

## Unisense microsensors in medical biofilm research

### O<sub>2</sub> and N<sub>2</sub>O microprofiles in sputum samples from cystic fibrosis patients with chronic *Pseudomonas aeruginosa* lung infection

The application note is based on the research and article by:  
Kolpen et al. (2014)

The application note is written by:  
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### Introduction

With Unisense microsensors and the Unisense MicroProfiling System you can complete microprofiles in biofilms with extreme positioning accuracy (precision < 10 μm) and high spatial resolution to obtain valuable information about the microenvironment.

Kolpen et al. (2014) used the Unisense O<sub>2</sub> and N<sub>2</sub>O microsensors to measure microprofiles in sputum samples from cystic fibrosis patients with chronic *Pseudomonas aeruginosa* infection. *P. aeruginosa* is the major cause of chronic lung infection of cystic fibrosis patients where the bacteria live as biofilm aggregates in the lungs. The biofilms can persist for years in the airways of the patient despite an active immune response and antibiotic therapy. The measurements in the paper by Kolpen et al. provided new insights about the microenvironment and growth of the *P. aeruginosa* biofilm which may lead to new treatment strategies.

### Laboratory setup

The authors used Unisense O<sub>2</sub> and N<sub>2</sub>O microsensors with a tip diameter of 25 μm (OX-25 and N<sub>2</sub>O-25). The microsensors were connected to a Unisense amplifier and mounted on the motorized Unisense MicroProfiling System (Figure 1). The glass vials containing the freshly expectorated sputum samples were kept at 37 °C during the measurement. The sensors were first positioned manually at the upper surface of the sputum sample using the micromanipulator (MM33) to define the surface of the sample as the zero depth. Then the motorized MicroProfiling System was used to do automated microprofiles vertically through the sputum samples.

The SensorTrace Profiling software controlled the movement of the microsensors and the authors used a step size of 100 or 200 μm, a waiting time between measurements of 3 seconds for the O<sub>2</sub> profile and 5 seconds for the N<sub>2</sub>O profile, and a delay time between each cycle of profile measurements of 10 seconds. Data acquisition and analysis were done with the SensorTrace Profiling software that logs the measured analyte concentration together with the depth position of the micromanipulator.

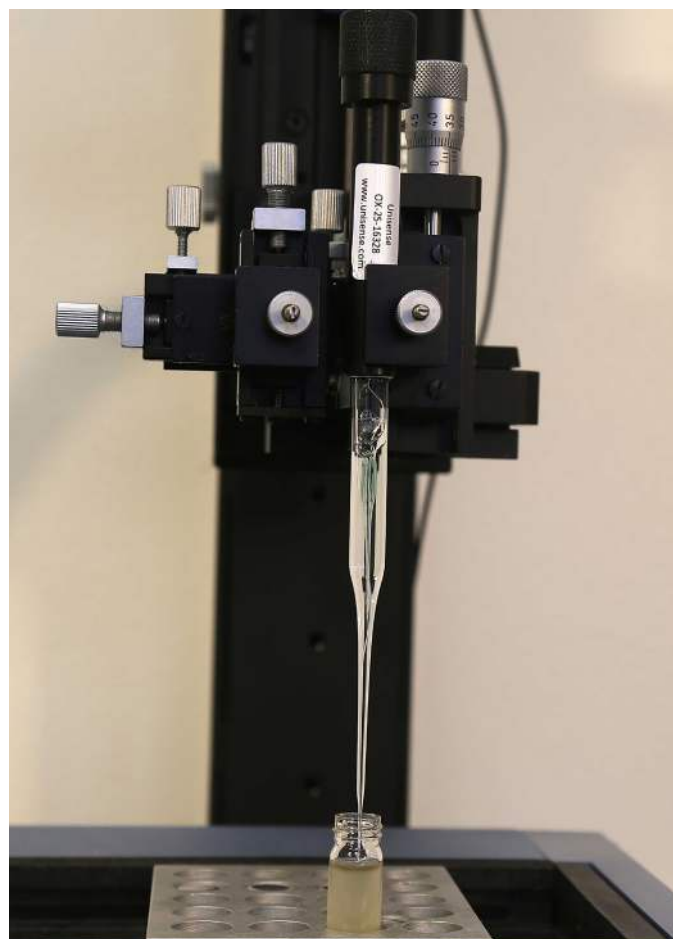


Figure 1: MicroProfiling setup with a Unisense microsensor (25 μm tip diameter) mounted in the Unisense micromanipulator (MM33) measuring in a sputum sample.

Photo: Kindly provided by Mette Kolpen.



O<sub>2</sub>

N<sub>2</sub>O

H<sub>2</sub>S

NO

H<sub>2</sub>

pH

Redox

Temp

EP

## Results and conclusion

Based on the  $O_2$  and  $N_2O$  microsensors profiling measurements, the authors demonstrated that sputum samples from patients with chronic *P. aeruginosa* infection consist of an upper oxygenated zone and a lower anoxic zone below around 3 mm from the sputum surface (Figure 2).  $N_2O$  production from the bacteria was mainly confined to the lower anoxic part and a maximum median concentration of  $41.8 \mu\text{M } N_2O$  was found. Significantly less  $N_2O$  was found in control sputum samples from cystic fibrosis patients without infection.  $N_2O$  is an intermediate in the denitrification pathway and the data indicated that *P. aeruginosa* may acquire energy for growth from denitrification when  $O_2$  is absent. Using the Unisense microsensors to obtain  $O_2$  and  $N_2O$  concentration gradients with high spatial resolution, the authors could explore the microenvironment in the sputum and they demonstrated  $N_2O$  production in clinical samples from infected cystic fibrosis patients for the first time.

The results are published in Kolpen et al. (2014) Nitrous oxide production in sputum from cystic fibrosis patients with chronic *Pseudomonas aeruginosa* lung infection. PLOS ONE Vol 9, Issue 1.

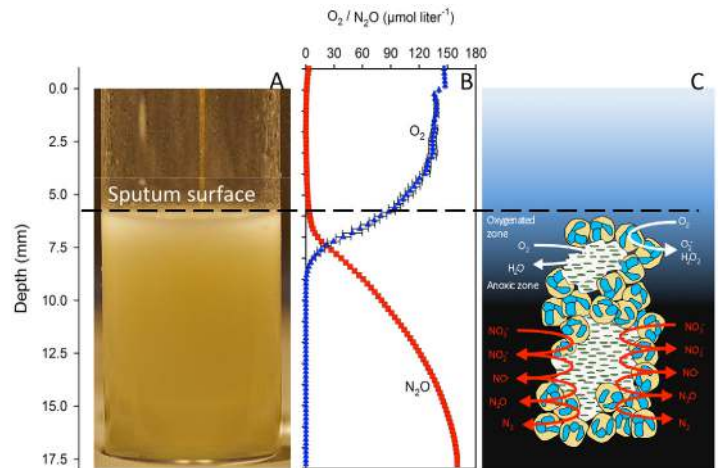


Figure 2: A) Unisense microsensor inserted into a sputum sample from a cystic fibrosis patient with chronic lung infection. B)  $O_2$  microprofiles (mean and SD of three profiles measured in the beginning of experiment) and  $N_2O$  microprofile measured 6–7 h after the beginning. C) Schematic model showing the metabolic mechanisms in the oxygenated and anoxic zones in the sputum sample.

Figure adapted from Kolpen et al. 2014.

## Suggested products

- $N_2O$ -25 sensors
- Microprofiling System
- Single or MultiChannel UniAmp
- SensorTrace Profiling (uSense Profile)



## Related publications

Kolpen et al. (2016) Reinforcement of the bactericidal effect of ciprofloxacin on *Pseudomonas aeruginosa* biofilm by hyperbaric oxygen treatment. *International Journal of Antimicrobial Agents* 47 (2016) 163–167.

Kolpen et al. (2014). Nitric oxide production by polymorphonuclear leucocytes in infected cystic fibrosis sputum consumes oxygen. *Clinical and Experimental Immunology*. 177. 310–319.

Cowley et al. (2015) Pediatric cystic fibrosis sputum can be chemically dynamic, anoxic, and extremely reduced due to hydrogen sulfide formation. *mBio* Volume 6, Issue 4.

Fox et al. (2016) Anaerobic bacteria grow within *Candida albicans* Biofilms and induce biofilm formation in suspension cultures. *Current Biology* 24, 2411–2416.

Pabst et al. (2016) Gel-entrapped *Staphylococcus aureus* bacteria as models of biofilm infection exhibit growth in dense aggregates, oxygen limitation, antibiotic tolerance, and heterogenous gene expression. *Antimicrobial Agents and Chemotherapy*, Volume 60, Number 10.

Madsen et al. (2015) Facultative Control of Matrix Production Optimizes Competitive Fitness in *Pseudomonas aeruginosa* PA14 Biofilm Models. *Applied and Environmental Microbiology*, Vol 81, Number 24.