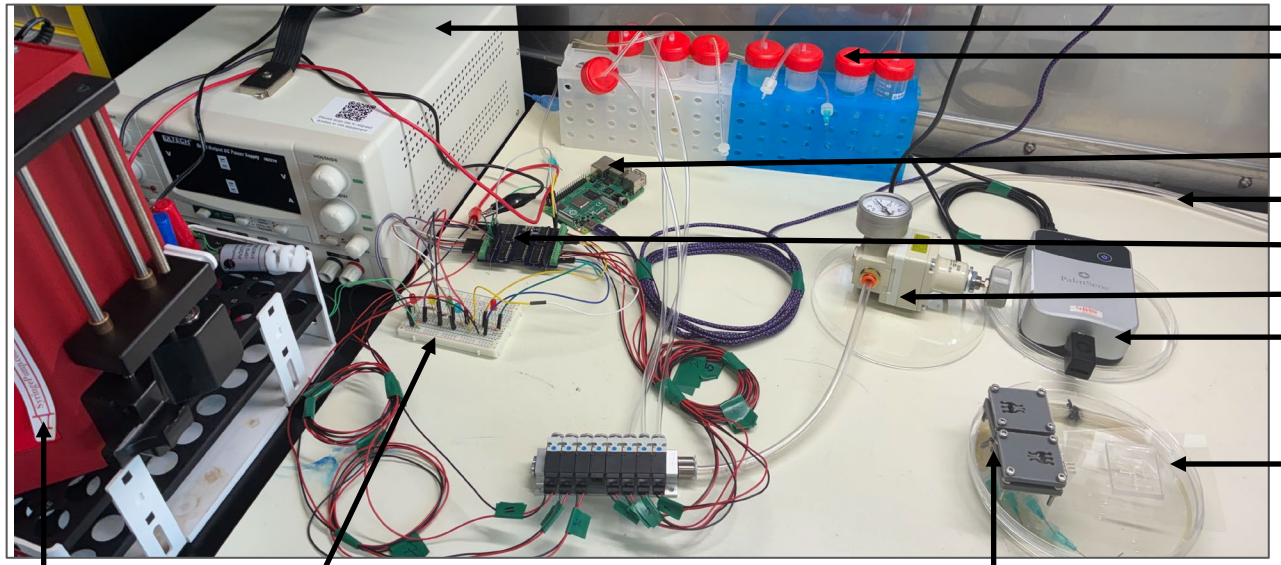
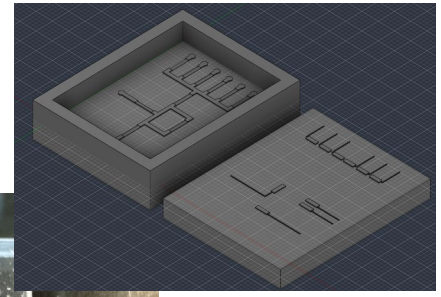
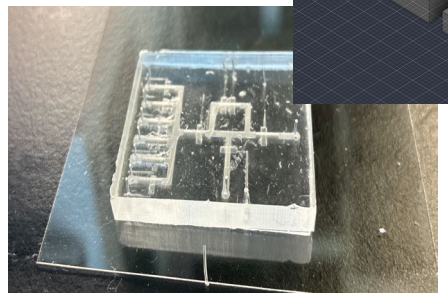
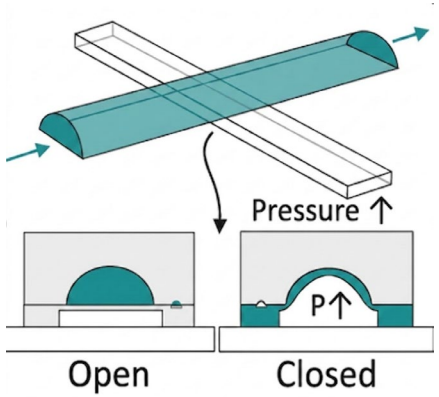
Bioloop Hardware Components



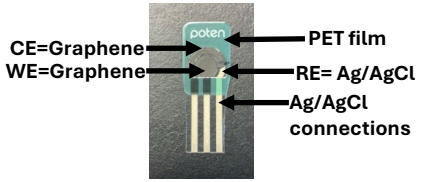
Routing Valve Chip

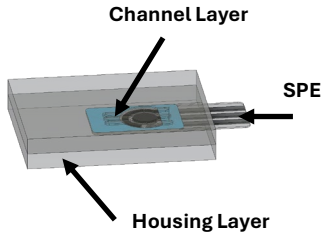


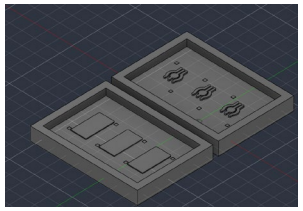


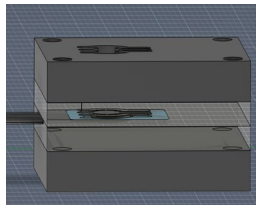


SPE Microfluidic Chip and Clamp









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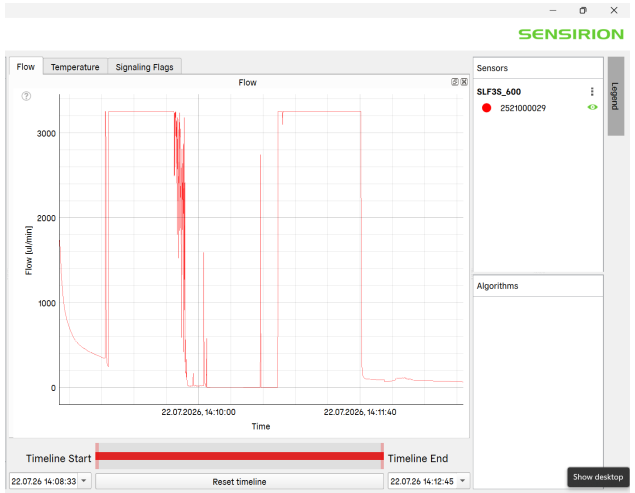
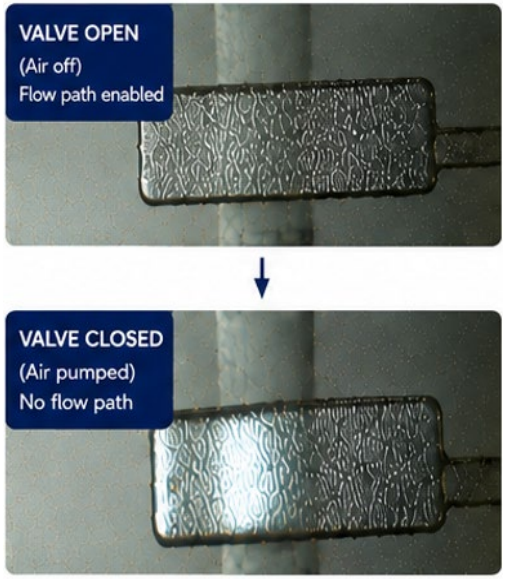
Verification

1. Flow Characterization



- Real-time flow monitoring with **Sensirion SLF3S** sensor
- Step response and stability analysis for each fluid path
- Identifies dead volume, valve leakage, and switching delays

2. Valve Actuation Visualization



- Direct observation of quaker valve actuation with **microscope**
- Confirm complete sealing and opening
- Confirm reliable switching for all reagents

3. Integrated System Testing

Key Verification Criteria

- ❑ Stable reproducible flow delivery
- ❑ Leak-free valve operation
- ❑ Accurate timing and actuation
- ❑ Reliable EIS during perturbations

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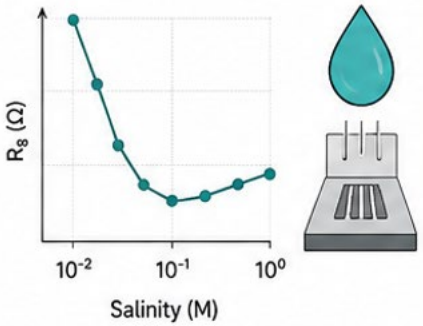
Validation

1 Environmental Salinity Monitoring

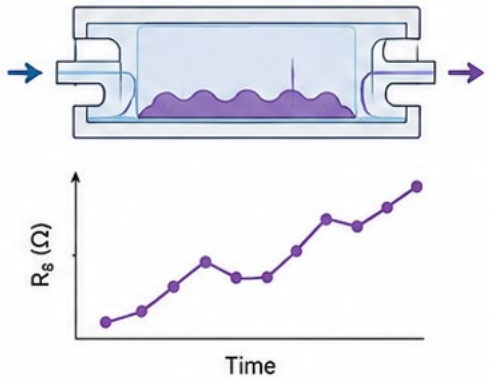


Real-time EIS tracking enables accurate salinity measurement and feedback.

2 Electrode Health and Automated Quality Control

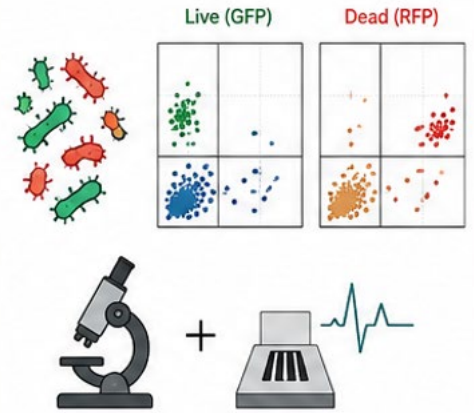
Continuous health assessment and automated pass/fail decisions ensure reliable data.

3 Biofilm Growth Monitoring and Environmental Perturbation



EIS tracks biofilm development and response to controlled environmental changes.

4 Preliminary Viability Assessment



Combining fluorescence imaging with EIS for early insights into cell viability.

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Environmental Salinity Monitoring

Objective

Demonstrate automated prediction of environmental ionic strength from EIS using an adaptive conductivity calibration model.

1. **CALIBRATE**
   Use NaCl standards of known ionic strength (0.01–0.60 M)
2. **MEASURE**
   Collect triplicate EIS spectra at each concentration and estimate solution resistance (R_s)
3. **CONVERT**
   Convert R_s to conductivity using the experimentally determined cell constant (K_{cell})
4. **FIT MODEL**
   Calculate molar conductivity (Λ_m) and update the empirical conductivity model
5. **PREDICT**
   Use the updated calibration curve to predict ionic strength of unknown environmental samples

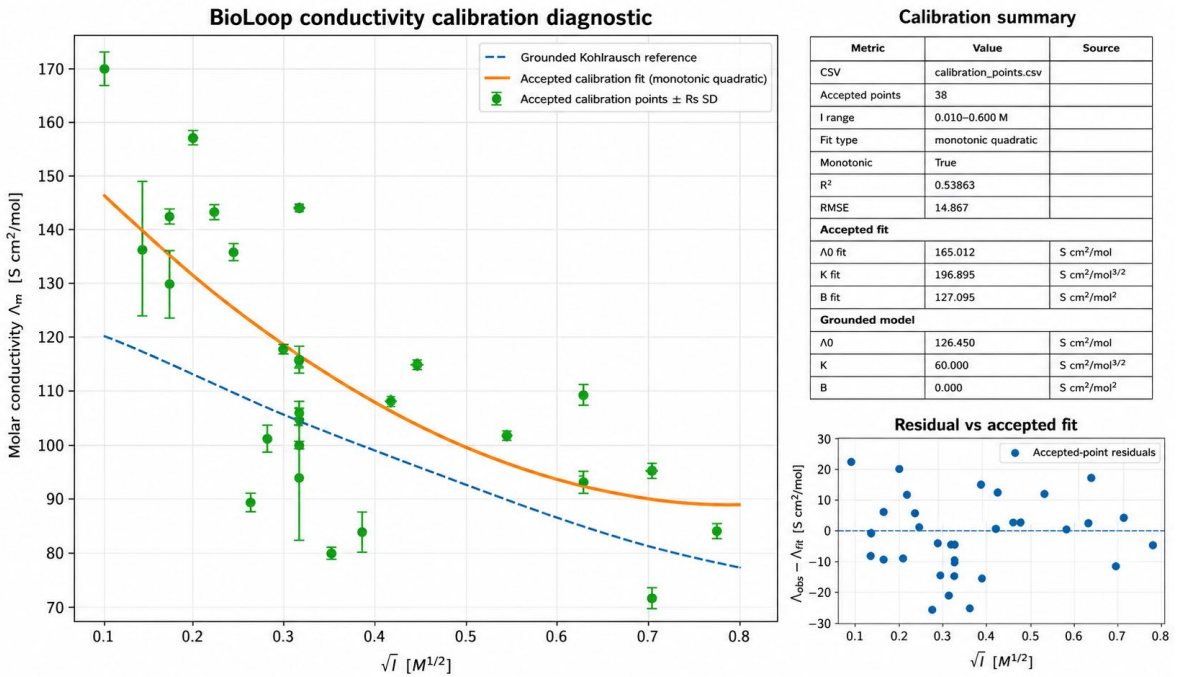
ADAPTIVE CALIBRATION

BioLoop continuously refines its conductivity model using user-approved calibration measurements.

- Compensates for sensor-to-sensor variability
- Improves prediction accuracy over time
- Maintains traceability through accepted calibration points

Future Validation

- ❑ Learn concentration-dependent correction term (BI)
- ❑ Expand calibration beyond NaCl to environmentally relevant ionic mixtures
- ❑ Implement continuous online model refinement during autonomous operation



Metric	Value	Source
CSV	calibration_points.csv	
Accepted points	38	
I range	0.010–0.600 M	
Fit type	monotonic quadratic	
Monotonic	True	
R^2	0.53863	
RMSE	14.867	
Accepted fit		
Λ0 fit	165.012	S cm²/mol
K fit	196.895	S cm²/mol$^{3/2}$
B fit	127.095	S cm²/mol²
Grounded model		
Λ0	126.450	S cm²/mol
K	60.000	S cm²/mol$^{3/2}$
B	0.000	S cm²/mol²

Figure 1. Diagnostic plot generated during salinity test using Bioloop. The blue dotted line represents the physically grounded Kohlrausch equation for NaCl solutions with coefficients $\mathrm{K_{NaCl}} \approx 60 \frac{Scm^2}{mol}\left(\frac{mol}{L}\right)^{-0.5}$ and $\Lambda^0_{m,NaCl} \approx 126.45E - 4\frac{Sm^2}{mol}$. Experimental model is fit with coefficient B to account for the expected decrease in slope as ion concentration increases due to effects such as electrostatic repulsion of ion shells and increasing solution viscosity.

$$A_m = -KI^{0.5} + \Lambda_m^0 + BI$$

- Λ_m = molar conductivity (S cm²/mol)
- Λ_m^0 = limiting molar conductivity (S cm²/mol)
- K = Onsager empirical constant (S cm²/mol$^{3/2}$)
- B = empirical correction factor (S cm²/mol²)
- I = ionic strength (mol/L)

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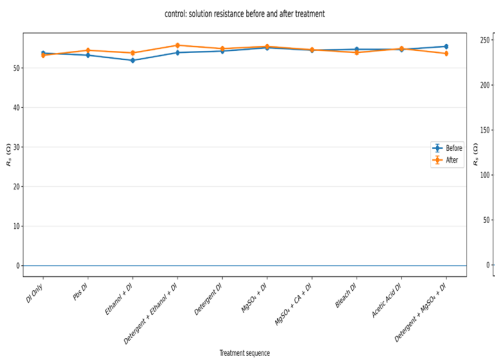
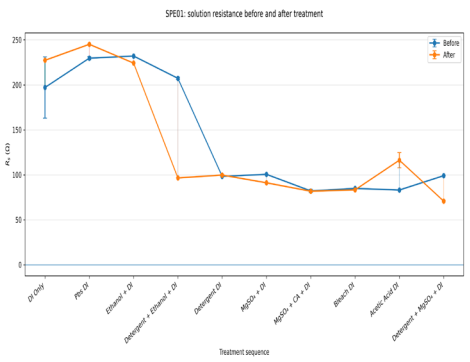
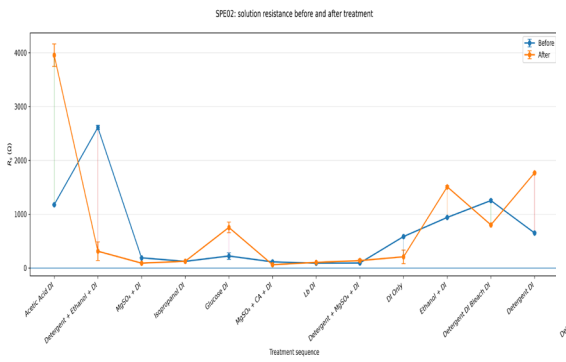
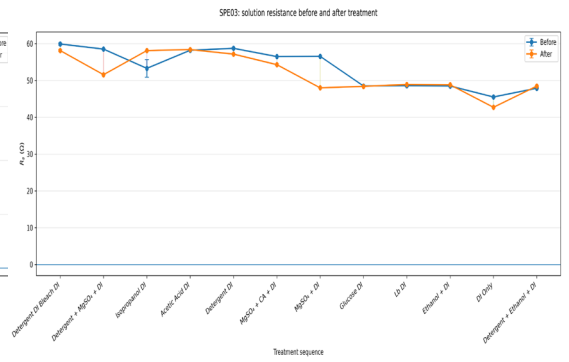
Electrode Health and Automated Quality Control

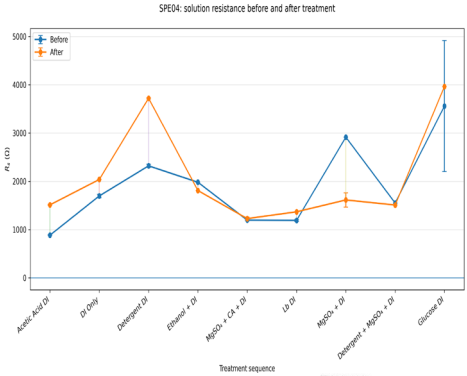
Demonstrate automated detection of electrode condition

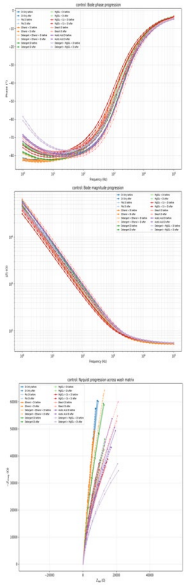


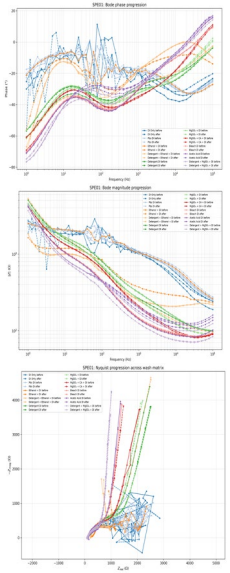


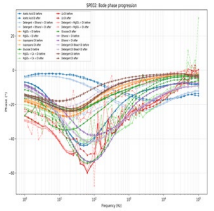


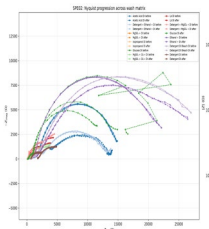


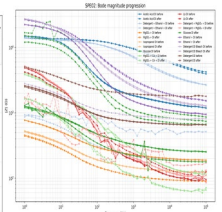


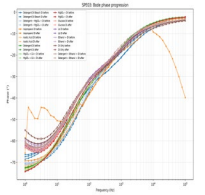


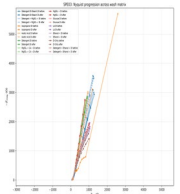


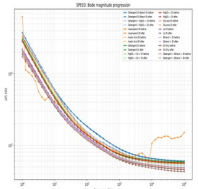


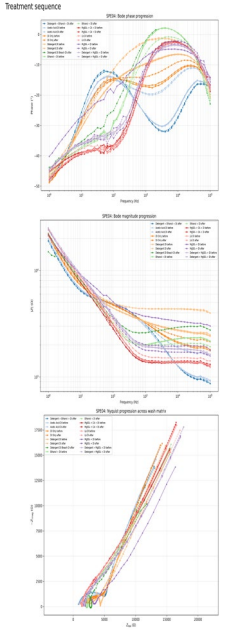












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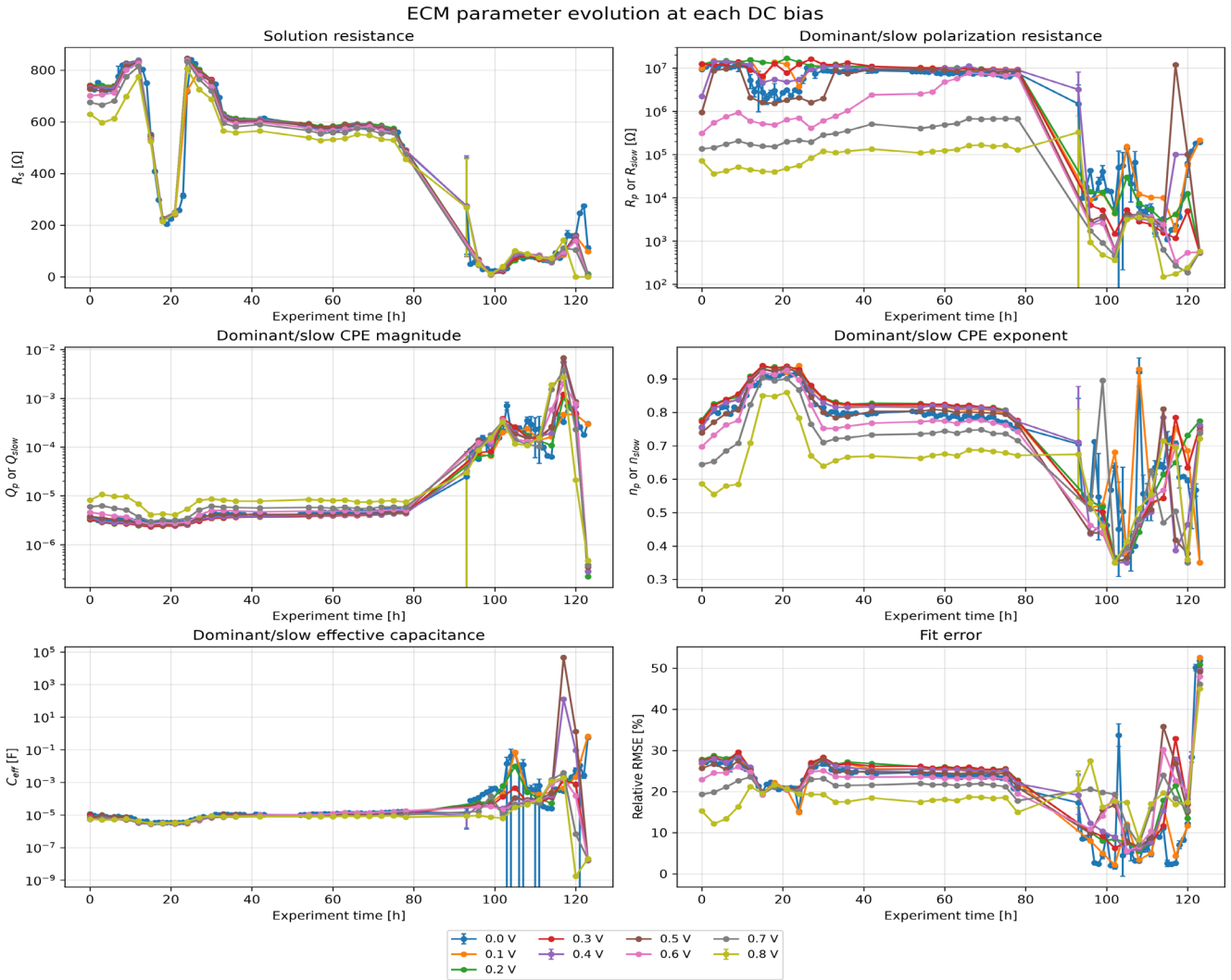
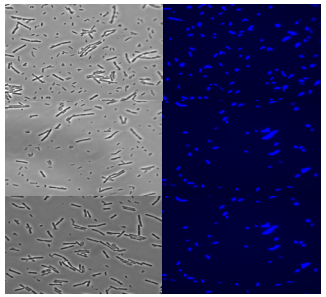
Bacterial Growth Monitoring and Environmental Perturbation

Exploratory demonstration of electrochemical monitoring during biofilm cultivation



Main Hypothesis: Environmental ionic conditions influence microbial physiology through changes in membrane potential, ion transport, and gene regulation.

Parameter fits over time. From left to right- top to bottom parameters are **Rs, Rpe, CPE, n, Ceff, and RMSE**.



77 hr timepoint data for bacterial growth on electrode experiment.

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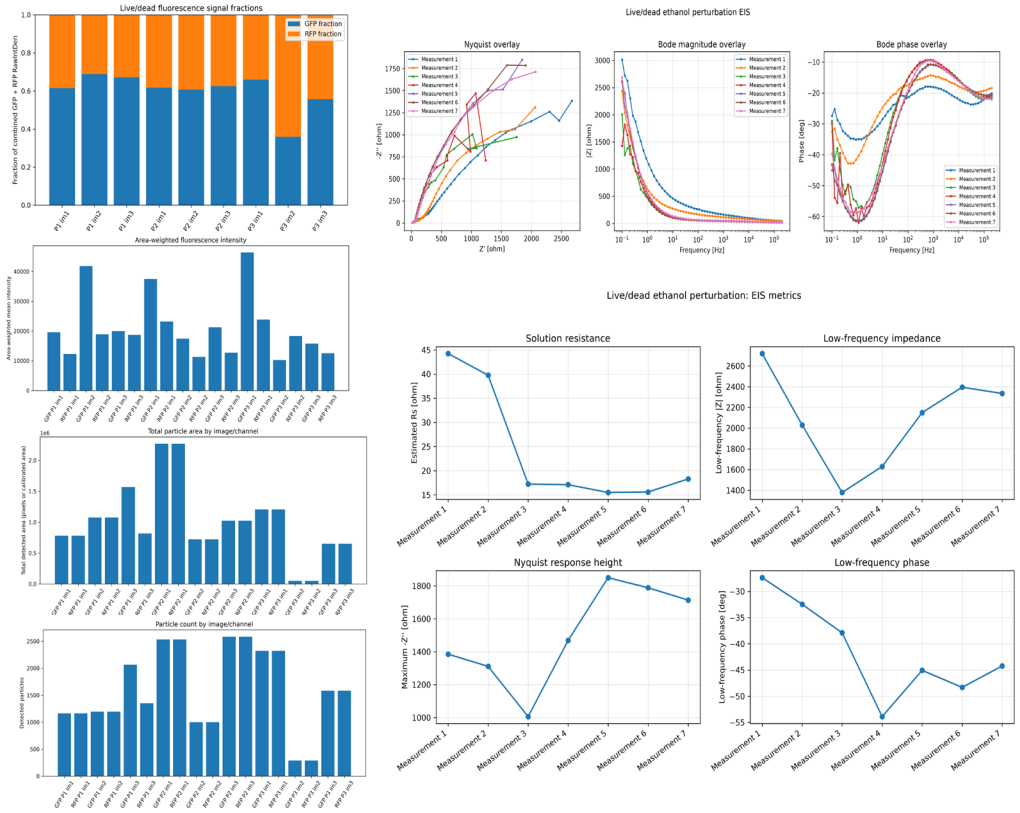
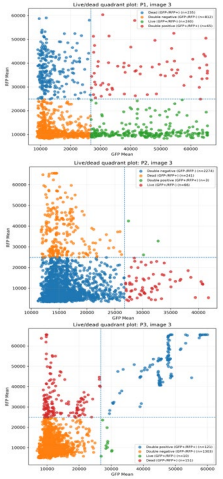
Preliminary Viability Assessment

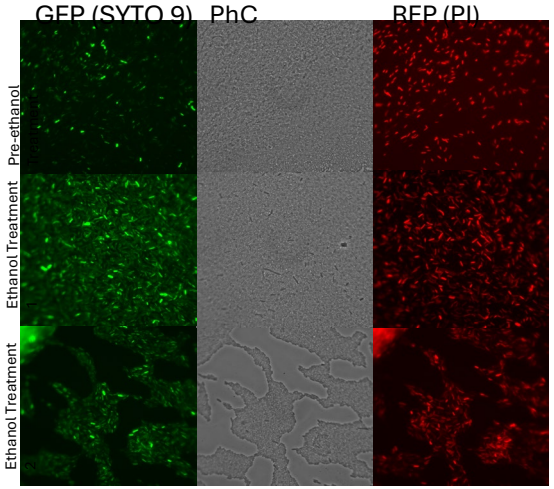
Evaluate whether repeated ethanol exposure produces measurable changes in bacterial impedance response while assessing viability using fluorescence microscopy

1. Load *Pseudomonas* cells into microfluidic chamber
2. Acquire EIS spectrum
3. Collect sample for Live/Dead staining
4. Image GFP / Phase Contrast / RFP
5. Treat reservoir with ethanol (1 s)
6. Incubate 3–5 min
7. Repeat for two treatment cycles



Preliminary EIS and fluorescent results for ethanol treatment study. From left to right shown are the Nyquist plot, bode magnitude plot, and bode phase plot. It is expected some experimenter error interfered with EIS scan quality.





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Future Directions and Applications

- BioLoop is currently at TRL 1–2. Future work will focus on verification, reliability, and platform performance
- Platform demonstrations will be iteratively refined and repeated to improve data robustness and experimental protocols
- BioLoop's integrated architecture provides a foundation for investigating multiparametric biofilm characterization, environmental sensing, and adaptive experimental feedback beyond conventional biomass monitoring.

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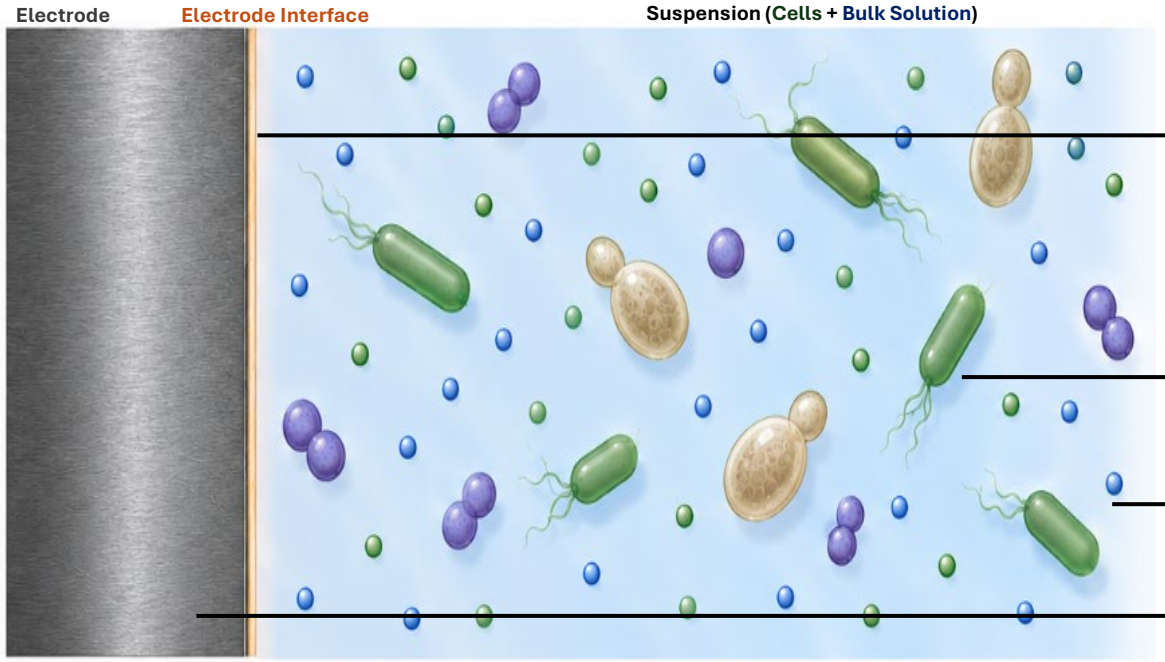
Electrochemical Impedance Spectroscopy (EIS) Modeling

Unattached Microbial Single Shell Suspension Model



Interface: Thin region containing electrical double layer, interfacial charge transfer, adsorbed ions and biomolecules.

Rct
Cdl

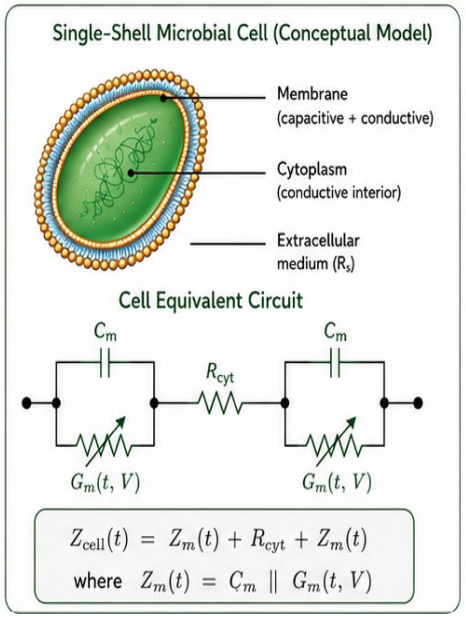
Microbial Cell (Bacteria, fungi, ect.)

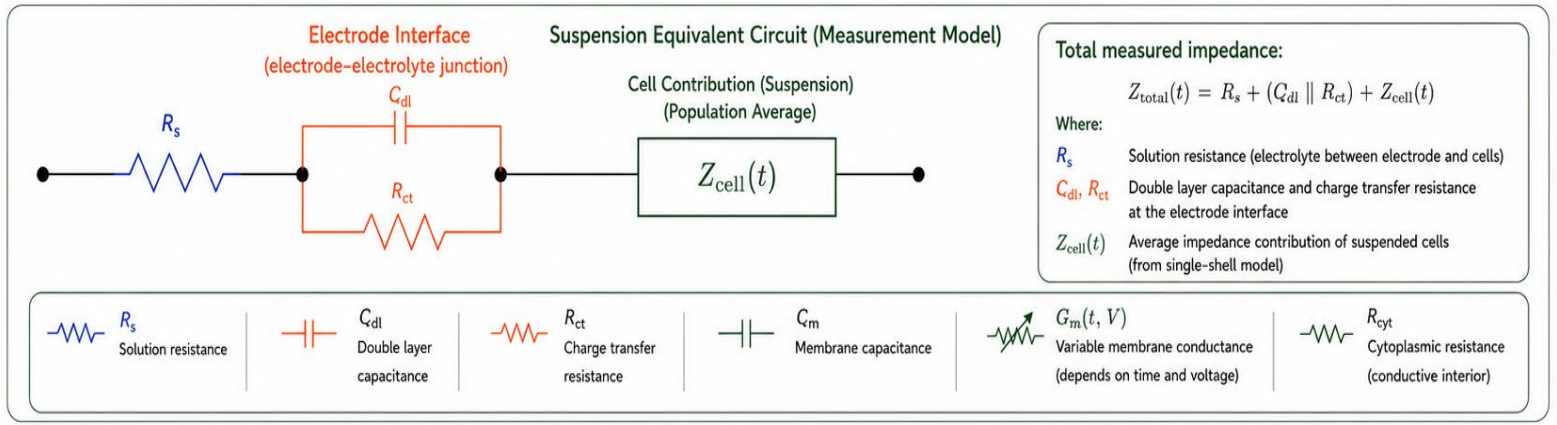
Gm, Rcyt, Cm

Ions, nutrients, metabolic products in solution

Rs

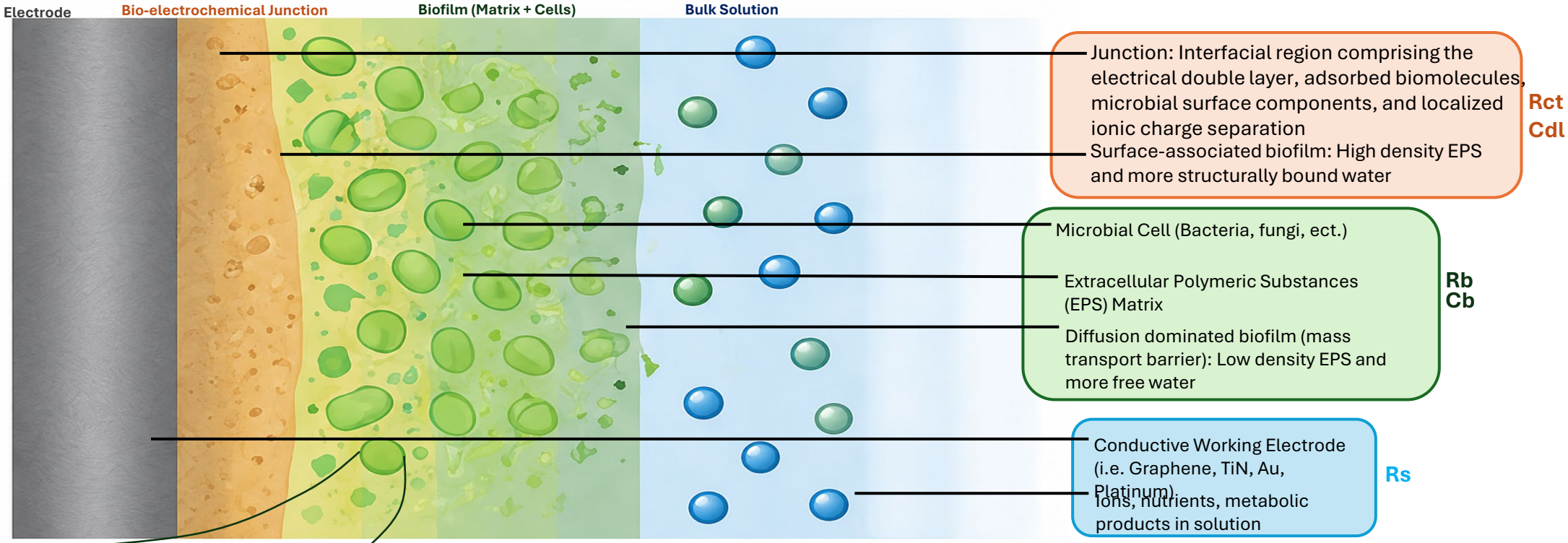
Conductive Working Electrode (i.e. Graphene, TiN, Au, Platinum)

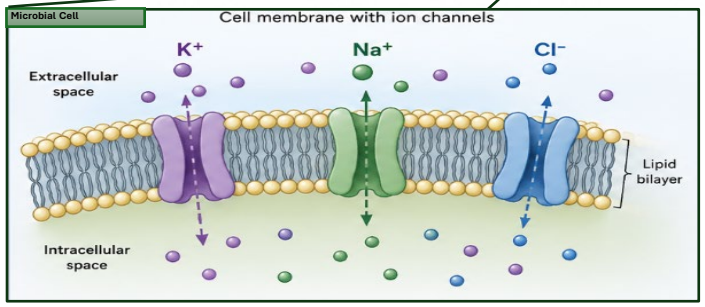


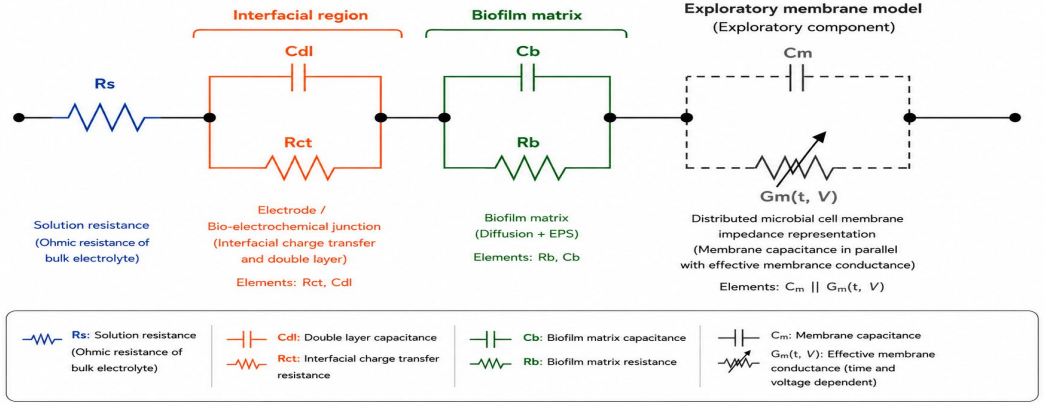


Electrochemical Impedance Spectroscopy (EIS) Modeling

Attached Microbial Biofilm Shell Model







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Acknowledgments

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- This work was performed at the Joint School of Nanoscience and Nanotechnology, a member of the National Nanotechnology Coordinated Infrastructure (NNCI), which is supported by the National Science Foundation (Grant ECCS-2025462)

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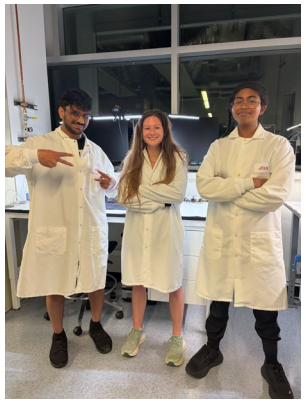
Acknowledgments



Questions?

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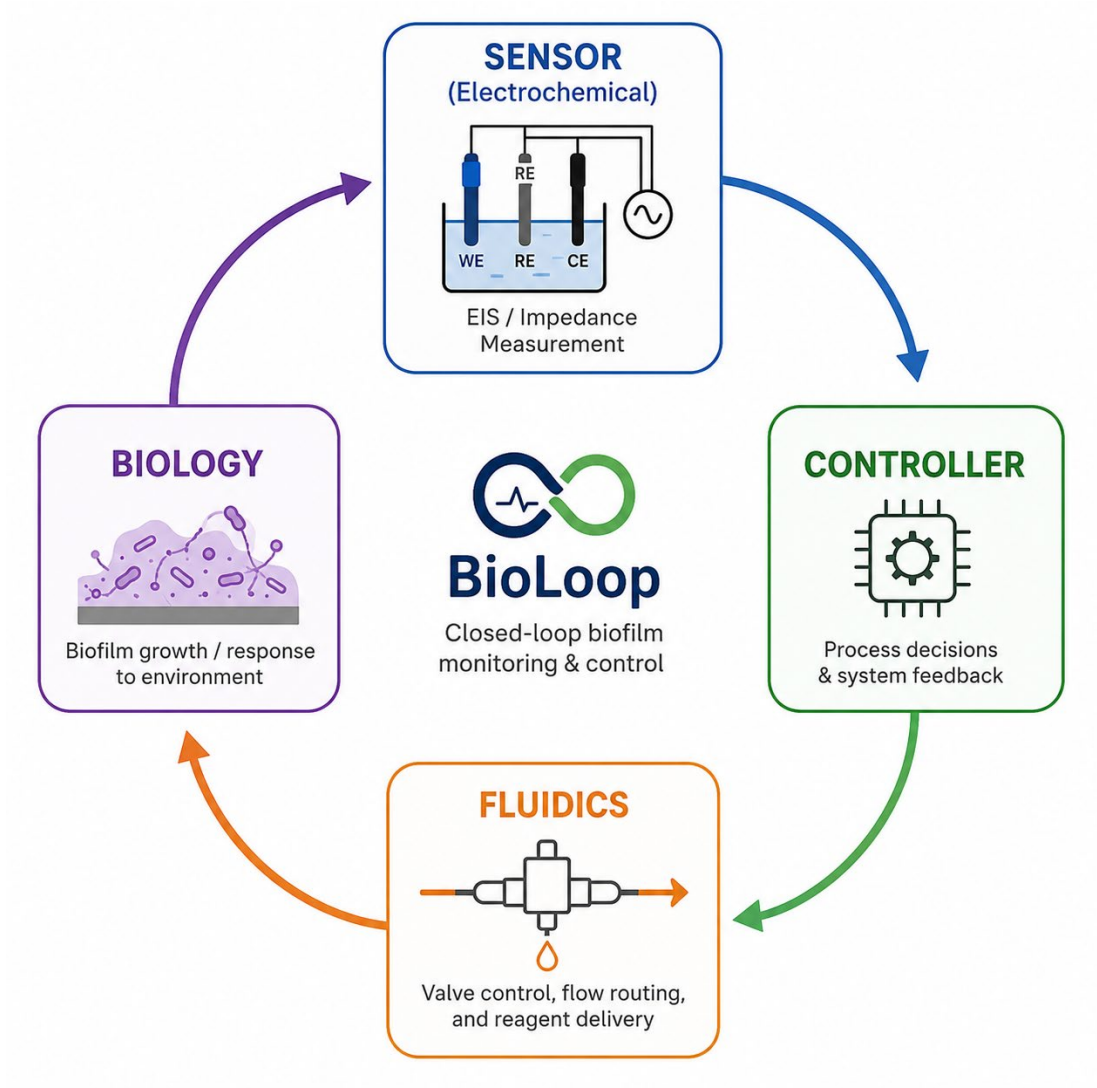
Appendix


SENSOR
(Electrochemical)
RE
WE
RE
CE
EIS / Impedance
Measurement
CONTROLLER
Process decisions
& system feedback
FLUIDICS
Valve control, flow routing,
and reagent delivery
BIOLOGY
Biofilm growth / response
to environment
BioLoop
Closed-loop biofilm
monitoring & control

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Proposed Solution

BioLoop integrates label-free monitoring with automated fluidics and system feedback to deliver continuous insight into biofilms.

1. HARDWARE INTERFACE

Syringe Pump (Serial) | Valve / I²C (MCP23017) | EmStat4X (Serial)

- Serial and I²C communication
- Device initialization and status check
- Real-time command execution

2. PROTOCOL ENGINE

```
protocol:
 name: biofilm_pilot
 steps:
   - seed: 3h
   - flow: 5 uL/min
   - duration: 8h
   - eis_scans: 3
   - interval: 1h
```

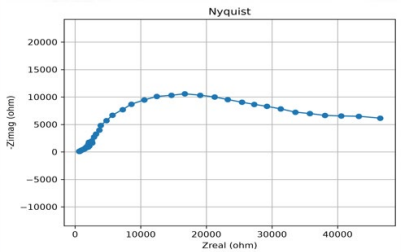
- YAML-based protocol definition
- Step scheduling and timing
- Flexible experimental design

3. EIS ACQUISITION

```
> OCP
> EIS
f 1e5 1e-2 51
ba 100u
pstat 3
pmax 100m
pmin 100p
wait 4
pck f r j
*
```

- MethodSCRIPT command generation
- Real-time data streaming
- Robust packet parsing

4. DATA PROCESSING



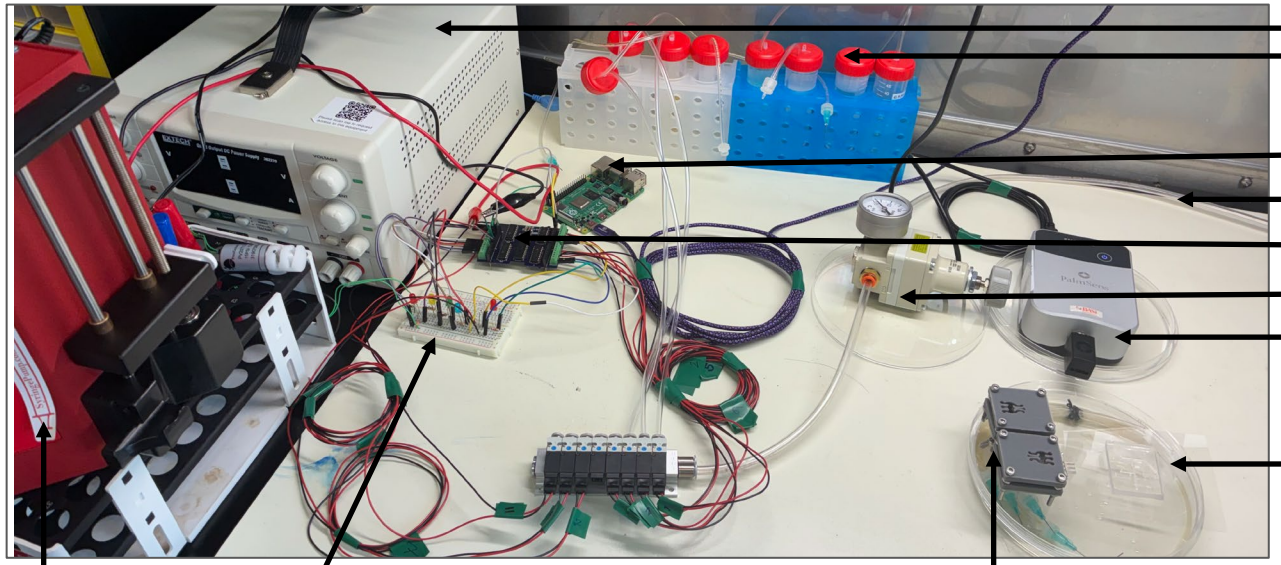
- Rs extraction and quality metrics
- Outlier detection and validation
- Concentration estimation (Kohlrausch)

5. FEEDBACK & CONTROL

Salinity: 0.156 M
Status: OPTIMAL
Action: Maintain flow

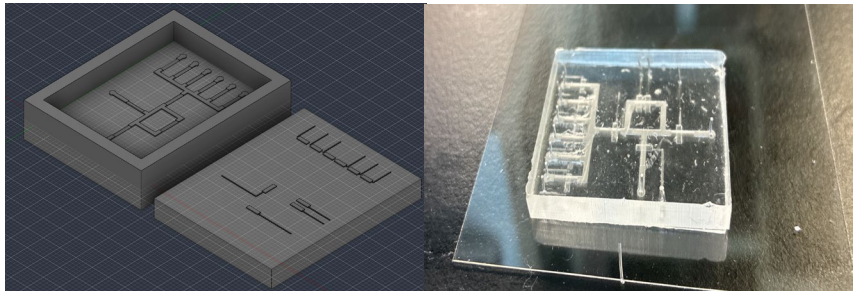
- Rule-based decision making
- Automated system adjustments
- Closed-loop environmental control

Bioloop Hardware Components



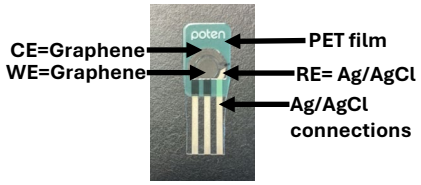
Routing Valve Chip

- PDMS microfluidic chip fabricated with SLA resin printed molds
- Quaker style valves with 2 PDMS layers:
  - Flow Layer: 0.1mm height semicircular channels through which fluids are routed
  - Control Layer: 0.1 mm height rectangular channels orthogonal to flow channels through which air is pumped

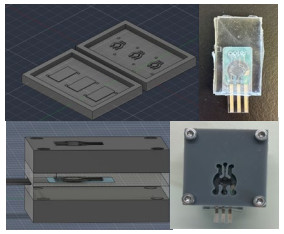


SPE Microfluidic Chip and Clamp

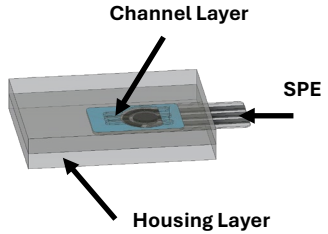
- Poten SPE: commercial electrode



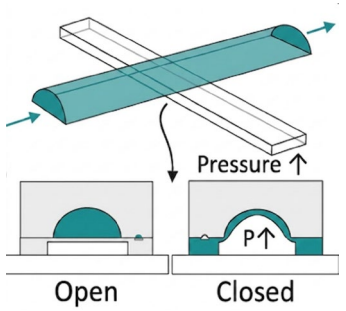
- Clamp is two SLA resin printed squares which are screwed together to compress the microfluidic chamber and prevent leakage



- 2 PMDS chips bound together around poten SPE:
  - **Channel layer**: contains geometry for optimizing flow over poten electrode
  - **Housing layer**: contains 0.5 mm extrusion cut to house poten SPE



- Mechanism:
  - OPEN: solenoid valve closed, flow channel paten, atmospheric pressure in control layer
  - CLOSED: solenoid valve open, flow channel occluded, positive pressure in control layer



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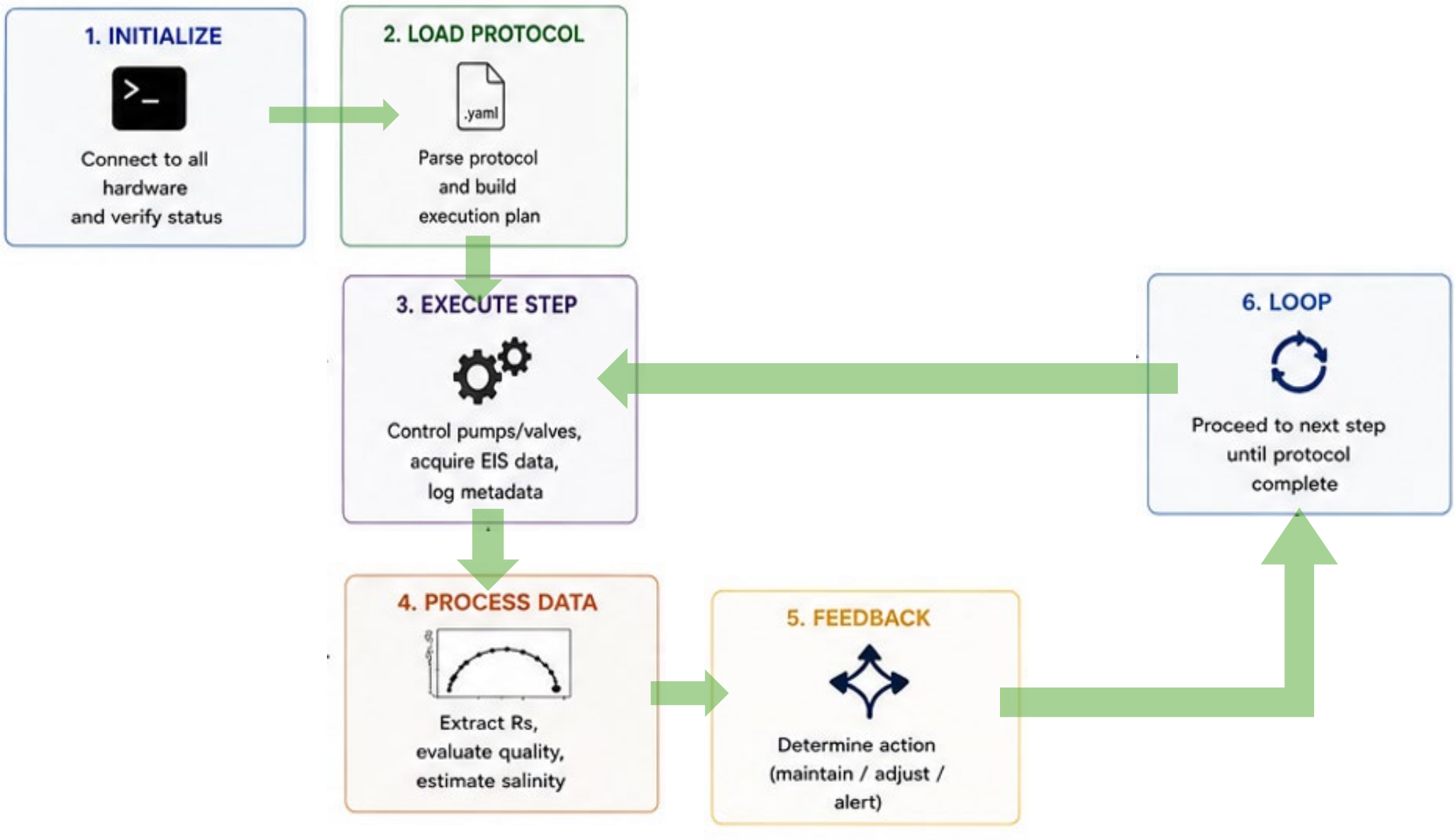
Bioloop Platform: Software



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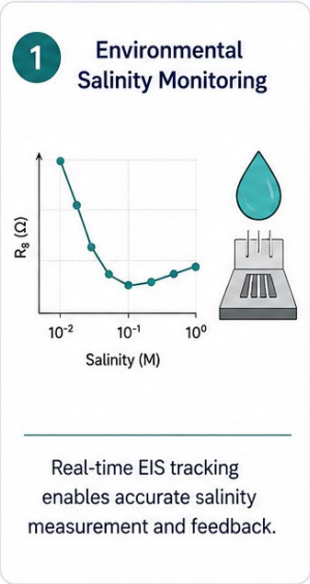
Validation

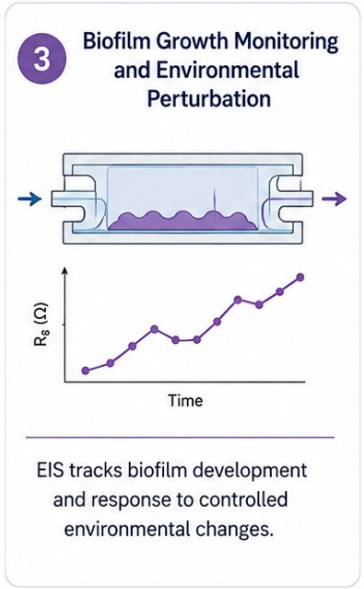


Future Validation

- ❑ Learn concentration-dependent correction term (BI)
- ❑ Expand calibration beyond NaCl to environmentally relevant ionic mixtures
- ❑ Implement continuous online model refinement during autonomous operation

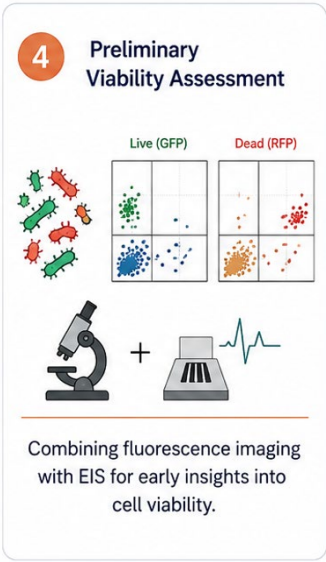
Future Validation

- ❑ Reduce treatment matrix to most effective restoration protocols
- ❑ Validate with larger electrode sample sizes
- ❑ Integrate automated treatment selection and EIS feedback into Bioloop platform



Future Validation

- ❑ Replicating long term experiments collecting additional microscopy timepoints and biomass measurements
- ❑ Continuing exploring memory effect of ionic system perturbation and ECM parameter fitting for biofilm
- ❑ Integrating ionic strength model and OD bacterial concentration calibration model to better demonstrate biofilm growth, microbe environment, and microbe quantity



Future Validation

- ❑ Verify EIS acquisition and data logging to ensure all treatment measurements are captured
- ❑ Confirm microfluidic flow rate and ethanol exposure timing
- ❑ Refine fluorescence image processing, including threshold selection and particle segmentation
- ❑ Increase the number of biological and technical replicates
- ❑ Correlate impedance-derived features with quantitative live/dead fluorescence metrics to develop predictive viability models
- ❑ Expand testing to additional antimicrobial treatments and exposure conditions
- ❑ Introduce frequency variable treatment protocols

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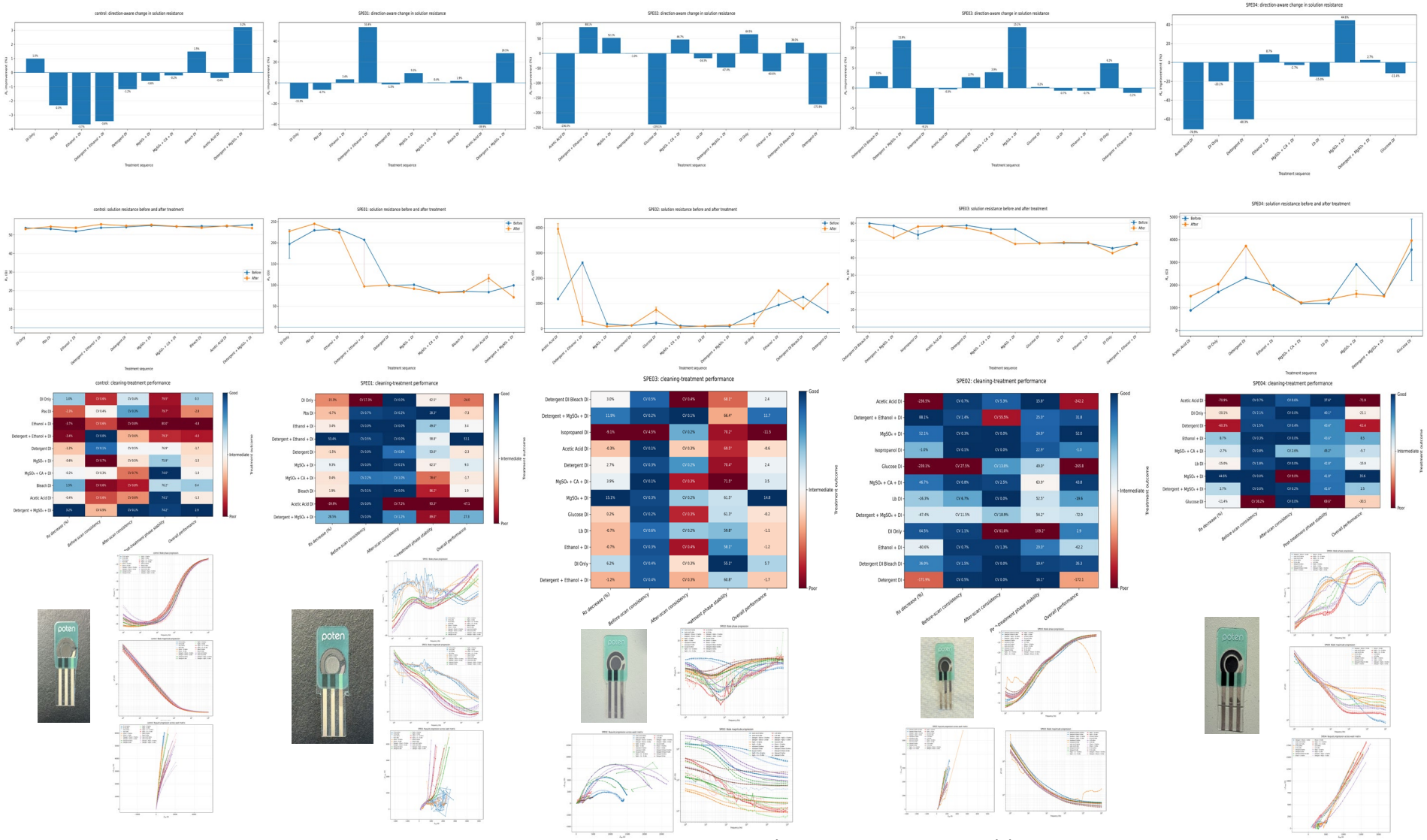
Electrode Health and Automated Quality Control



Demonstrate automated detection of electrode condition

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Electrode Health and Automated Quality Control

Table 1. Treatment Recommendation Summary

Treatment	Typical Rs Response	Typical Electrical Behavior	Overall Assessment
DI → DI	Minimal change	No meaningful change	Negative control
PBS → DI	Minimal change	No meaningful change	Matrix control
Ethanol → DI	Moderate Rs decrease	Improved electrical behavior on degraded electrodes	Effective restoration treatment
Detergent → Ethanol → DI	Large Rs decrease	Consistently improved electrical behavior across multiple electrodes	Most effective cleaning treatment
Detergent → DI	Variable Rs response	Minor improvement on some electrodes	Limited benefit alone
$MgSO_4$ → DI	Moderate Rs decrease	Improved electrical behavior on damaged electrodes	Effective electrochemical conditioning
$MgSO_4$ + Chronoamperometry → DI	Small Rs change	Improved spectral quality despite limited Rs change	Electrical conditioning without major resistance change
Bleach → DI	Small or inconsistent change	Limited improvement	Not recommended as primary treatment
Detergent → $MgSO_4$ → DI	Moderate to large Rs decrease	Consistently improved electrical behavior	Effective combined treatment

Table 2. Electrode Metrics Summary

Electrode	Initial Rs (Ω)	Final Rs (Ω)	ΔRs (Ω)	Overall Recovery (%)
Control	53.740 ± 0.318	53.662 ± 0.065	−0.078	+0.15%
SPE01	197.106 ± 34.072	70.806 ± 0.871	−126.300	+64.08%
SPE02	1175.332 ± 8.592	1767.054 ± 0.874	+591.722	−50.34%
SPE03	59.907 ± 0.287	48.462 ± 0.138	−11.445	+19.10%
SPE04	884.810 ± 6.479	3965.509 ± 0.000	+3080.699	−348.18 %

Future Validation

- ❑ Reduce treatment matrix to most effective restoration protocols
- ❑ Validate with larger electrode sample sizes
- ❑ Integrate automated treatment selection and EIS feedback into Bioloop platform

Table 3. Electrode Overall Assessment

Electrode	Overall Assessment
Control	Maintained ideal electrical behavior throughout testing.
SPE01	Recovered substantially toward ideal electrical behavior.
SPE02	Resistance improved, but ideal electrical behavior was not fully recovered.
SPE03	Maintained nearly ideal electrical behavior throughout the cleaning protocol.
SPE04	Displayed modest improvement in electrical behavior but remained degraded.

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Bacterial Growth Monitoring and Environmental Perturbation

Exploratory demonstration of electrochemical monitoring during biofilm cultivation

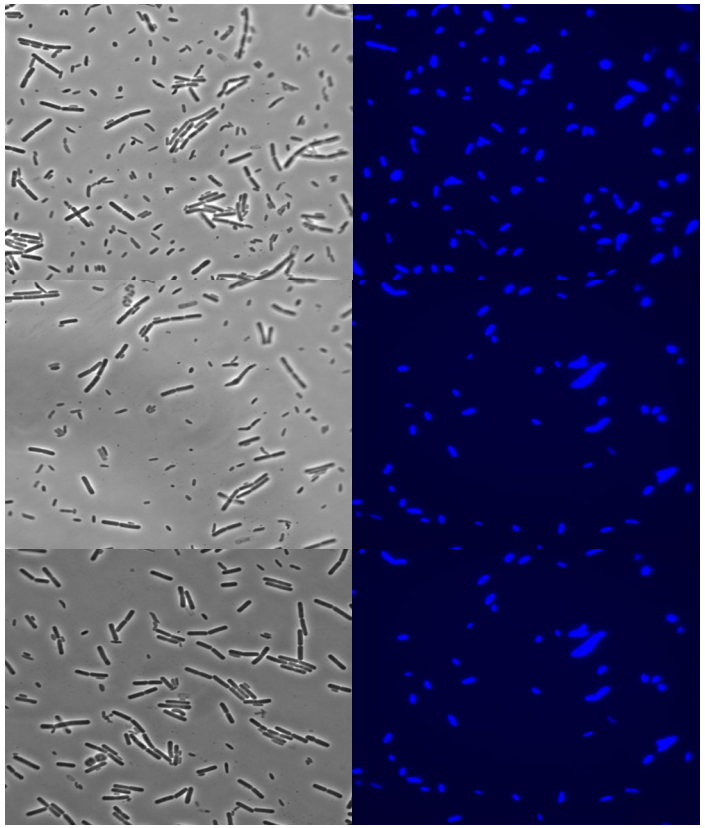
EXPERIMENTAL PROTOCOL

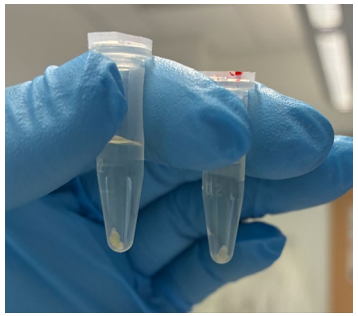
Biofilm Seeding
- *E. coli* S22
- LB Miller medium
- Static seeding: 3 hours
- Flow stopped during seeding

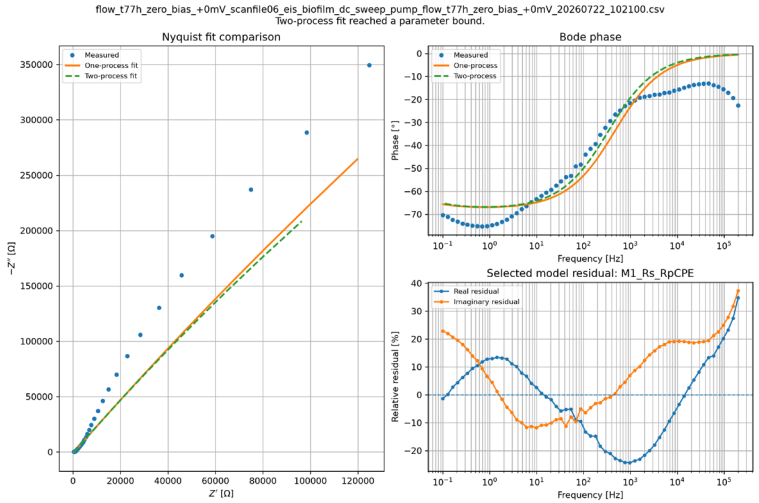
Continuous Perfusion
- Flow rate: 5 µL/min
- Medium: LB
- Continuous recirculation after seeding

EIS Acquisition
- Three replicate spectra at each time point
- Hourly measurements
- Frequency range: 100 kHz to 0.1 Hz
- OCP EIS and DC sweep: 0.1–0.8 V
- AC amplitude: 100 mV

Perturbation
- 0.5 M NaCl flushed into chip for 5 s
- EIS measurements performed
- LB medium restored after perturbation
- Hourly monitoring continues







77 hr timepoint data for bacterial growth on electrode experiment.

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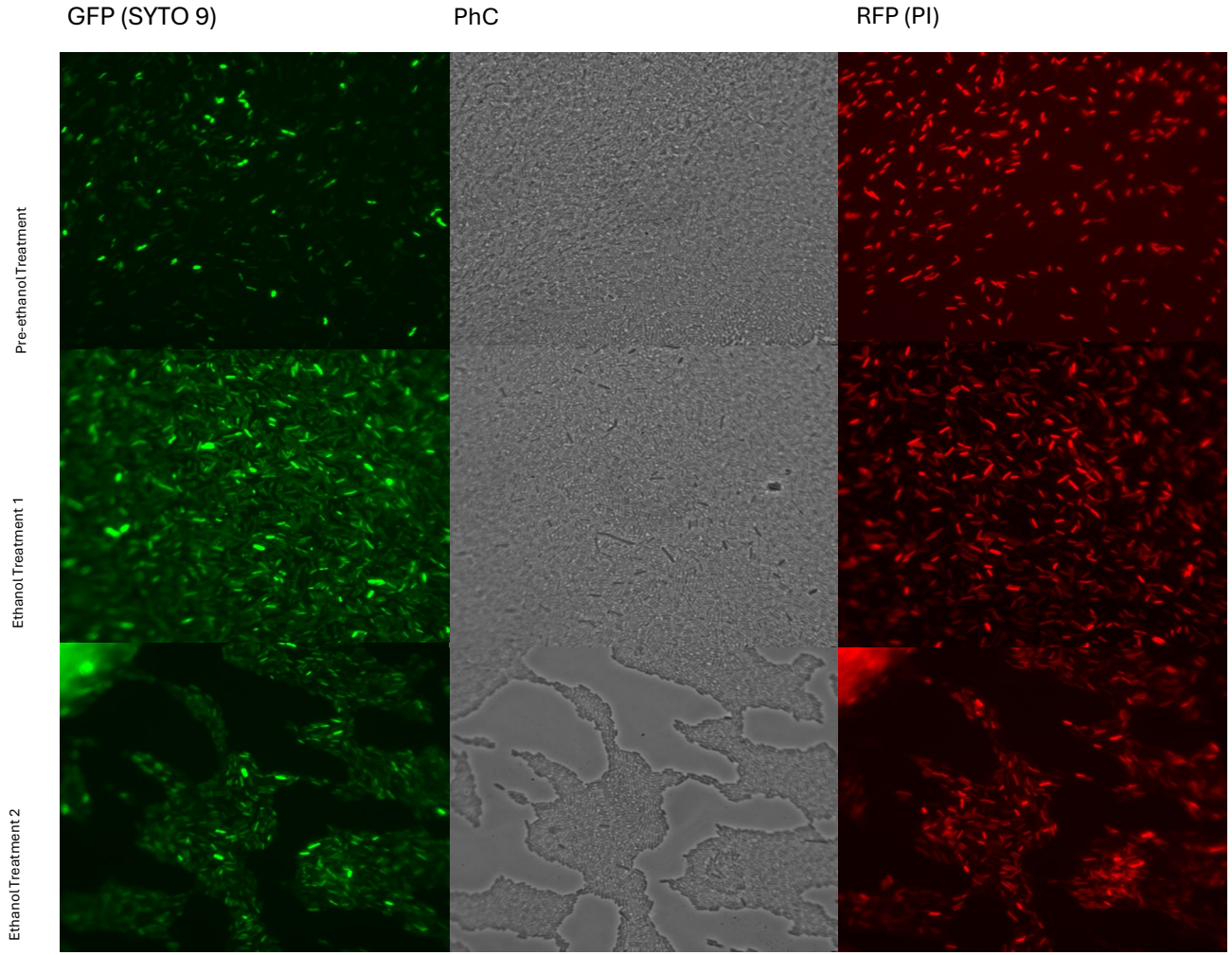
Preliminary Viability Assessment

Evaluate whether repeated ethanol exposure produces measurable changes in bacterial impedance response while assessing viability using fluorescence microscopy

1. Load *Pseudomonas* cells into microfluidic chamber
2. Acquire EIS spectrum
3. Collect sample for Live/Dead staining
4. Image GFP / Phase Contrast / RFP
5. Treat reservoir with ethanol (1 s)
6. Incubate 3–5 min
7. Repeat for two treatment cycles



Preliminary results for live dead cell assay. Pre-ethanol treatment, post treatment 1, and post treatment 2.