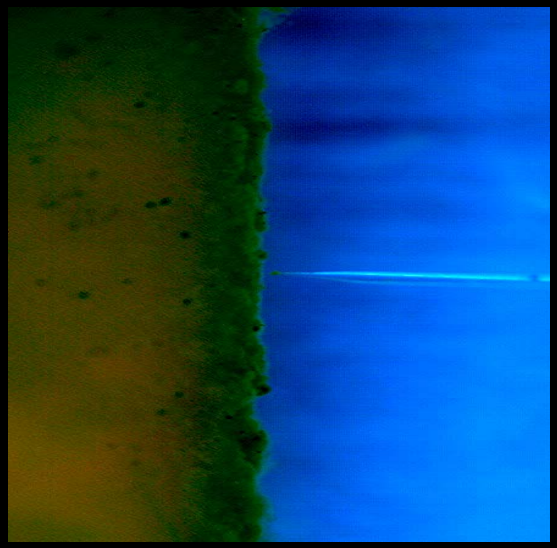
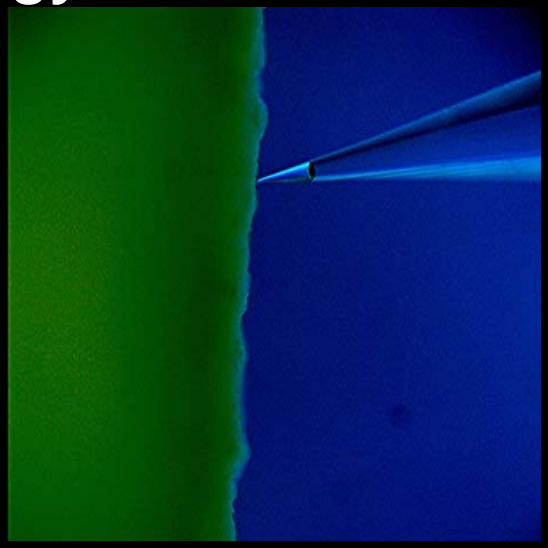
Self-referencing sensors for characterizing microbial biofilm physiology





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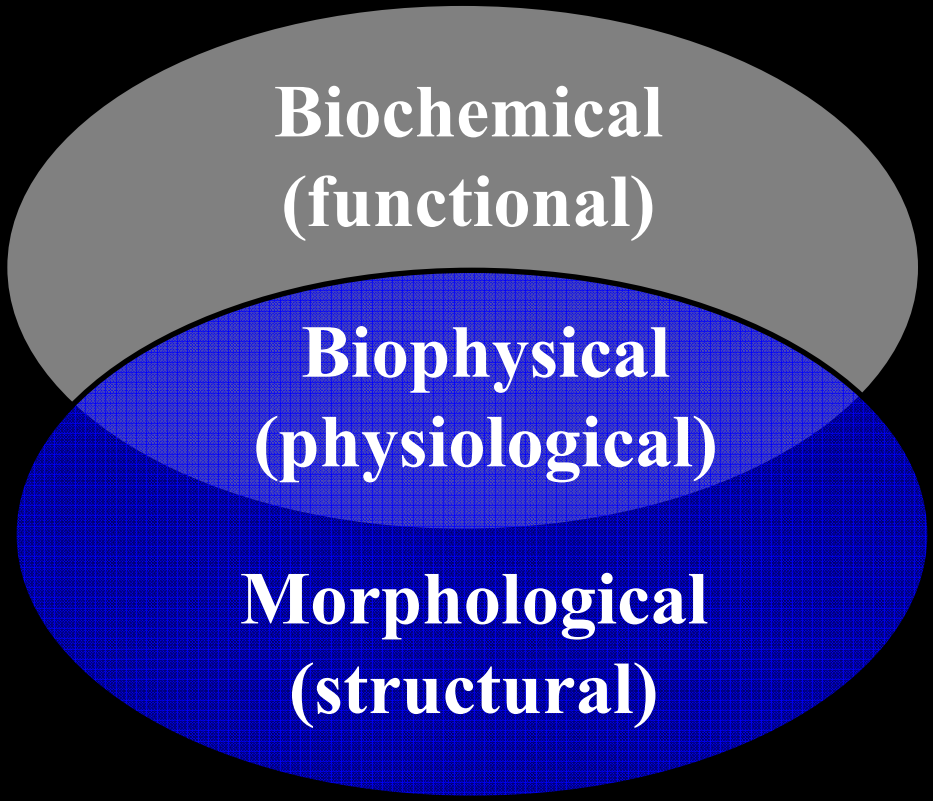
Biofilm Physiology

- Function
  - Ion/nutrient flux
  - Oxygen flux
- Structure
  - Shared analyses
    - Atomic force microscopy
- Why is studying biofilm physiology important?
  - Wastewater treatment
  - Toxicity studies



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Techniques for characterizing biofilm physiology

- Respirometry
- Invasive concentration (activity) sensors
  - Oxygen, ammonium, nitrate, pH
- Classic biochemical techniques
  - *In vitro* detectors and assays

Non-invasive techniques with greater sensitivity and temporal resolution are required

PHYSIOLOGICAL SENSING FACILITY

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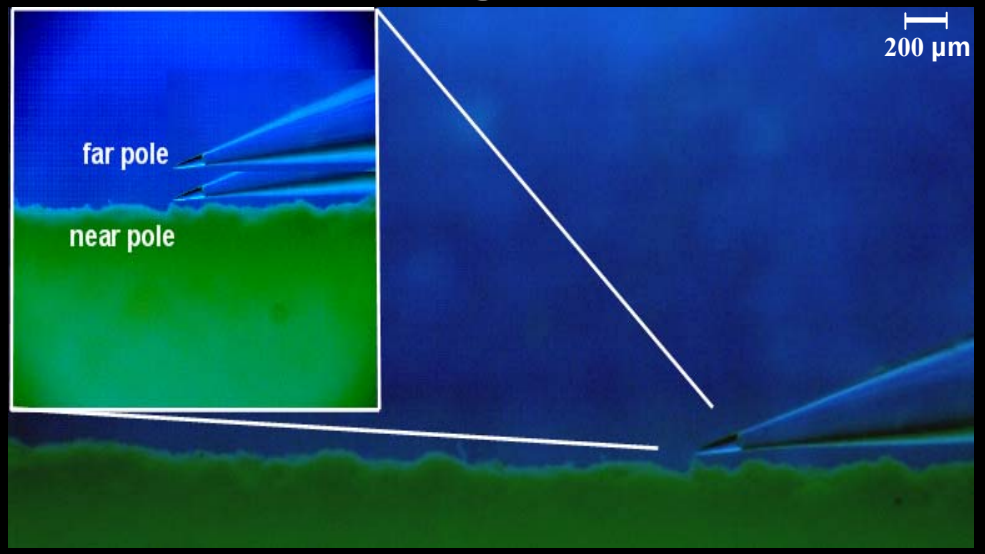
Self-referencing sensors

- Real time biophysical flux
  - Fixed excursion distance
  - Near pole
  - Far pole
- Corrects for mechanical motion through liquid solution
  - Reference measurement
    - ≈0.4 cm from surface

dC = difference in concentration between near pole and far pole

$$J = -D\frac{dC}{dr}$$

dr = excursion distance

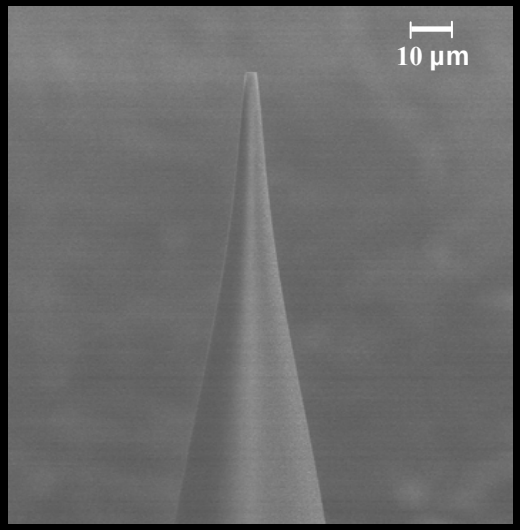


***N. europaea* biofilm immobilized on silicon membrane**

PHYSIOLOGICAL SENSING FACILITY

Benefits of self-referencing technique

- Very low detection limit (fmol/cm^2-sec)
- Non-invasive (non-destructive)
- Real time temporal resolution
- Direct quantification of analyte flux
  - Dynamic measurement



SEM of electrode tip

- Multiple sensor approach for measuring flux
  - cumulative signal error
- One sensor, one source of error

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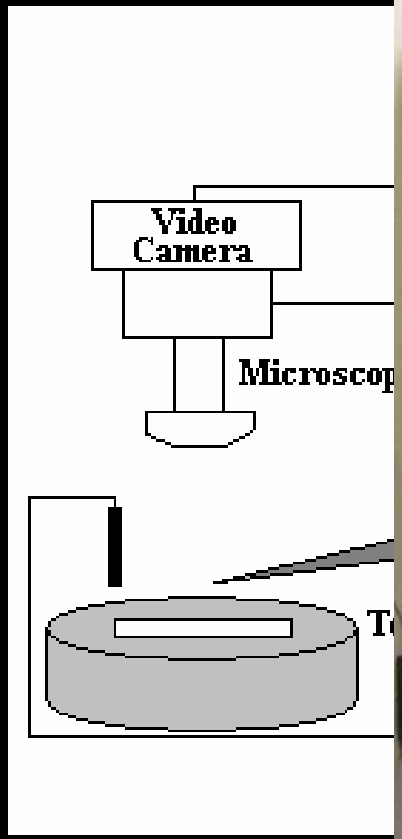
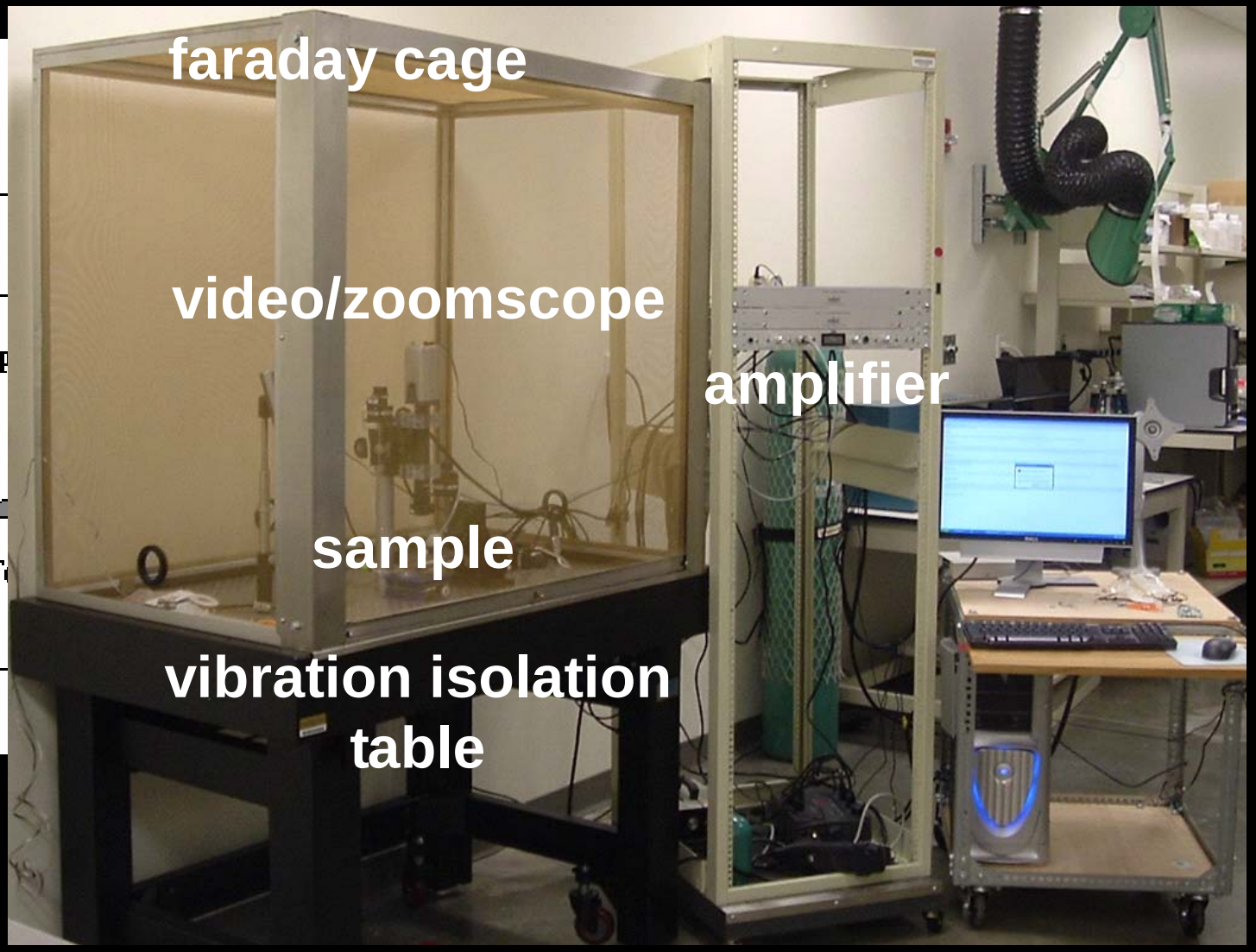
Self-referencing sensor hardware





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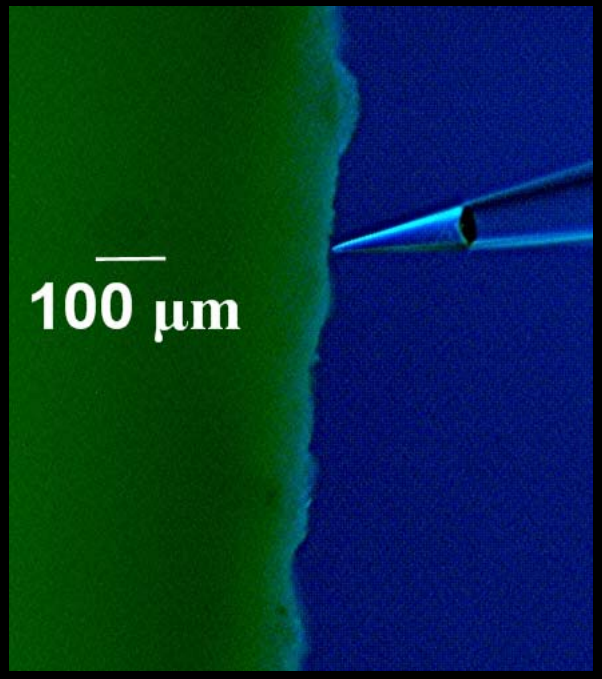
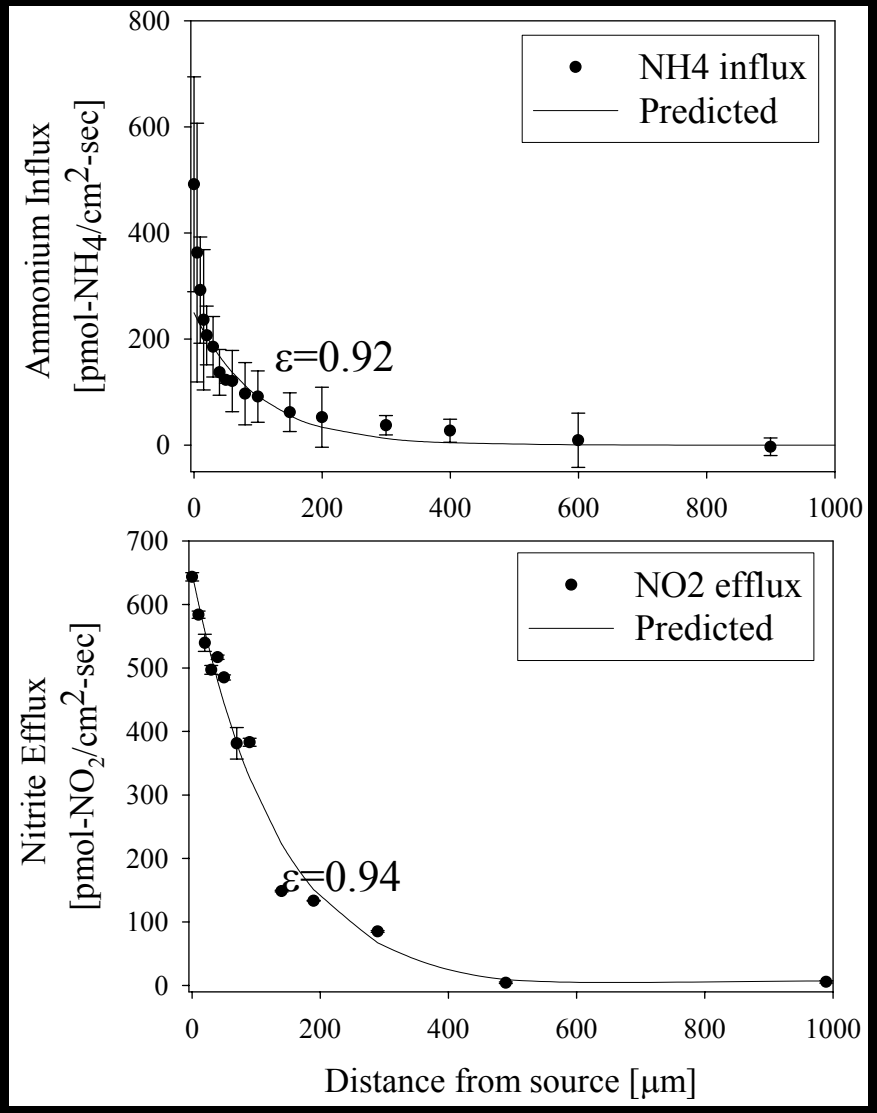
Sensor calibration (dynamic)

- Step-back experiments on *N. europaea* biofilm



***N. europaea* biofilm immobilized on silicon membrane**



Classic physiological study

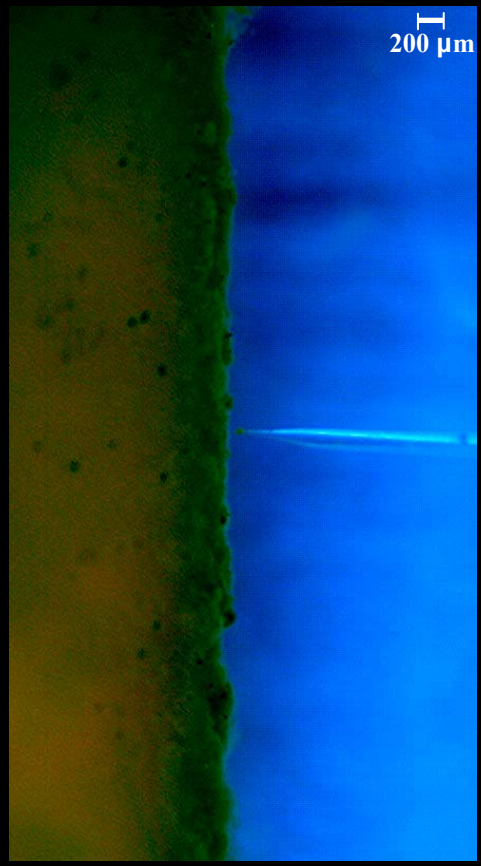
- Ammonia oxidizing bacteria
  - *Nitrosomonas europaea*
    - Ammonia monooxygenase (AMO)
      - membrane bound protein
      - copper at active site
    - Hydroxylamine oxidoreductase (HAO)
      - Produces electrons required by AMO

$$NH_4 \xrightarrow{pK_a = 9.2} NH_3 + H^+$$

$$NH_3 + O_2 + 2H^+ \xrightarrow{AMO} NH_2OH + H_2O \xrightarrow{HAO} NO_2^- + 5H^+ + 4e^-$$

AMO inhibition

- Alternative substrates
  - AMO can oxidize over 40 substrates
- Mechanism-based (e.g., acetylene)
- Reversible inhibition
  - Visible light
  - Copper chelating agents
    - Thiourea, carbon disulfide

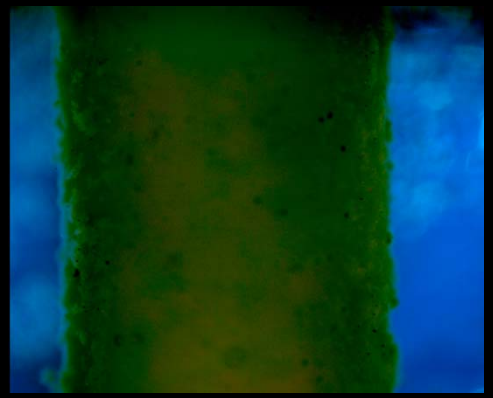


Mixed culture nitrifying biofilm immobilized on silicon membrane

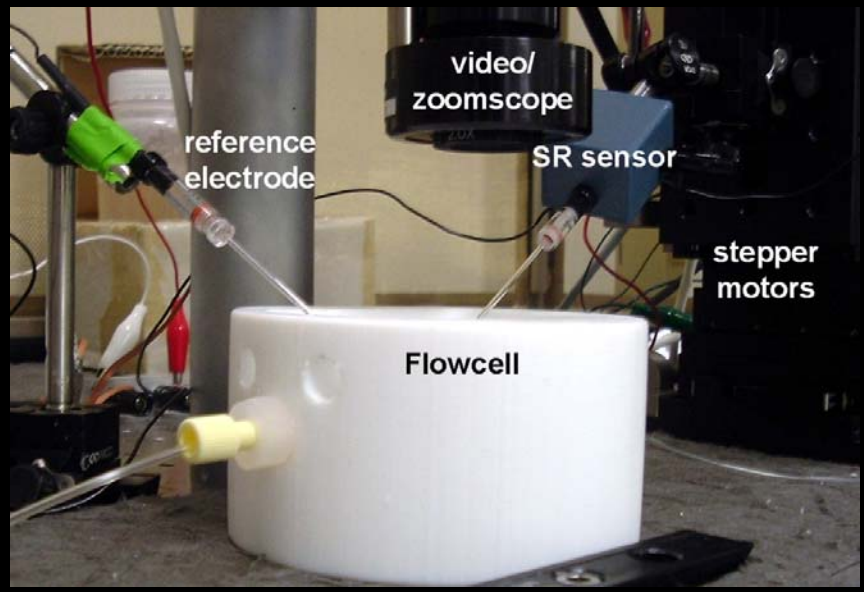
Cell harvesting and immobilization

- Microbes immobilized on oxygen-permeable silicon membranes
- Upflow membrane-aerated bioreactor
  - Lumen-side air flow
- Membranes transferred to flow cell for measurement

***N. europaea* biofilm grown in MABR**



MABR limit the formation of anoxic zones



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Experimental design

position 1

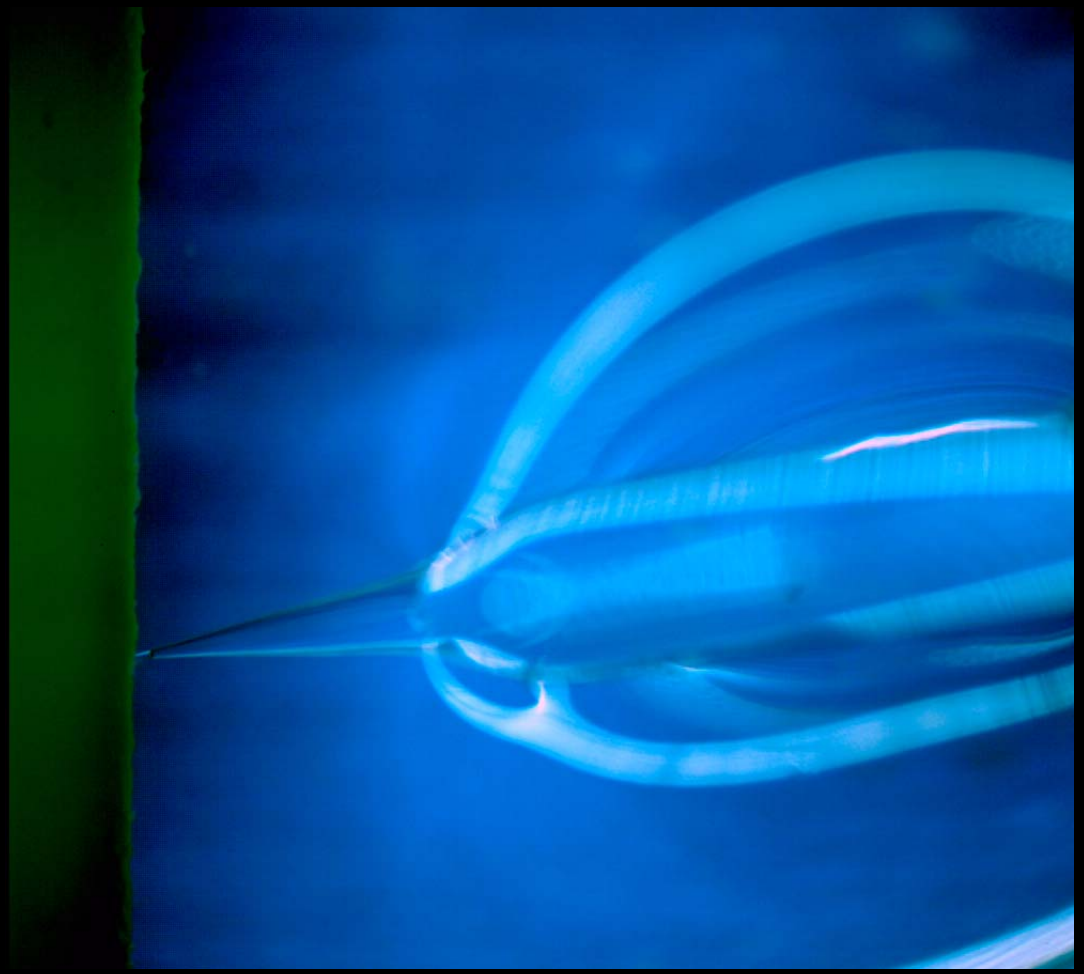
position 2

position 3

position 4

position 5

position 6



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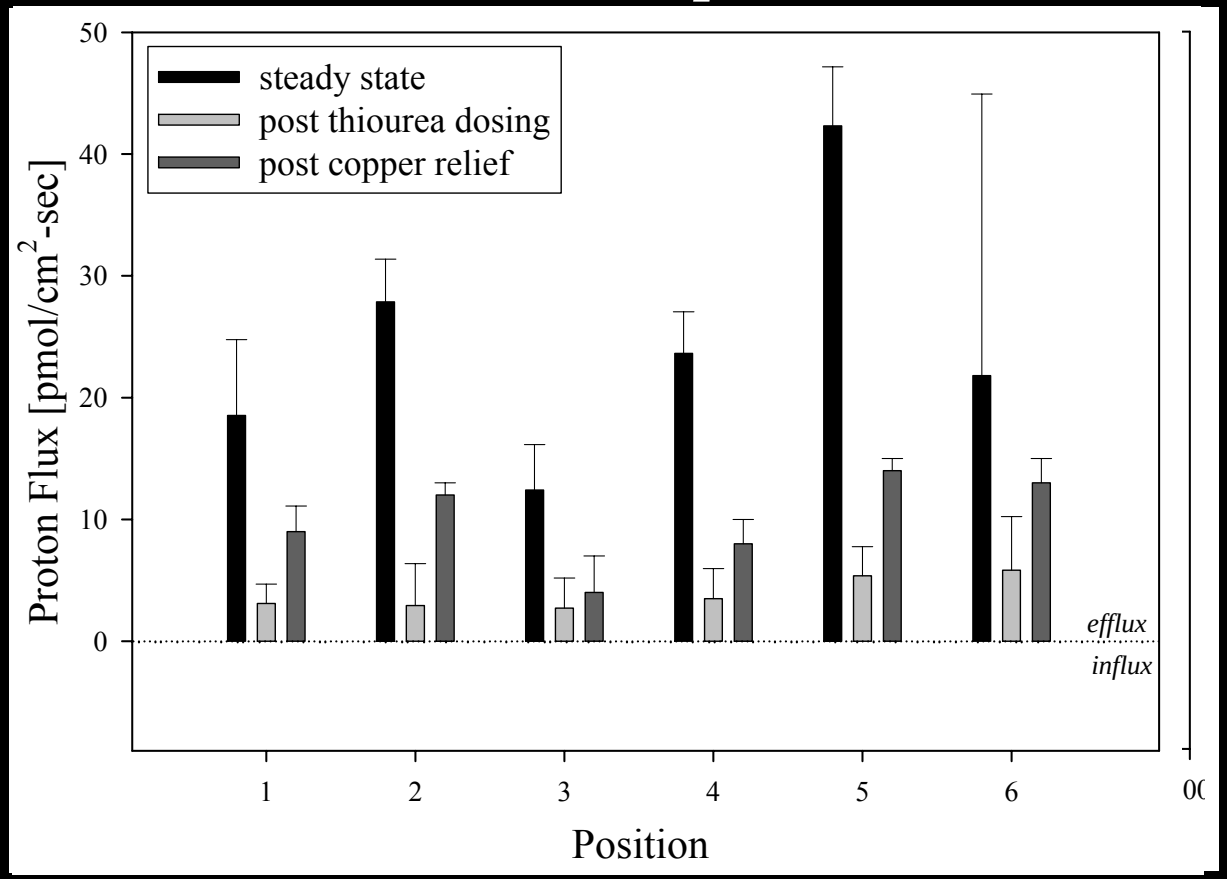
AMO inhibition study: Proton flux

Nitrosomonas europaea biofilm

pH at biofilm surface was 6.71 ± 0.10 c



Real time data collected at position 1

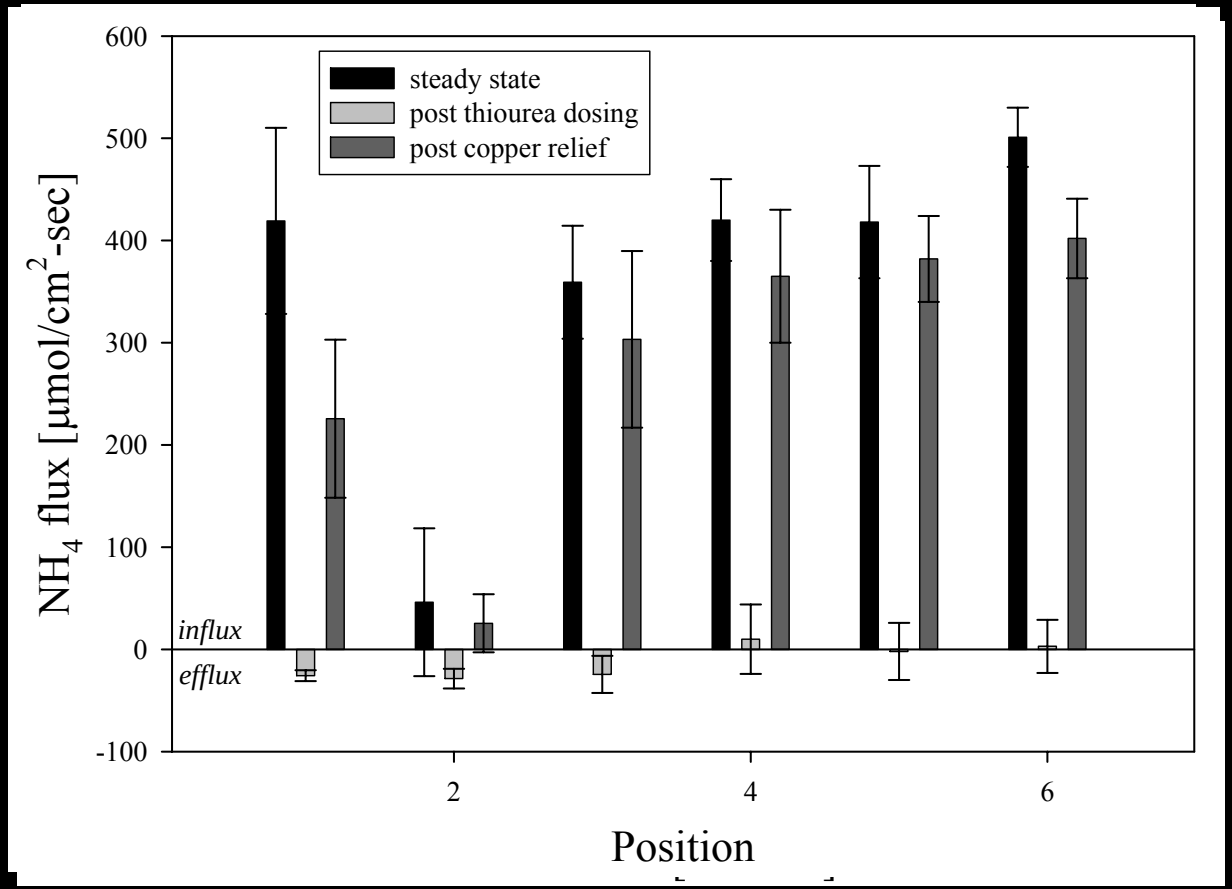
PHYSIOLOGICAL SENSING FACILITY

AMO inhibition study: Ammonium flux

Nitrosomonas europaea biofilm

Real time data collected at position 6

100 µM $CuCl_2$ sufficient for restoring steady state NH_4 influx



Conclusions

- Successfully demonstrated reversible inhibition of AMO by copper chelation in a biofilm
  - Non-invasive
  - Real time response
- More copper required to restore steady state oxygen and proton flux levels than required to restore NH_4 influx
  - Oxygen and copper are required for many cellular processes other than AMO activity
- Use of self-referencing sensors will allow non-invasive investigation of real time biofilm stress response to chemical toxin exposure

Ongoing studies

- Chemical toxicity in pure and mixed culture biofilms
  - Uncoupling of aerobic respiration
  - Glutathione gated-potassium efflux
    - Associated with deflocculation
      - Additionally measuring efflux of cross-linking ions (Ca^{2+}, Mg^{2+})
  - Aerobic biodegradation of respiratory-inhibiting compounds
  - Heavy metal exposure

Acknowledgements

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- Discovery Park
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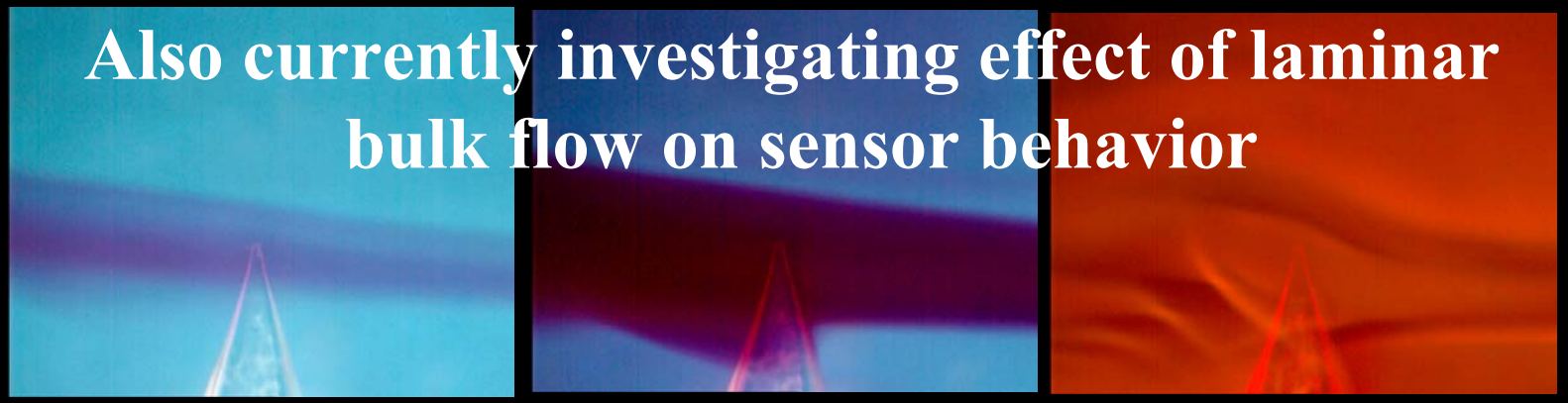
- Jon Shaff (Cornell, USDA)
- Aeraj ul Haque, Rameez Chatni (Electric. and Comp. Engr.)

Also currently investigating effect of laminar bulk flow on sensor behavior



AMO inhibition study: Oxygen flux

Real time data collected at position 1

Nitrosomonas europaea biofilm