Michael Kühl

Professor

Tel.: +45 353-21956
mkuhl@bio.ku.dk

Lars Rickelt

Chemical Engineer
Development and fabrication of optical sensors
Tel.: +45 353-21968
lfrickelt@bio.ku.dk

Marc Staal

Post-doctoral scientist
Microscopic oxygen imaging
Tel.: +45 353-21972
mstaal@bio.ku.dk

Karin E. Ulstrup

Post-doctoral scientist
Microenvironmental controls and photobiology of sea-ice microalgae
kulstrup@bio.ku.dk

Sana Suhela Dandan

MSc. student (2008-2009)
Distribution of host pigments and photosynthesis in corals
ssdandan@bio.ku.dk

Anni Glud

Technician (on leave)

Tel.: +45 353-21953
aglud@bio.ku.dk

Sheila I. Jensen

Ph.D. student (2007-2010)
In situ gene expression and ecophysiology of thermophilic cyanobacteria
Tel.: +45 353-21968
sijensen@bio.ku.dk

Lars Liengaard Jensen

Ph.D. student (2008-2011)
Nitrous oxide emission associated with seasonal flooding-drying cycles in Pantanal, a tropical wetland
liengaard@bio.ku.dk

Erik Trampe

MSc. student (2008-2009)
Functional heterogeneity of photosynthesis in plankton
etrampe@bio.ku.dk

Isabel Jimenez-Denness

External Ph.D. student
University of Technology, Sydney
Thermal microenvironment of corals
isabel.jimenez@student.uts.edu.au

Undergraduate students

B.Sc. student (external, Feb-June 09)
Özge Er
Anaerobic biofilms in soft tissue fillers
Practical work done at the Faculty of Health Sciences, Univ. of Copenhagen

B.Sc. student (March-June 09)
Rasmus Nørregaard
Role of hyaline hairs for solute exchange in Fucoid macroalgae

Project student (external, Feb-May 09)
Bibi Emilie Friis Ziersen
Microrespirometry on coral zooxanthellae over a depth gradient
Practical work done in Australia

B.Sc. student (external, Feb-June 09)
Kasper Claus Thomsen
Topic
Practical work done at the Faculty of Health Sciences, Univ. of Copenhagen

B.Sc. student (March-June 09)
Mads Dahl
Temperature and pH effects on corals

B.Sc. student (external)
N. N.

Part of *Aquatic Microbiology Group* in the *Aquatic Biology Section* *Dept. of Biology*, *Univ. Copenhagen*

Research Topics

- *Microbial life and biogeochemistry at interfaces.*
- *Microenvironmental controls of cell behavior, interaction and activity in microbial communities and symbioses.*
- *Imaging and microsensor techniques for mapping physical and chemical microenvironments and for quantifying transport processes and metabolic activity at high spatio-temporal resolution.*

www.mbl.ku.dk/mkuhl

Biofilm Ecology and Microenvironmental Analysis

Funded projects:

FNU: *Photobiology of surface-associated microbial communities.*

FNU: *Dynamics and function of reactive oxygen in natural microbial communities.* NEW

FI/FUU: *In situ gene expression and ecophysiology of thermophilic cyanobacteria in hot spring microbial mats*

FTP: *Microscopic oxygen imaging.* (2D & 3D distributions)

NSF: *Do Species Matter in Microbiology?*

ARC: several grants related to symbioses and biofilms

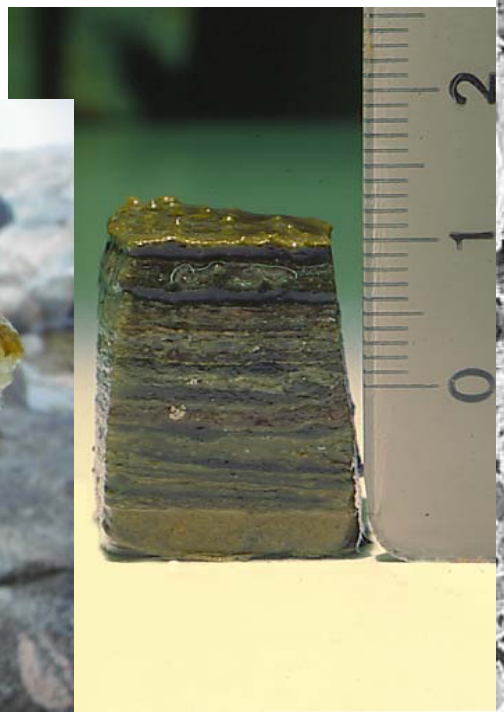
Experimental tools:

Microsensors, nanosensors and advanced imaging.


Hypersaline lakes/lagoons


Coral reefs and symbioses


Hot Springs




Surface of sediment
Biofilm
Sediments

Measuring microbial activity at the appropriate scale ?

Microsensors

Electrochemical

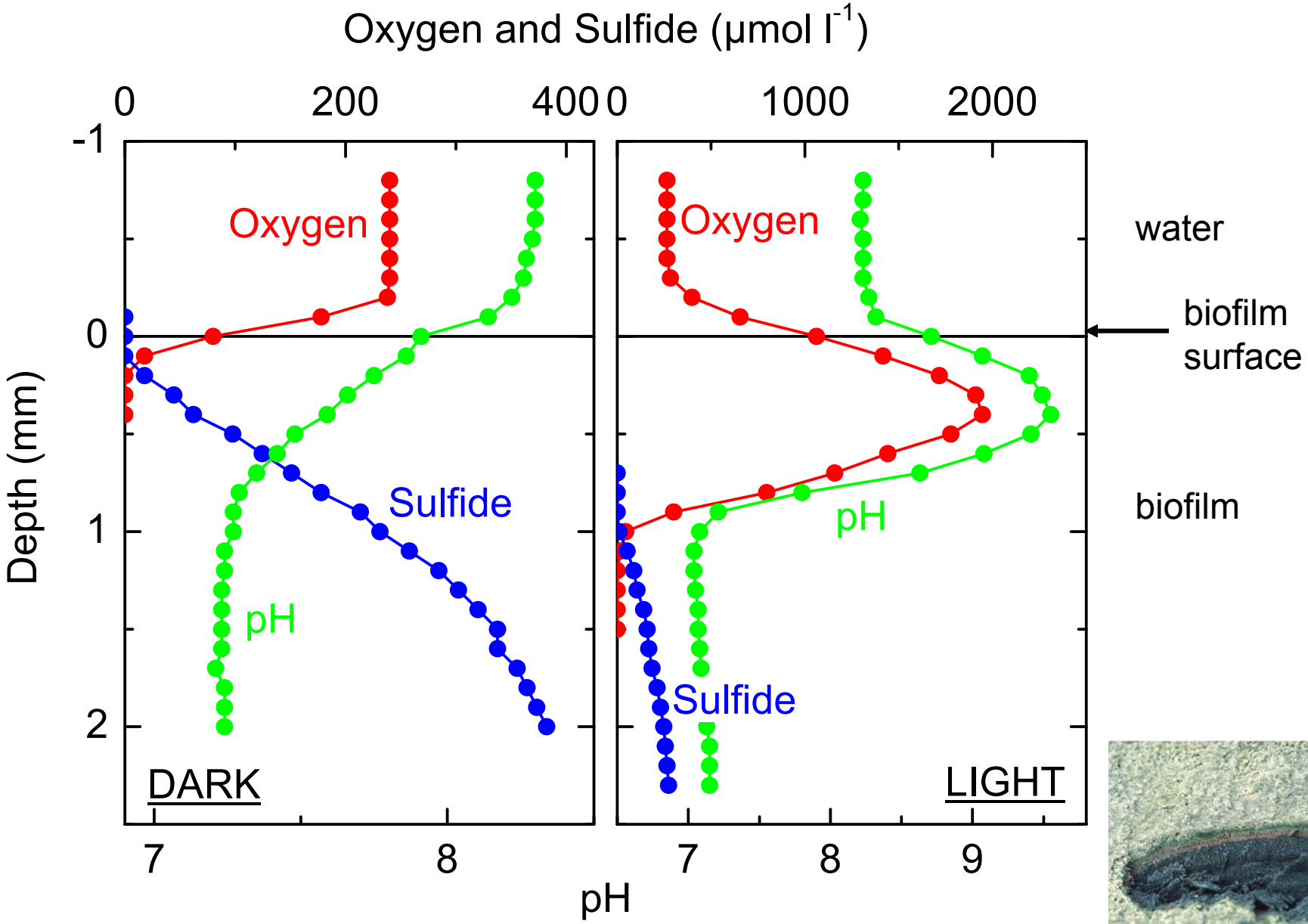
- *Amperometric* sensors for:
  O_2, H_2S, N_2O, NO, H_2, H_2O_2
  flow, diffusivity
- *Potentiometric* sensors for:
  pH, CO_2, S^{2-} (solid state)
  pH, CO_3^{2-}, NO_2^-, NO_3^-, NH_4^+, Ca^{2+} (LIX-based)
- *Voltammetric* sensors for:
  Reduced Fe & Mn, O_2, S_{tot},…
- *Microbiosensors* for:
  NO_3^-/NO_2^-, CH_4, Glucose, BOD, VFA

Fiber-optic

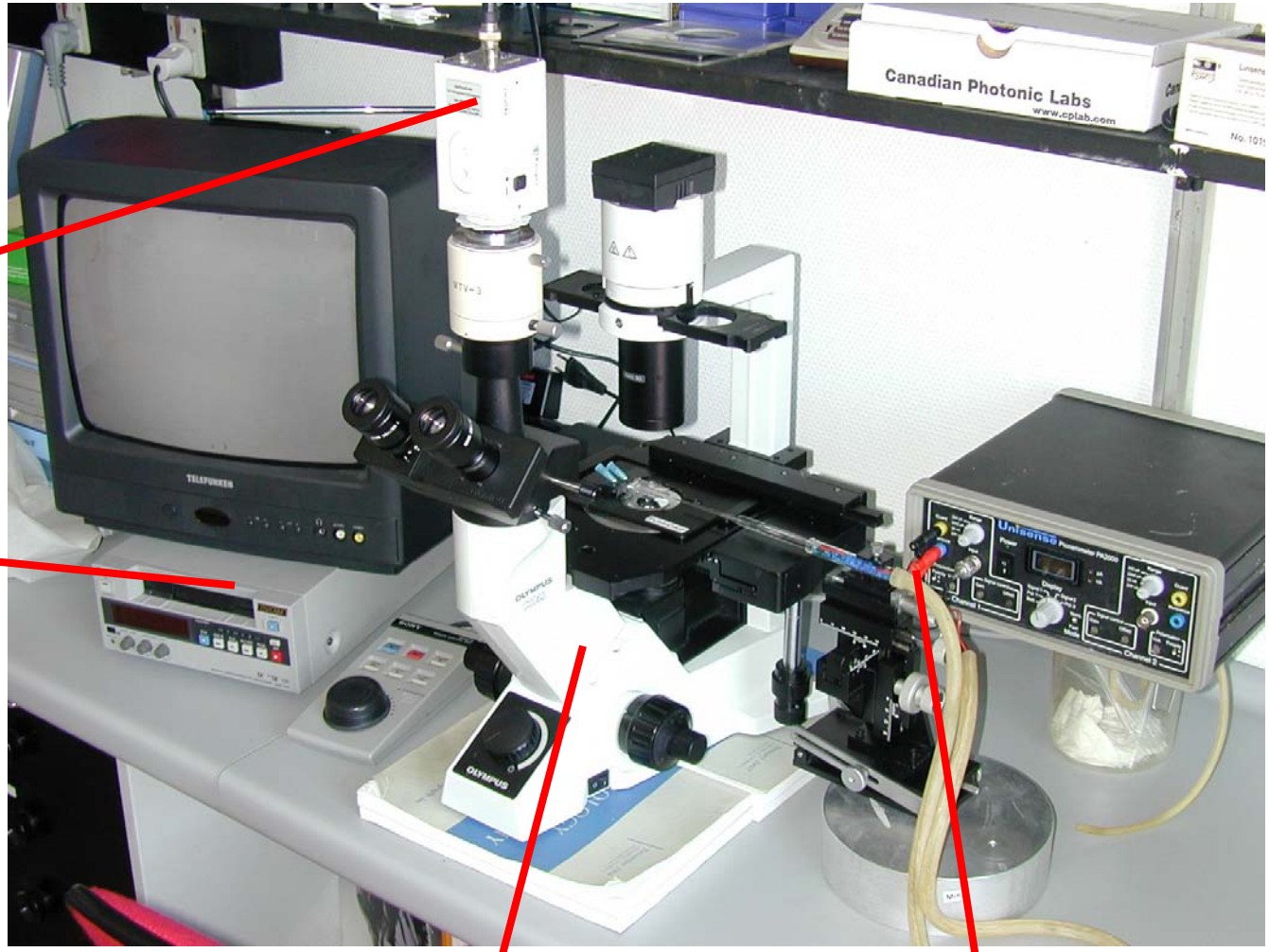
- *Microprobes* for:
  - radiance, irradiance, scalar irradiance (UV-NIR light)
  - Surface detection
  - Pigment fluorescence
  - Diffusivity/Flow
- *Micro-opt(r)odes* for:
  O_2, pH, CO_2, temperature

Kühl & Revsbech 2001, Revsbech 2005,Kühl 2005

Microsensor analysis of biofilms



Gradient-capillary-cell-tracking-setup



video camera

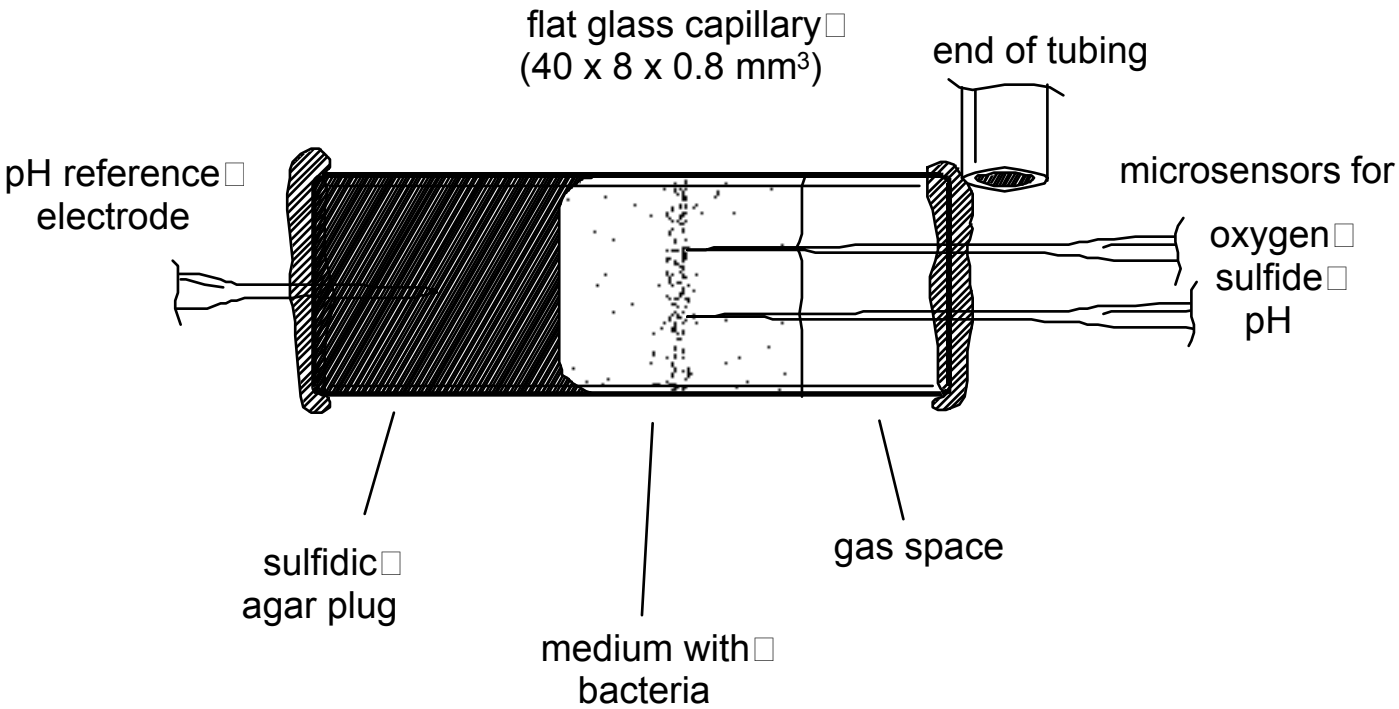
video recorder

inverted microscope

oxygen-microelectrode with Picoammeter

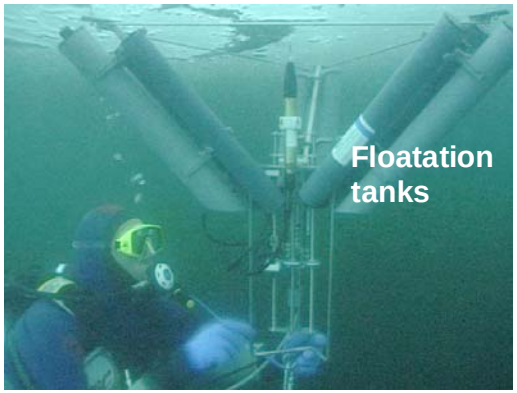
Motility of Microorganisms in Response to Light, Oxygen, and Sulfide

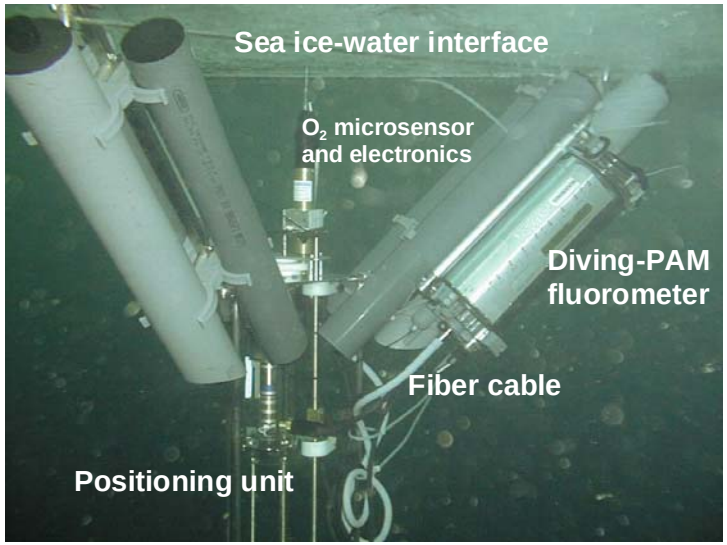
Gradient Capillary Setup



The setup is mounted on a light microscope which allows computer-aided cell tracking via digital video recordings

In-situ oxygen and fluorescence measurements below sea ice

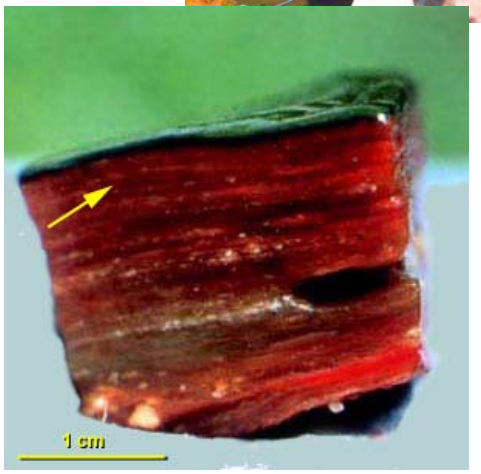




Kühl et al. 2001

In Situ Analysis of Metabolic Switching in Unicellular Thermophilic Cyanobacteria Inhabiting Hot Spring Microbial Mats

Octopus and Mushroom Spring Yellowstone National Park, USA



In situ nitrogenase activity, gene expression and oxygen penetration Octopus Spring June 23, 2005.

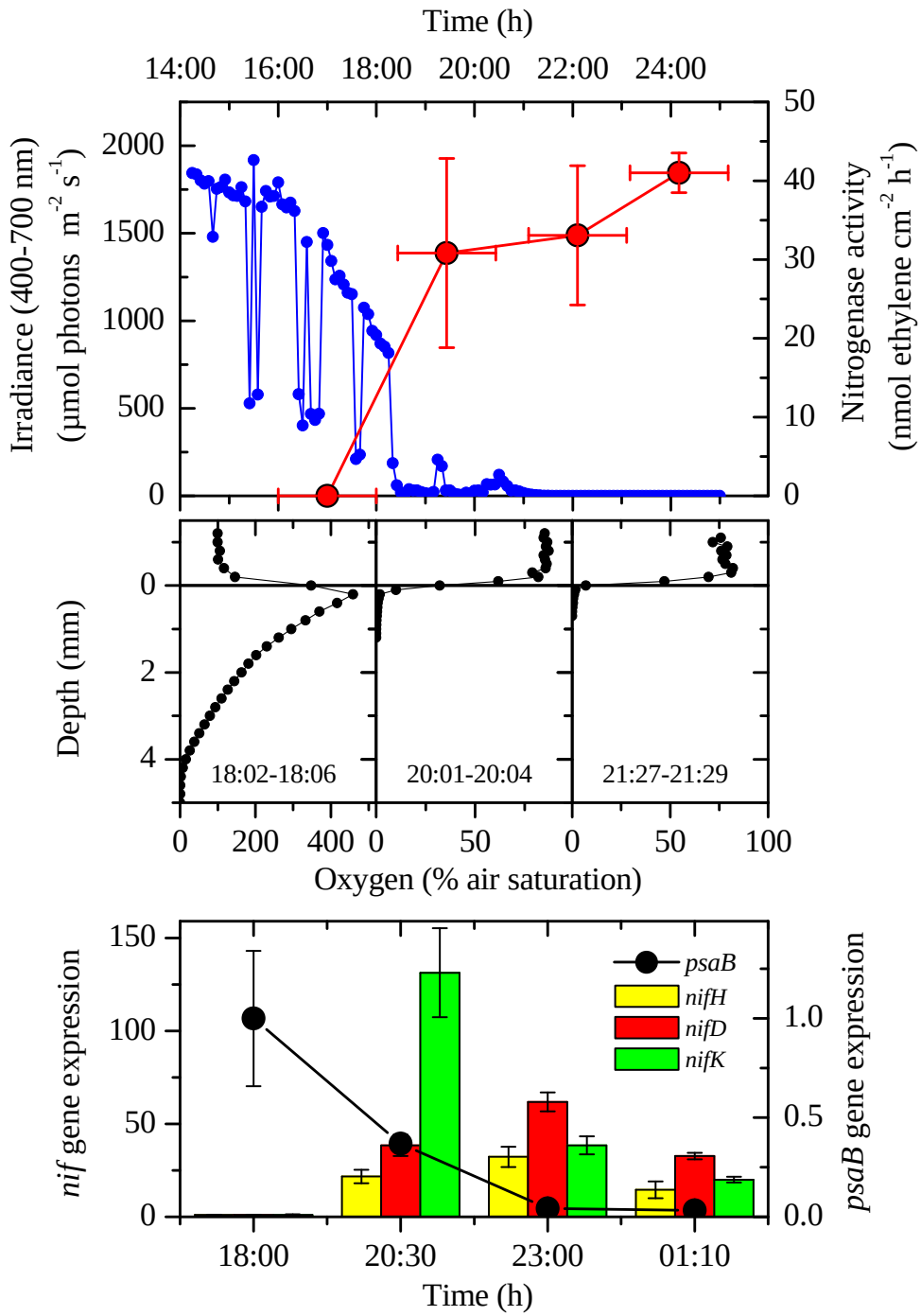
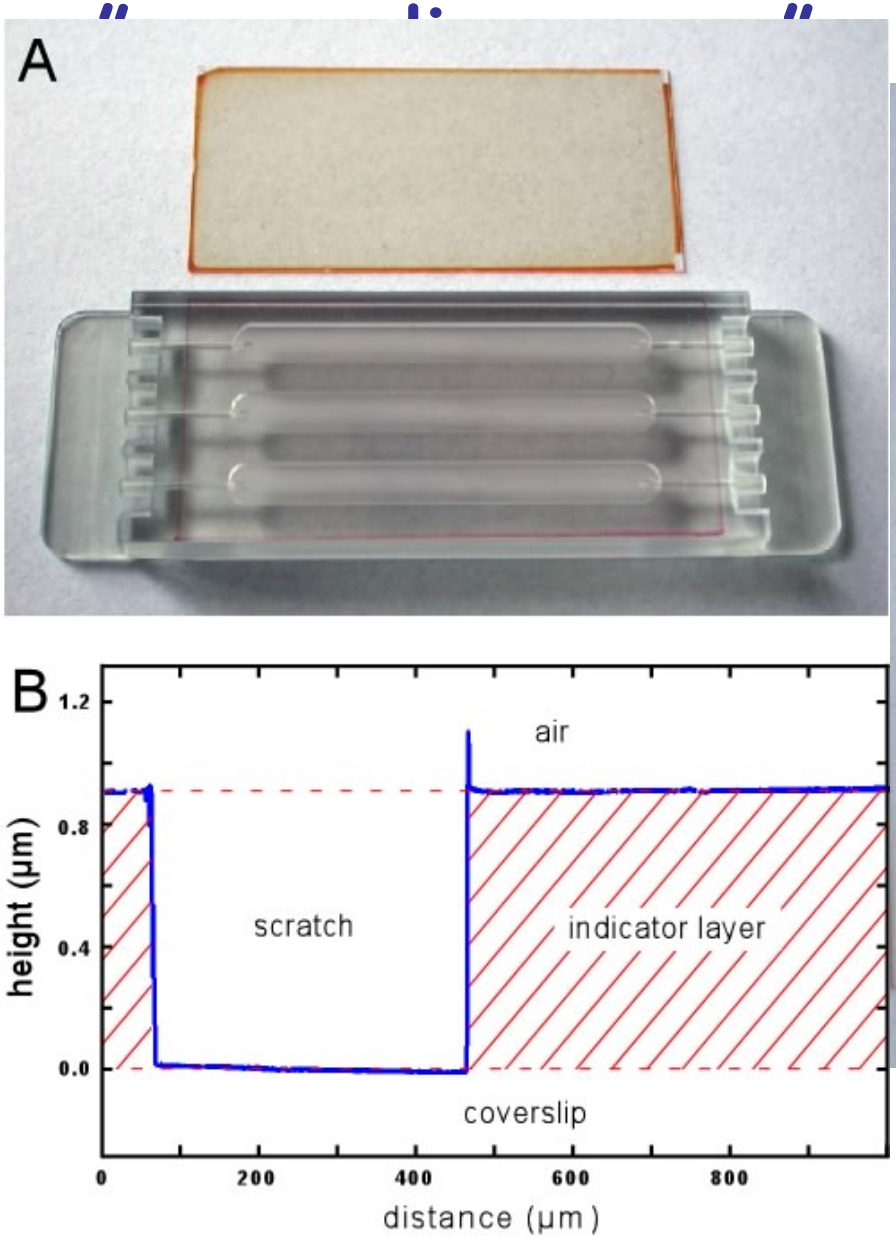


Figure 3: *In situ* nitrogenase activity, gene expression and oxygen penetration measured in the Octopus Spring microbial mat at decreasing irradiance on June 23, 2005. Top: Incident irradiance (blue) and nitrogenase activity (red) as a function of time. Middle: Depth distribution of oxygen concentration in the microbial mat at 934, 29, and 0.0 µmol photons m-2 s-1 respectively. Bottom: qPCR examination of *nifHDK* and *psaB* transcript levels at times indicated. Symbols/columns with error bars indicate the mean ± standard deviation.

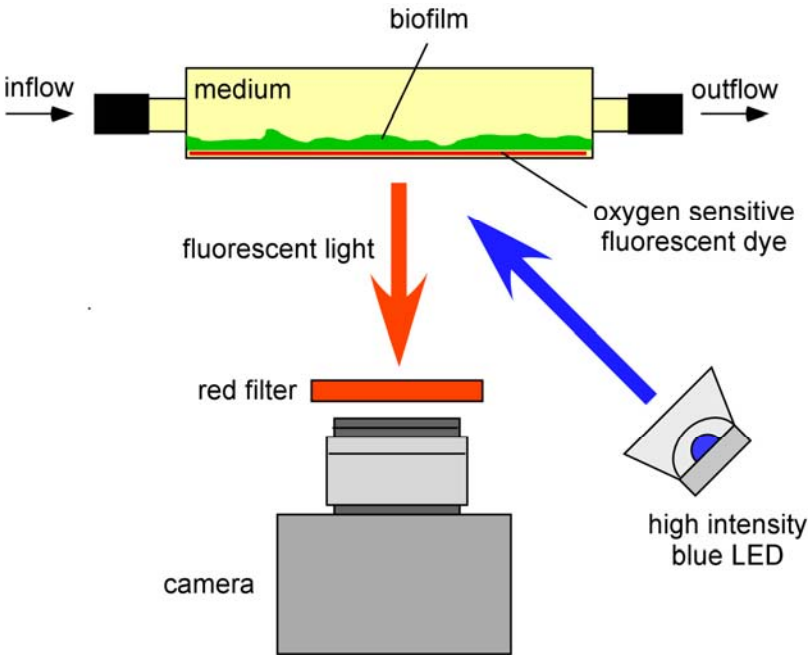
Steunou et al. 2006

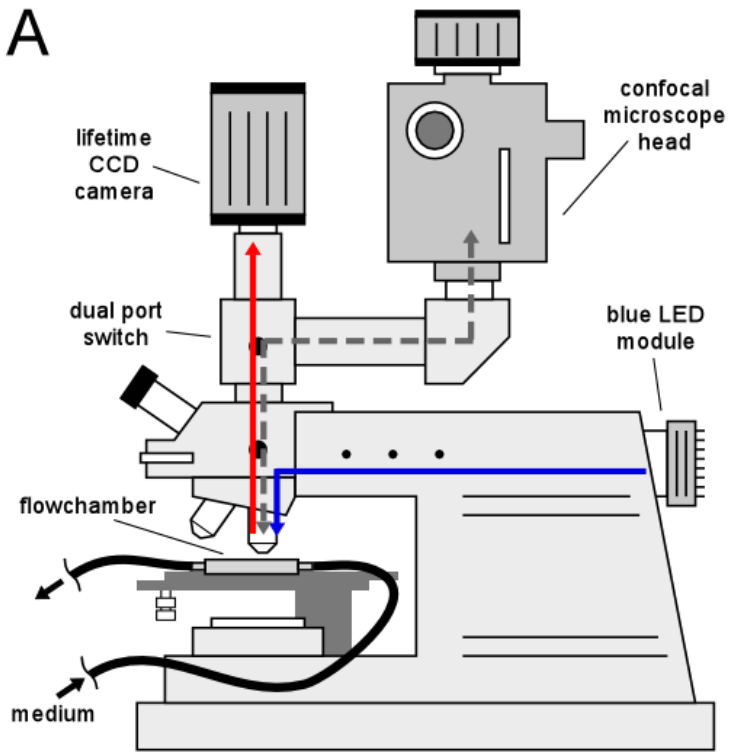
Planar oxygen nano-optode

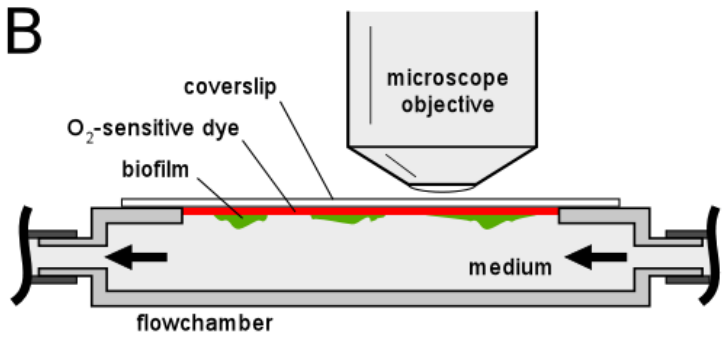


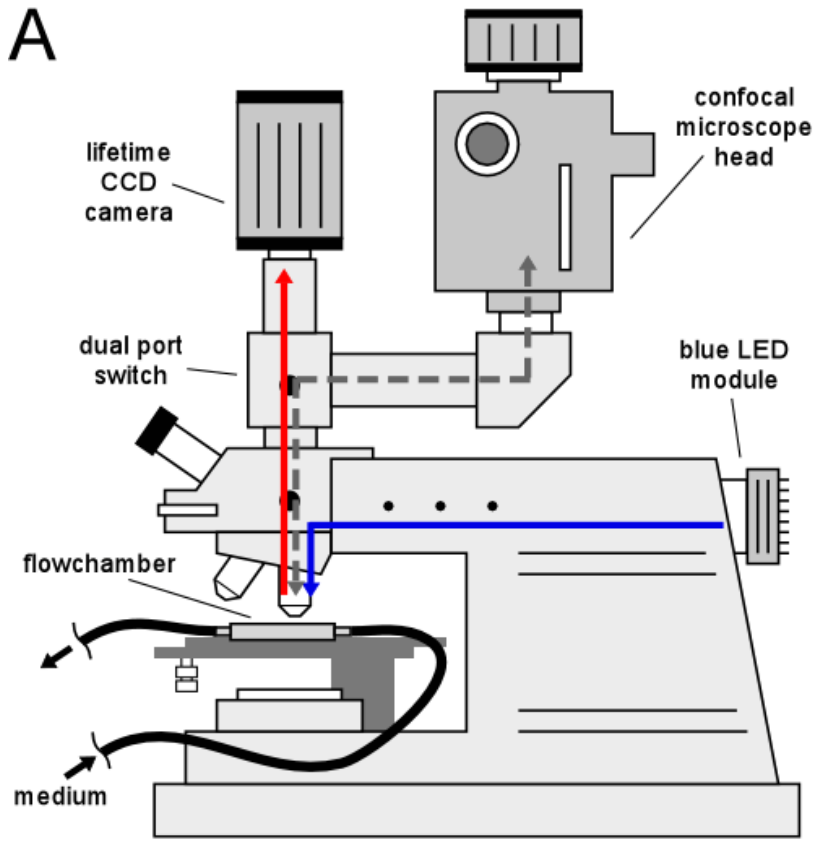
Kühl et al. 2007

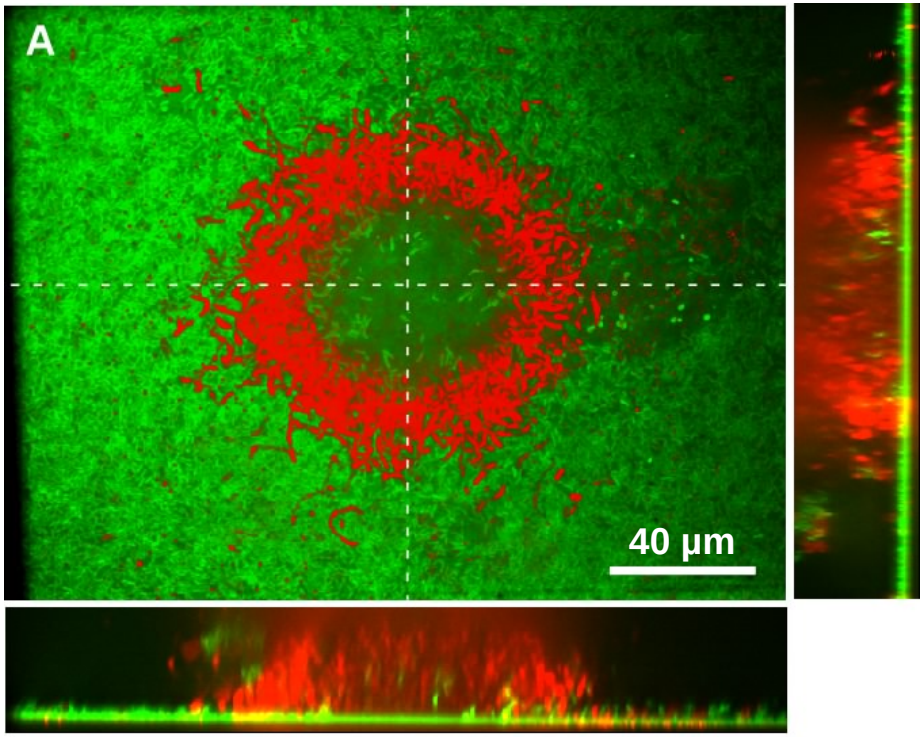
Flexible use with growth chambers

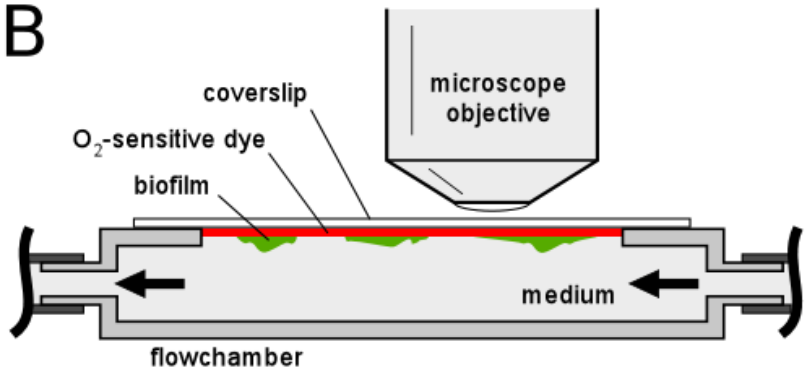


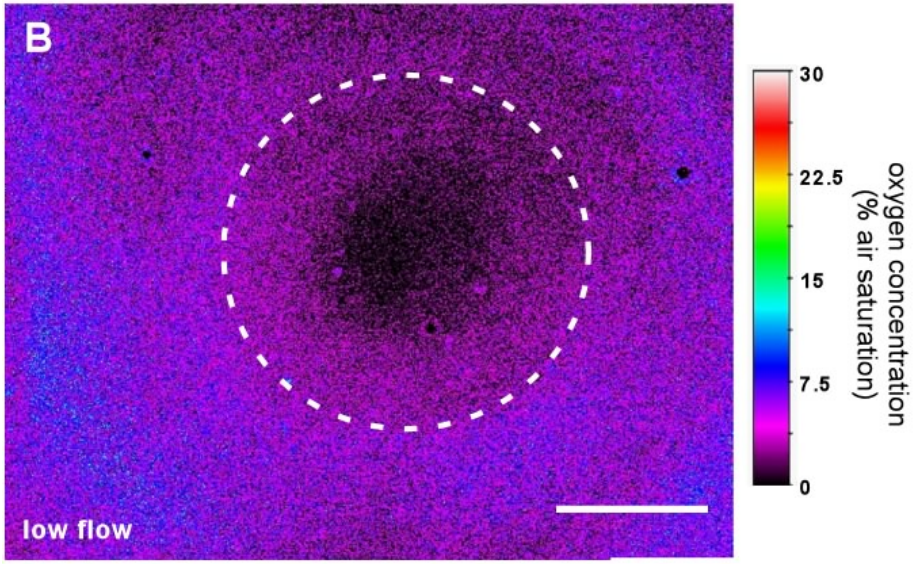




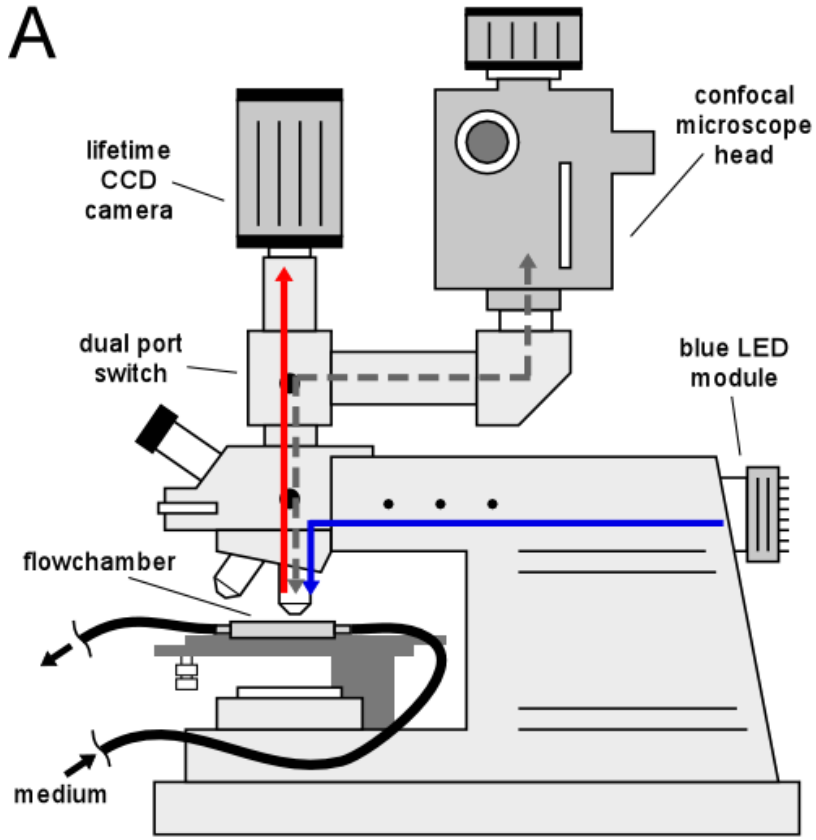


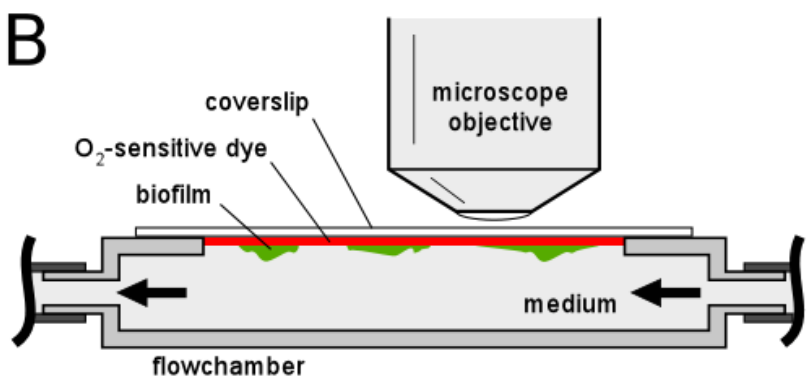


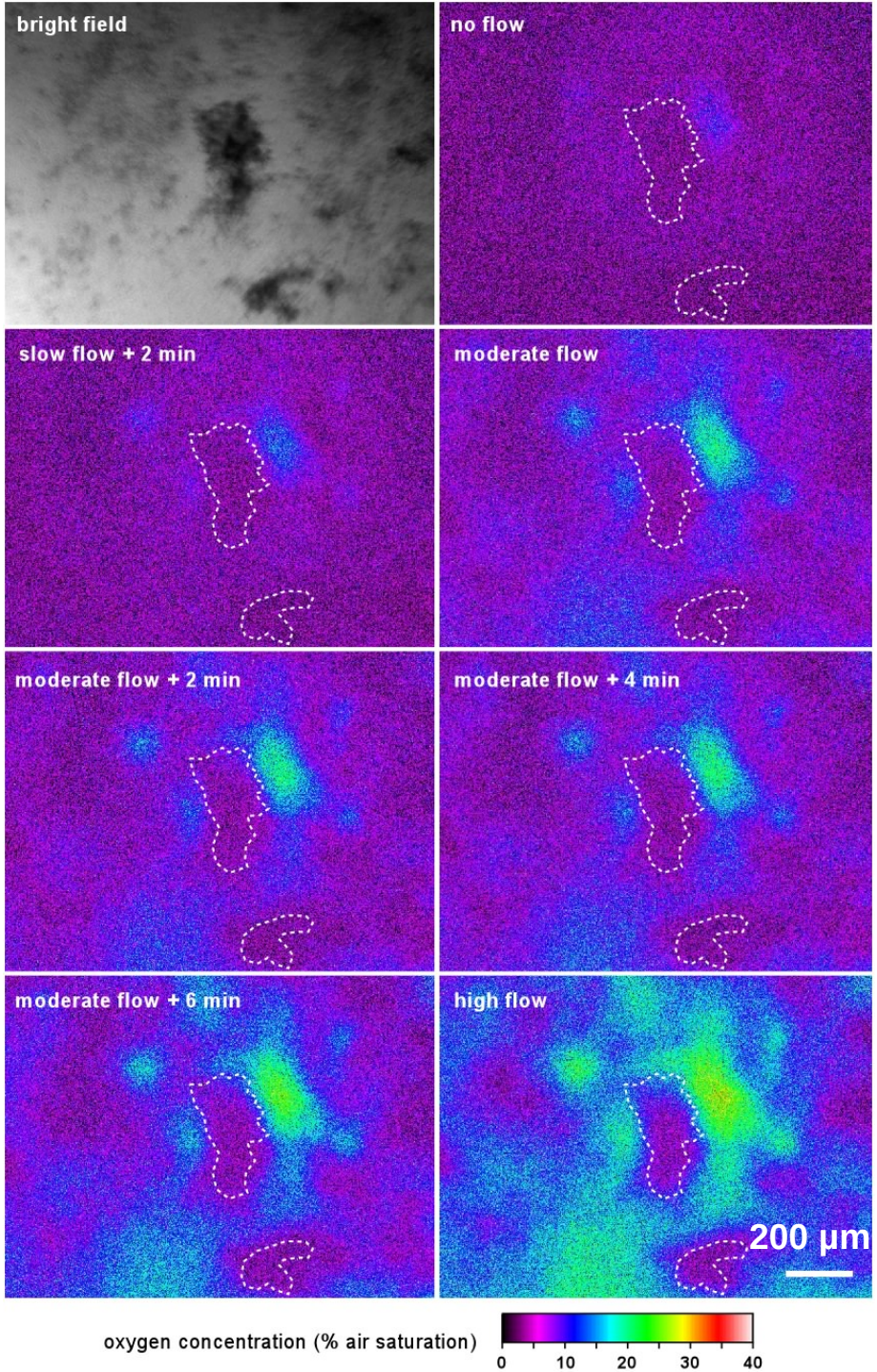




Kühl et al. 2007







Kühl et al. 2007

Biofilm Ecology and Microenvironmental Analysis

Some relevant recent publications:

- **Kühl, M.**, and Polerecky, L. (2008) Functional and structural imaging of phototrophic microbial commmunities and symbioses. *Aquatic Microbial Ecology* 53: 99-118.
- **Kühl, M.**, **Rickelt, L. F.**, and **Thar, R.** (2007) Combined imaging of bacteria and oxygen in biofilms. *Applied and Environmental Microbiology* 73: 6289-6295.
- Steunou, A. S., Bhaya, D., Bateson, M., Melendrez, M., Ward, D. M., Brecht, E., Peters, J. W., **Kühl, M.** and Grossman, A. (2006) *In situ* analysis of nitrogen fixation and metabolic switching in unicellular thermophilic cyanobacteria inhabiting hot spring microbial mats. *Proceedings of the National Academy of Sciences* USA 103: 2398-2403.
- Ward, D. M., Bateson,, M. M., Ferris, M. J., **Kühl, M.**, Wieland, A., Koeppel, A., and Cohan, F. M. (2006) Cyanobacterial ecotypes in the microbial mat community of Mushroom Spring (Yellowstone National Park , Wyoming) as species-like units linking microbial community composition, structure and function. *Philosophical Transactions of the Royal Society B* 361: 1997-2008.
- **Kühl, M.** (2005) Optical microsensors for analysis of microbial communities. *Methods in Enzymology* 397: 166-199.
- **Kühl, M.**, Chen, M., Ralph, P. J., Schreiber, U., and Larkum, A. W. D. (2005) A niche for cyanobacteria containing chlorophyll *d*. *Nature* 433: 820.
- Thar, R., and **Kühl, M.** (2003) Bacteria are not too small for spatial sensing of chemical gradients: An experimental evidence. *Proceedings of the National Academy of Sciences USA* 100: 5748-5753.

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