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$O_2$ $N_2O$ $H_2$ NO $H_2S$ Redox Temp Opto pH EP

The Microsensor Company

# Introduction to Microsensors


February 2022
Tage Dalsgaard

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# Online Biogeochemistry Workshop

**Wednesday 23 February**

14:30-15:30 CET - Introduction to Microsensors

15:45-16:45 CET - Lab-based Studies

**Thursday 24 February**

14:30-15:30 CET - Field Studies

15:45-16:30 CET - Demonstration of Field Microprofiling System

16:45-17:30 CET - Demonstration of Activity Calculation - Software

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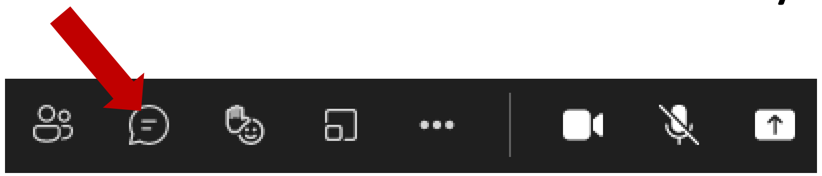
# A few rules before we get started

1. Please turn off your microphone

2. Questions: During the lecture please use chat.
After the lecture you can unmute and ask.



Very application-specific questions may be better answered in a private session afterwards.

You will get access to all the presentations as PDF´s + recordings shortly after the workshop.

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# About me

- Tage Dalsgaard, Ph.D. in biogeochemistry/microbiology
- Application Scientist
- With Unisense for 4 years
- Previously Senior Scientist at National Environmental Research Institute and Aarhus University, Denmark
- >25 years of academic research experience in microbiology and biogeochemistry (marine N and C cycling)

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# Outline

- About Unisense
- Sensor production
- Amperometric microsensors
  - $O_2$ electrochemical sensor in detail
  - Other sensors ($H_2S$, $N_2O$, NO, $H_2$)
- Potentiometric microelectrodes
- Optode for $O_2$
- Adaptation and customization
- Choosing the right tip size
- Calibration

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# About Unisense

- Established in 1998
- Largely owned by scientists
- Employees: 23 (11 with Ph.D.)
- > 20 years of experience in developing, constructing, and applying microsensors
- ISO 9001 certified
- Website: [www.unisense.com](http://www.unisense.com)

ISO 9001 Certified
ExVeritas
UKAS MANAGEMENT SYSTEMS 8613


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# About Unisense

- Sell and collaborate worldwide
- Network of +5000 customers
- Distributor network
- 2 annual workshops in Denmark, annual workshop in Asia, plus advanced Field Systems training
- >1000 guest for training and instructions
- More than 5.000 scientific email Q&As per year
- More than 2500 peer-reviewed publications with our sensors

ISO 9001 Certified ExVeritas UKAS MANAGEMENT SYSTEMS 8613

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# Workshops at Unisense

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# Support team

- Line Daugaard, Ph.D., Product Manager
- Tage Dalsgaard, Ph.D., Application Scientist
- Lars Hauer Larsen, Ph.D., Chief Scientific Officer
- Andrew Cerskus, Ph.D., Sales Manager US/CA
- Thomas Rattenborg, Ph.D., CEO/CCO
- Mikkel Holmen Andersen, Ph.D., Chief Technical Officer
- Michael Nielsen, Ph.D., Senior Application Scientist
- Maria Hedegaard, Sales and Marketing Assistant
- Mette Gammelgaard, Administration and shipping
- Ole Pedersen, Prof., University of Copenhagen










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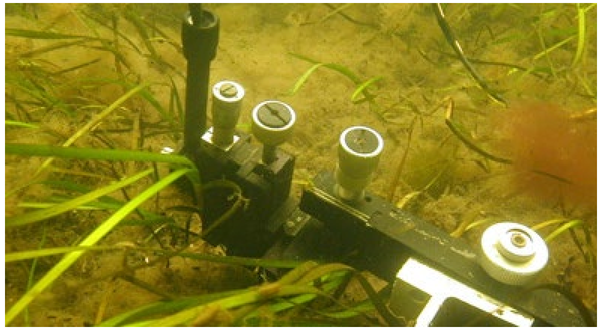
# Why use microsensors?

- High spatial resolution (as tip size)
- Non-destructive/minimally invasive
- Do not disturb chemical gradients
- Fast response
- Real-time measurements
- Low analyte consumption
- Low sensitivity to stirring (minimal artefact due to turbulence/diffusivity gradients)





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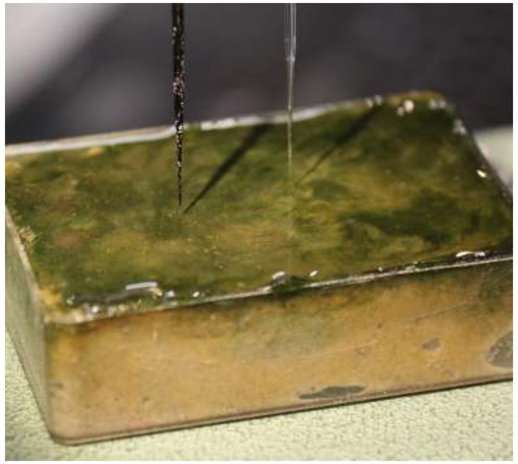
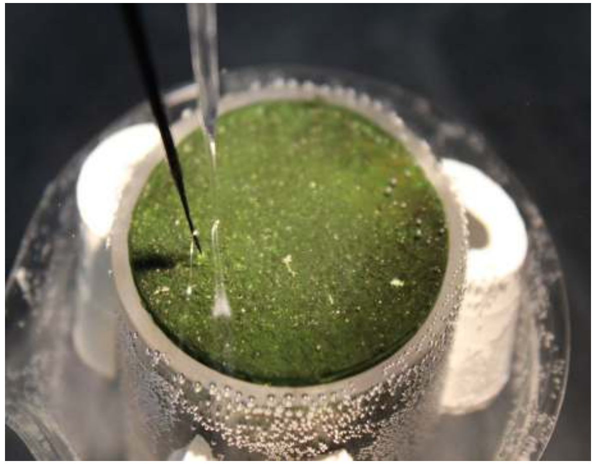
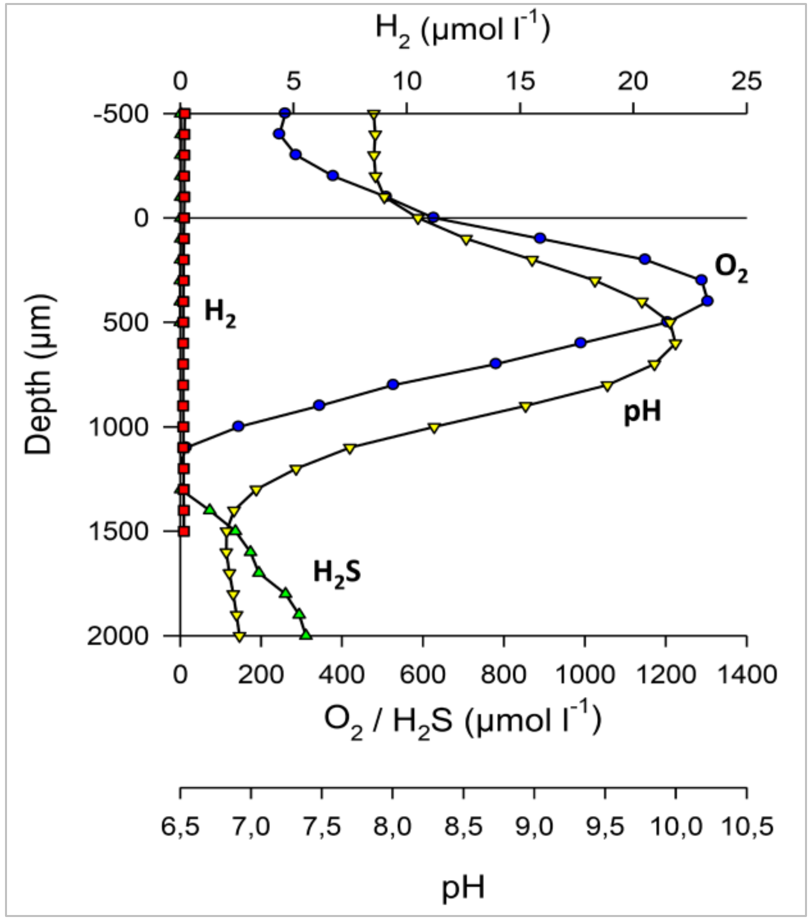
# Microbial mats








Nielsen, M., N. P. Revsbech, and M. Kühl. 2015. Microsensor measurements of hydrogen gas dynamics in cyanobacterial microbial mats. Front. Microbiol. 6: 1–12. doi:10.3389/fmicb.2015.00726

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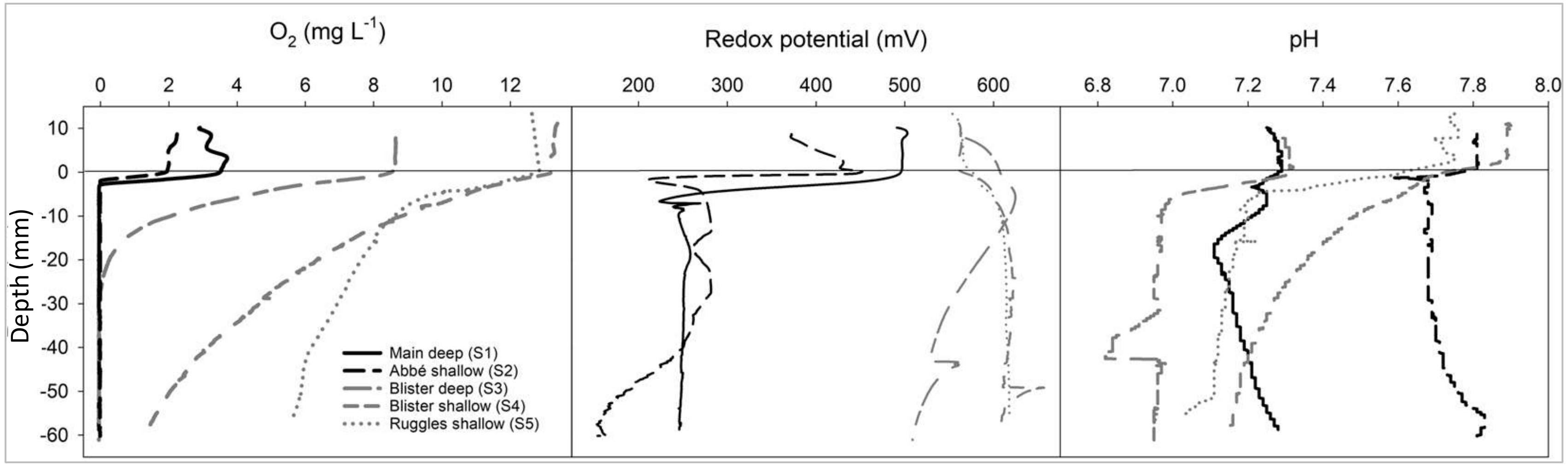
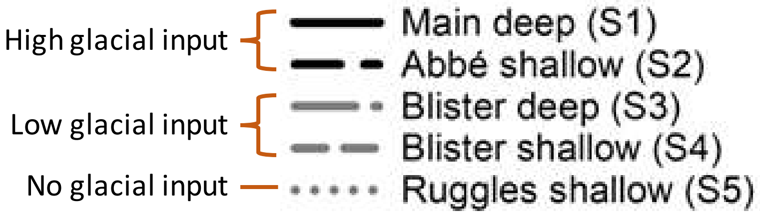
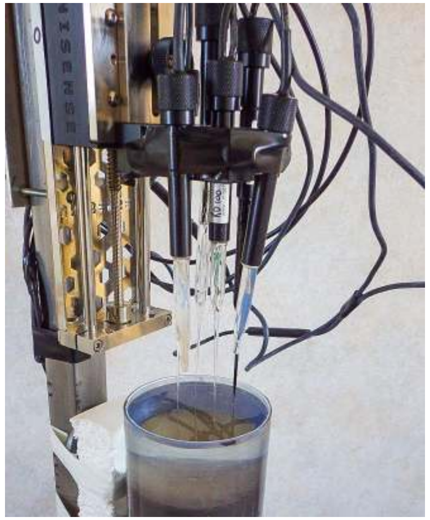
# Effects of run-off in Arctic Lake Hazen



St. Pierre, K. A., V. L. St. Louis, I. Lehnherr, and others. 2019. Scientific Reports **9**: 1–15.

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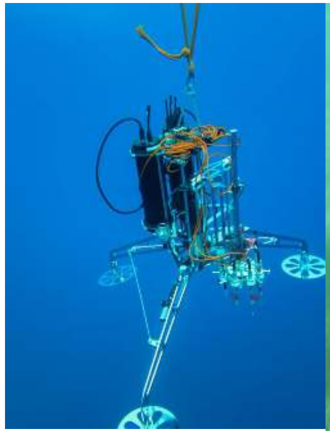
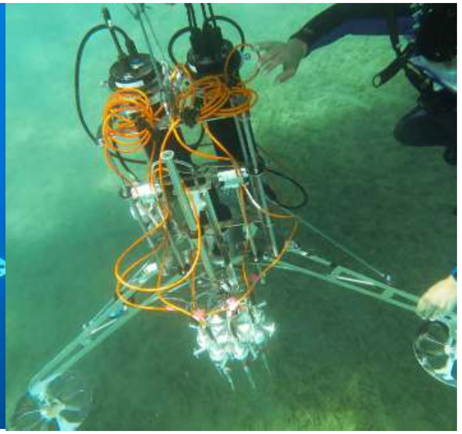
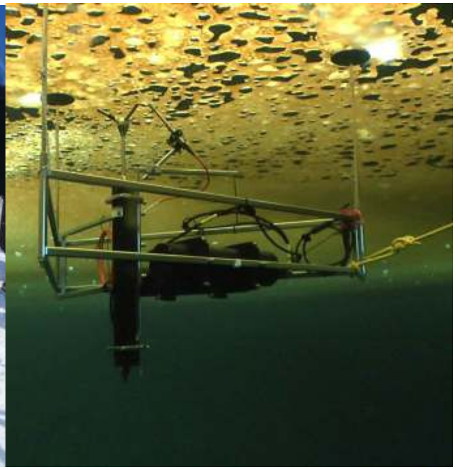
# In Situ measurements



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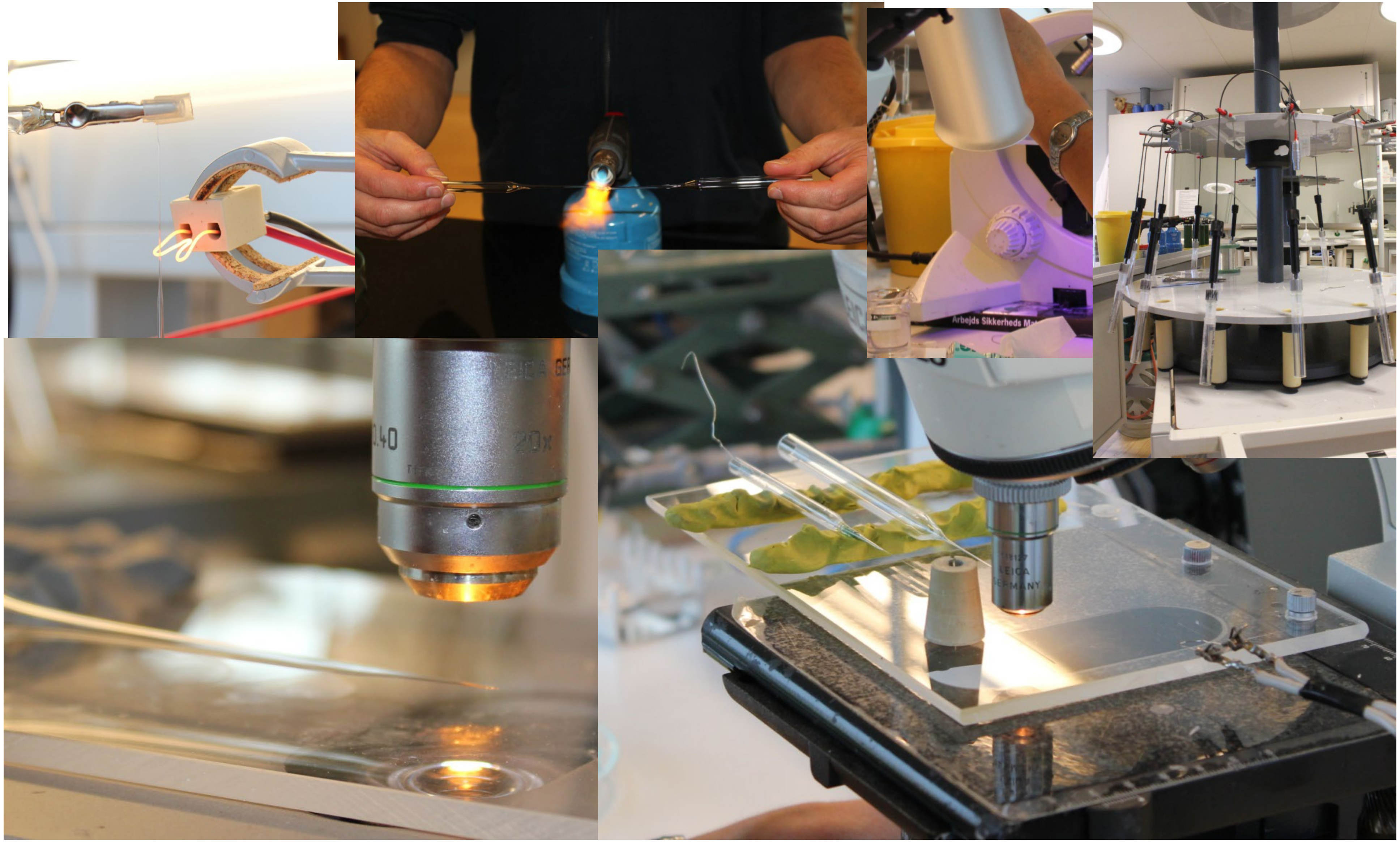
# Sensor Production



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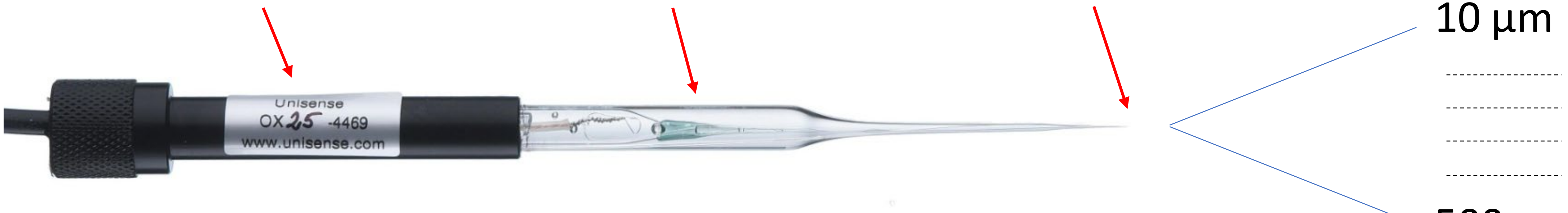
# The basic microsensor

Plastic shaft

Glass tube

Sensor tip




10 µm

500 µm

Amplifier


SensorTrace Suite

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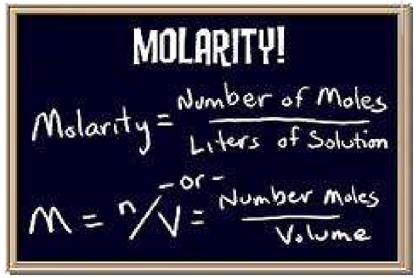
# Microsensors - adaptations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# Introduction to microsensors

## Types of microsensors

- Amperometric microsensor
  - Produce an electrical *current* (pA, $10^{-12}$)
  - Typically is a Clark-type sensor
  - Example: $O_2$, $N_2O$ and $H_2S$ sensors
- Potentiometric microelectrode
  - Produce a *voltage* signal (mV)
  - Reference electrode immersed in the SAME solution is required
  - Example: pH and Redox ($E_H$) electrode
- Micro optodes
  - Fluorophore at the tip exited with light
  - Fluorescence varies with $O_2$ concentration

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# Unisense microsensors and -electrodes

**Amperometric microsensors**

- Oxygen ($O_2$)
- Hydrogen sulfide ($H_2S$)
- Nitrous oxide ($N_2O$)
- Nitric oxide (NO)
- Hydrogen ($H_2$)
- STOX ($O_2$ down to 5 - 10 nM)

**Potentiometric microelectrodes**

- pH
- Redox potential ($E_H$, ORP)
- Electric potential

**Optical microsensors**

- Optode ($O_2$)

**Other microsensors**

- Temperature
- Diffusivity
- Flow

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# Amperometric microsensors – Clark type

Start with Oxygen sensor

- Principles
- Why use microsensors
- Continue with the other sensors


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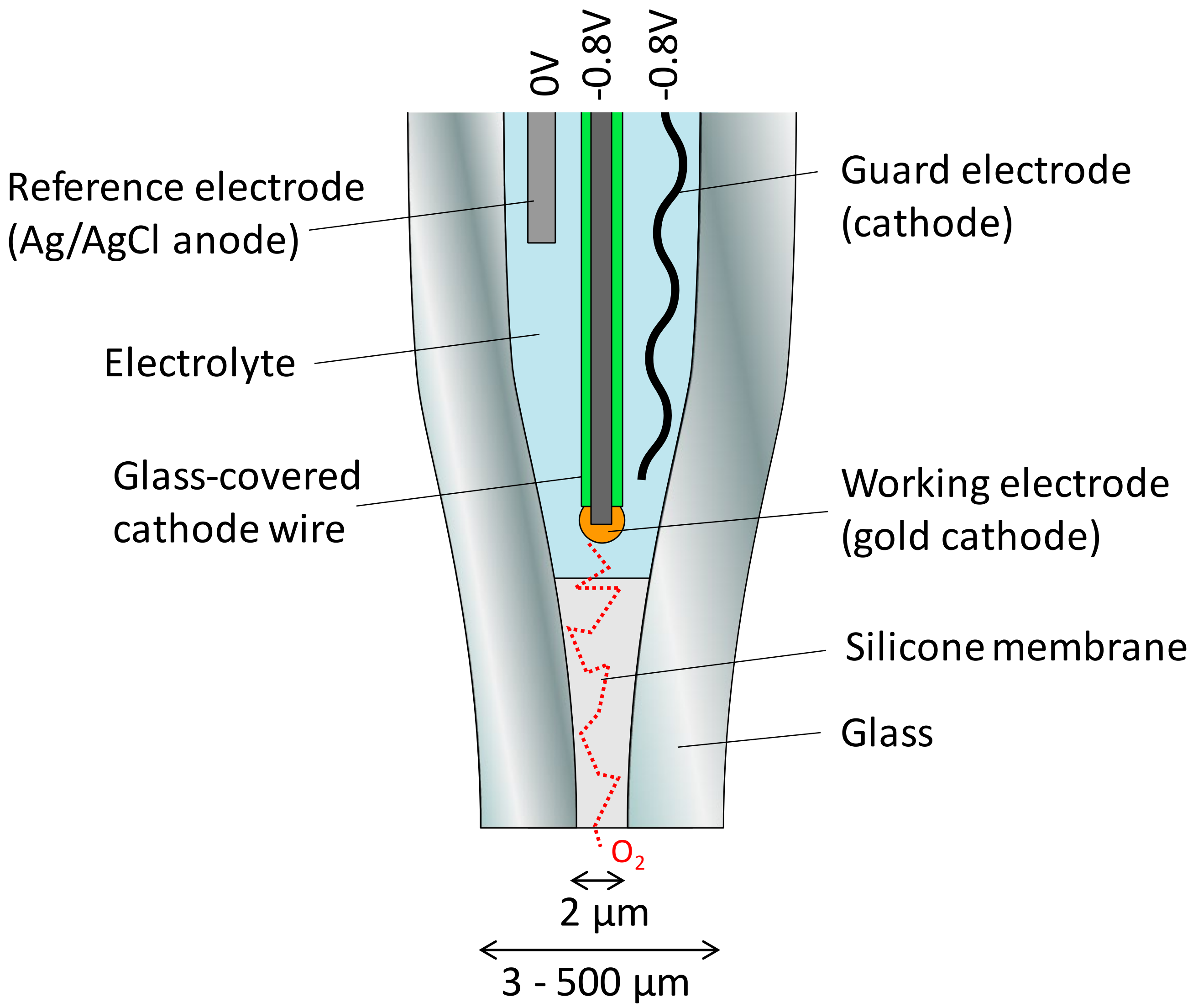
# Oxygen sensor principle



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# Oxygen sensor principle

## $O_2$ sensor electrochemistry

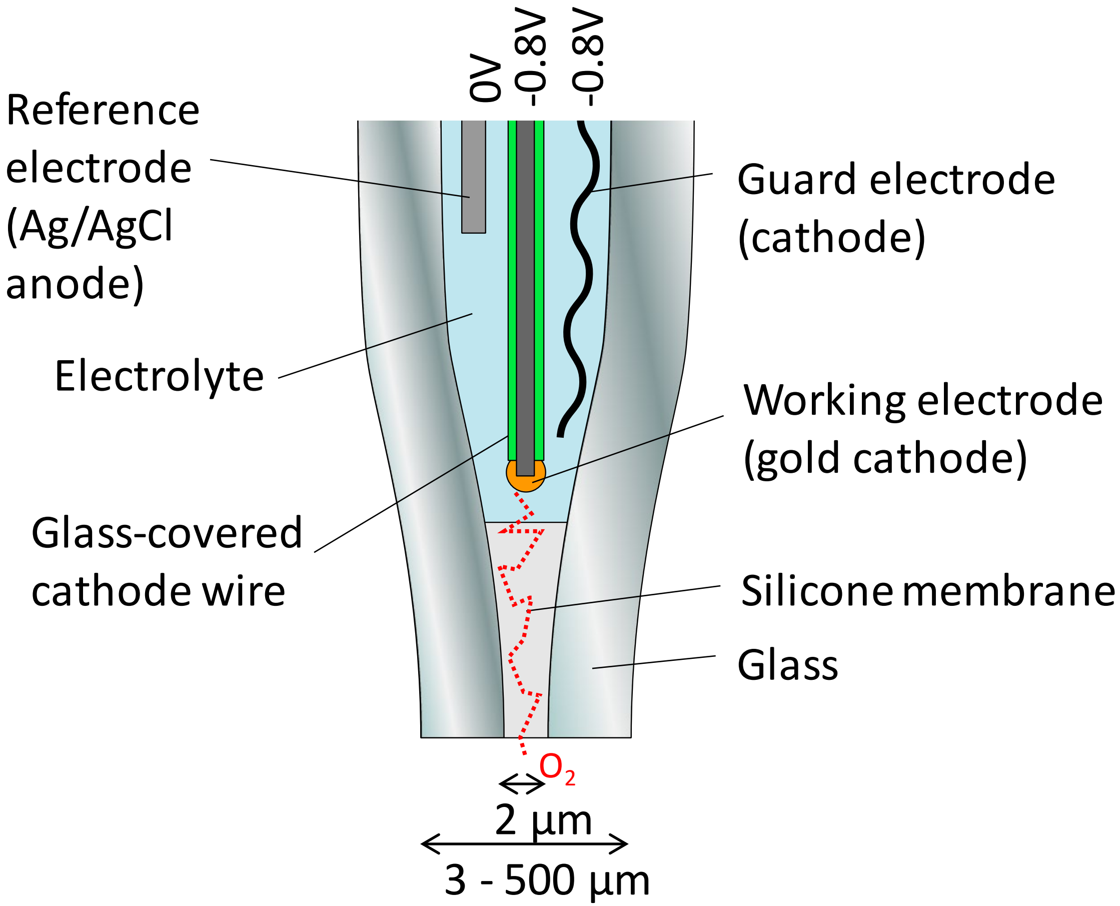
**(A)** $O_2 + 2H_2O + 2e^- \rightarrow H_2O_2 + 2OH^-$

**(B)** $H_2O_2 + 2e^- \rightarrow 2OH^-$

Cathode reaction: $O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$

Electrical current

Anode reaction: $4Ag \rightarrow 4Ag^+ + 4e^-$

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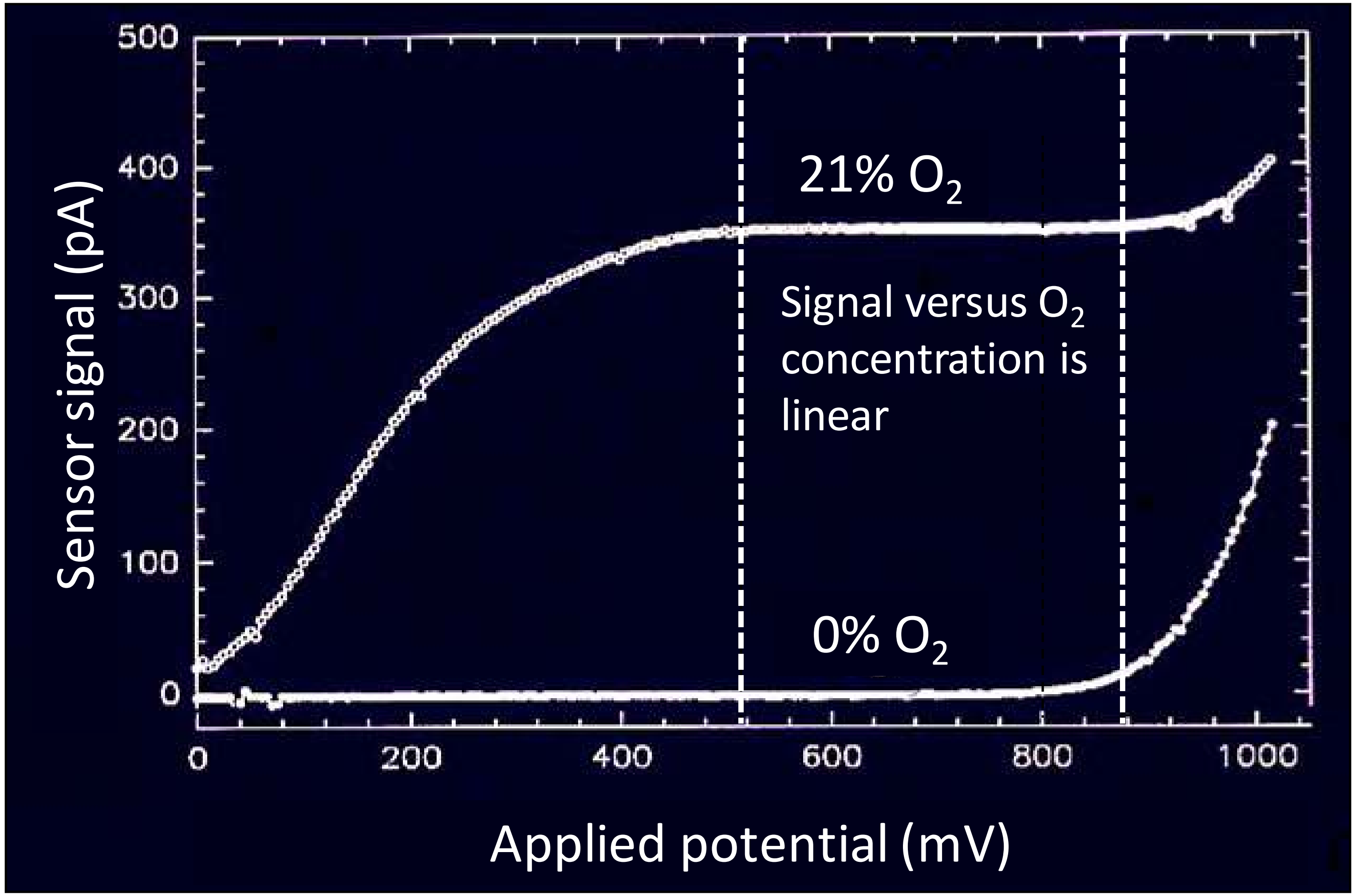
# Amperometric sensor - Signal versus polarization

Courtesy of Bill Armstron, Univ. of Hull, England

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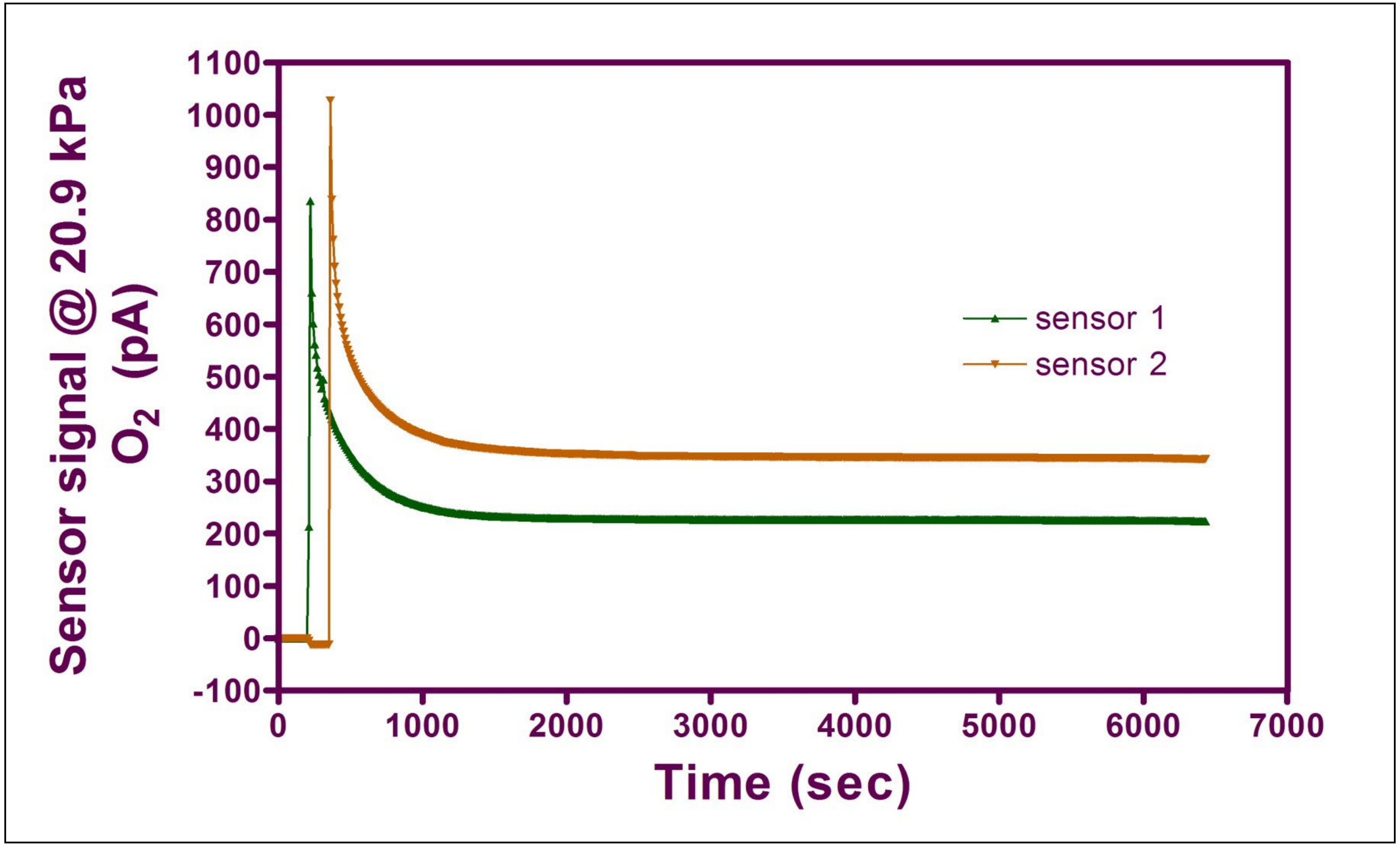
# Amperometric sensor pre-polarization

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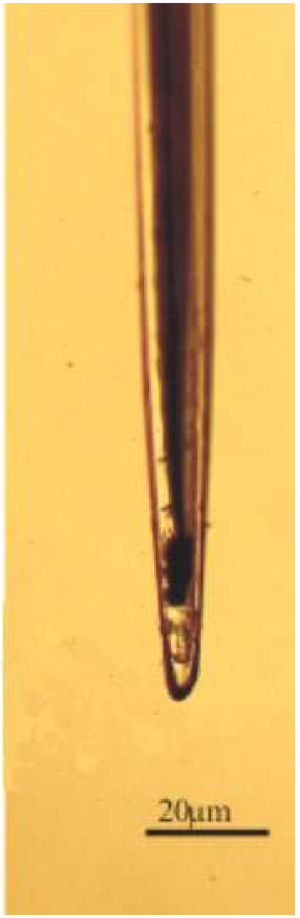
# Why use microsensors?

- High spatial resolution (as tip size)
- Non-destructive (sample can be measured undisturbed and repetitively)
- Low analyte consumption

*How long will it take for an $O_2$ microsensor with an output of 100 pA to consume all the $O_2$ in 1 ml of seawater?*

**34 years**

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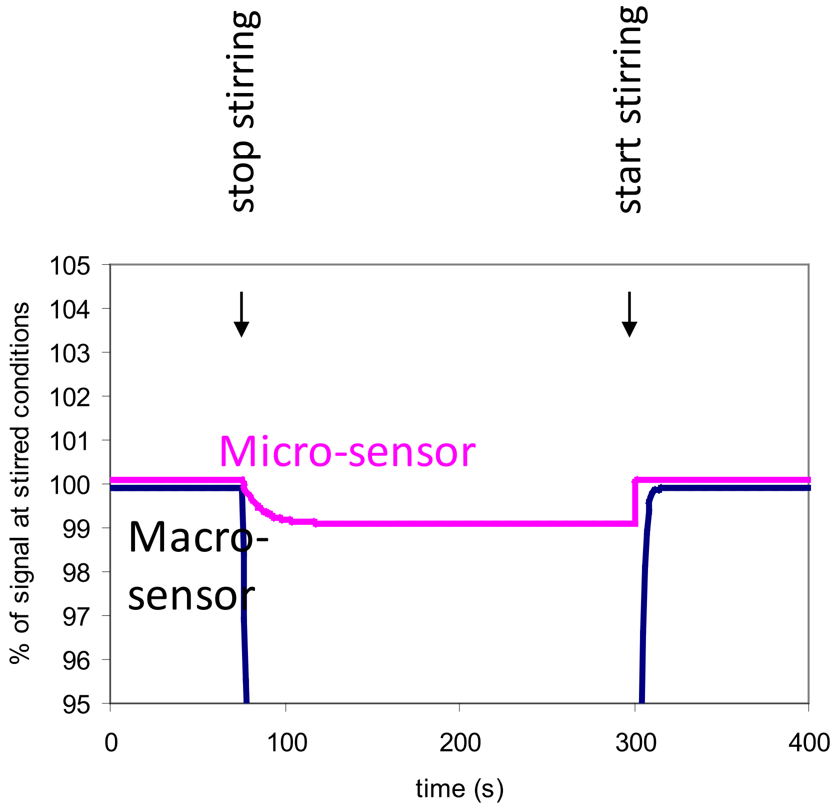
- Low sensitivity to stirring (minimal artefact due to turbulence/diffusivity gradients)

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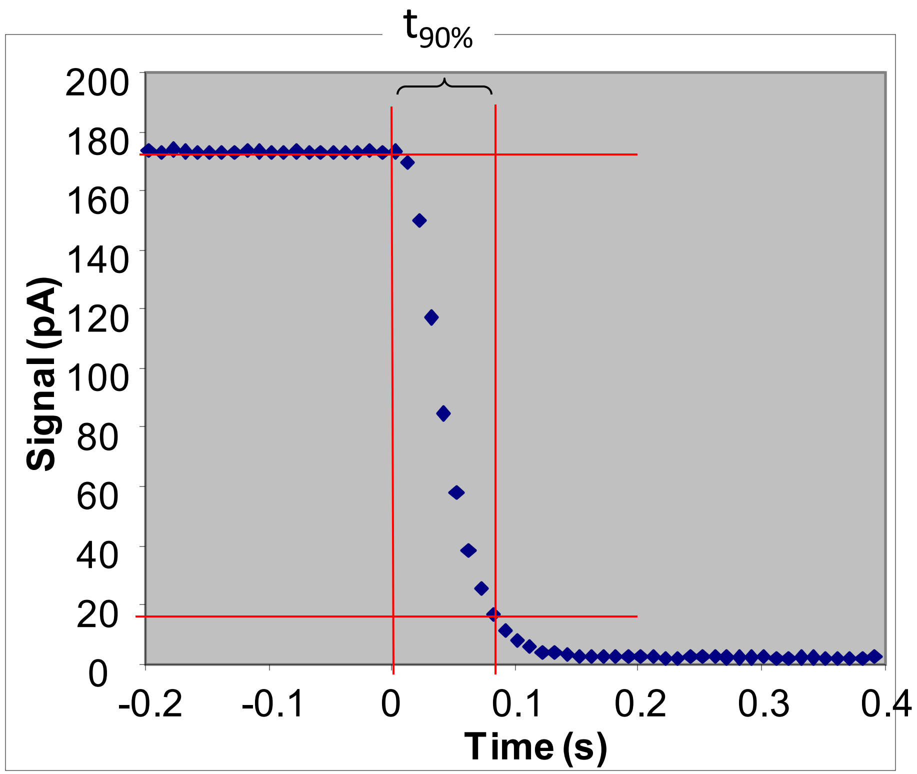
- Non-destructive (sample can be measured undisturbed and repetitively)
- Low analyte consumption
- Low sensitivity to stirring (minimal artefact due to turbulence/diffusivity gradients)
- Fast response

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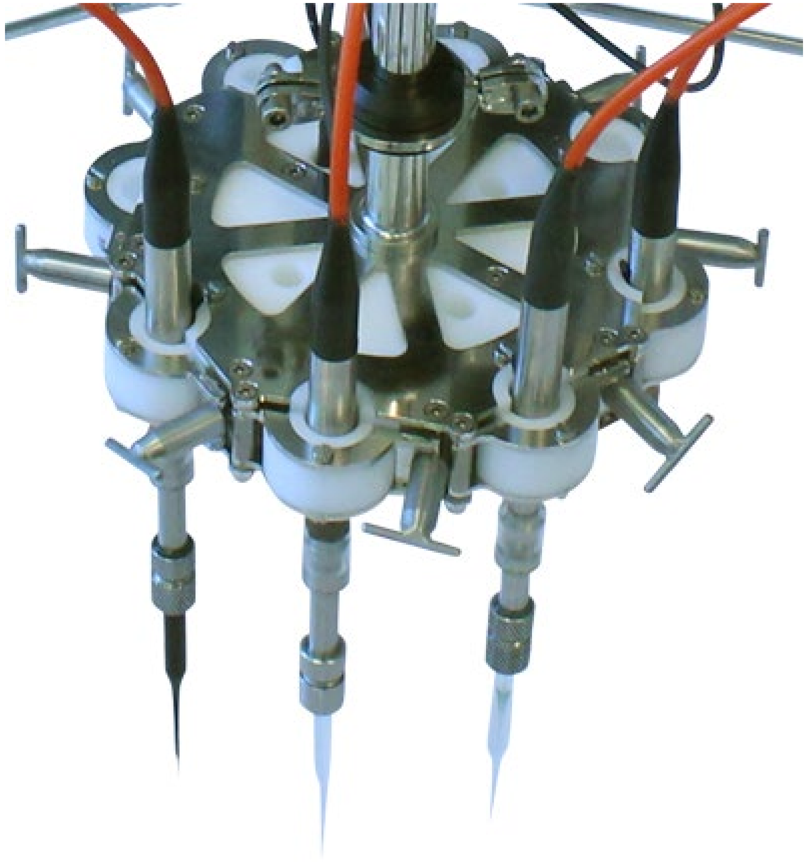
# Why use microsensors?

- High spatial resolution (as tip size)
- Non-destructive (sample can be measured undisturbed and repetitively)
- Low analyte consumption
- Low sensitivity to stirring (minimal artefact due to turbulence/diffusivity gradients)
- Fast response
- Pressure tolerant (6000 m)



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# Oxygen microsensor - Summary

- Amperometric sensor, a picoammeter is needed
- Tip size from 3 to 500 µm and needle versions
- 90% response time from <0.3 sec and up to 5 sec
- Stirring sensitivity from <1% and up to 5% or more
- Temperature sensitivity of 1-3% $°C^{-1}$
- Linear response, i.e. two-point calibration at air saturation and zero $O_2$
- Available as laboratory or *in situ* sensor

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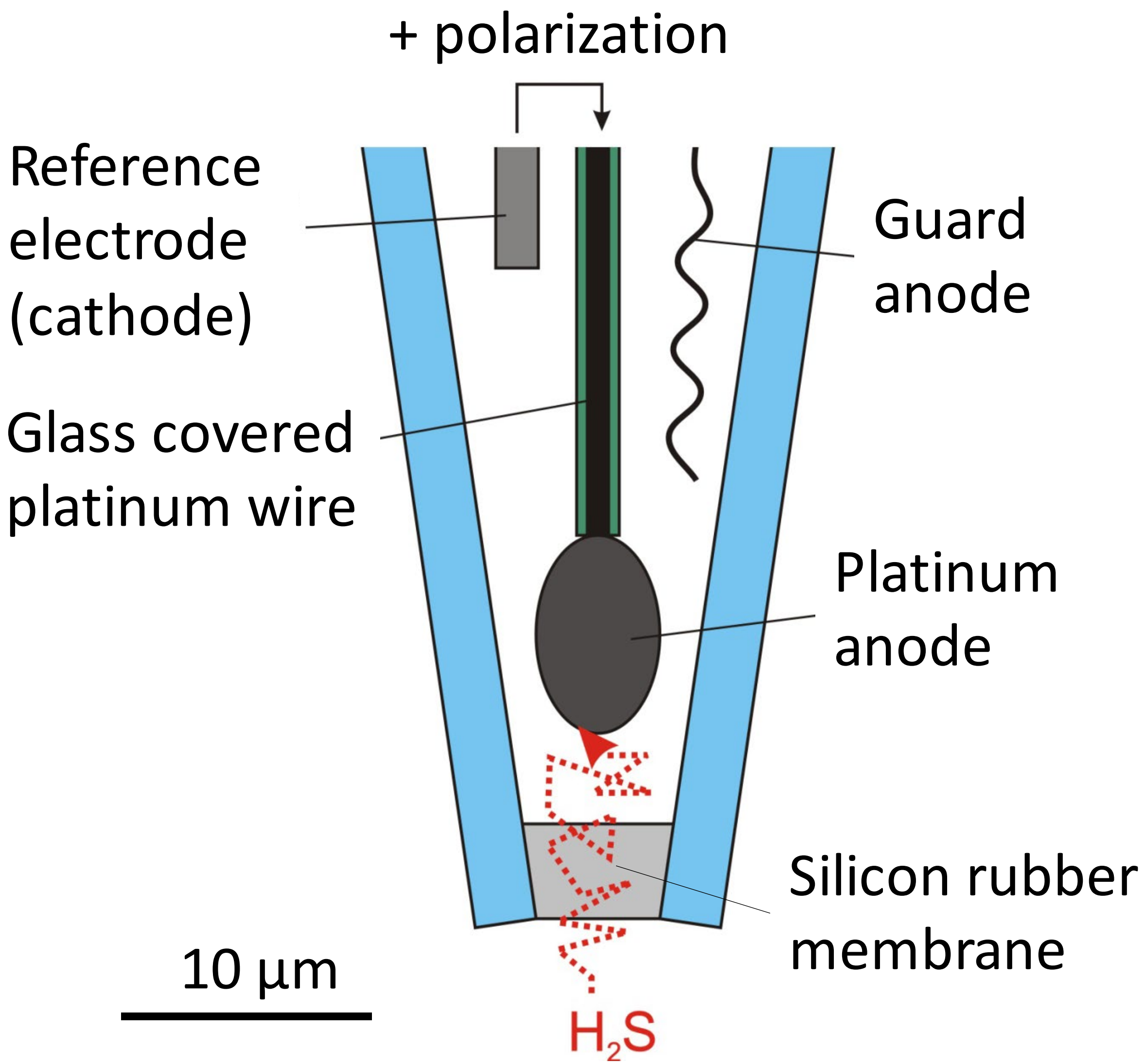
# Sulfide sensor principle




**2 types of $H_2S$ sensors**

- SULF type
- H2S type

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# Hydrogen sulfide sensor chemistry

**SULF-type (type-I)**

Principle

- Sulfide reacts on the anode to form $SO_4^{2-}$
- More sensitive (8 $e^-$ process)

Interferences

- Sensitive to $H_2$

**H2S-type (type II)**

Principle

- Sulfide is oxidized to elemental sulphur (2 $e^-$) via an iron-cyanide complex
- The iron-cyanide redox mediator reacts on the anode

Interferences

- Sensitive to light
- Painted black but may still be affected

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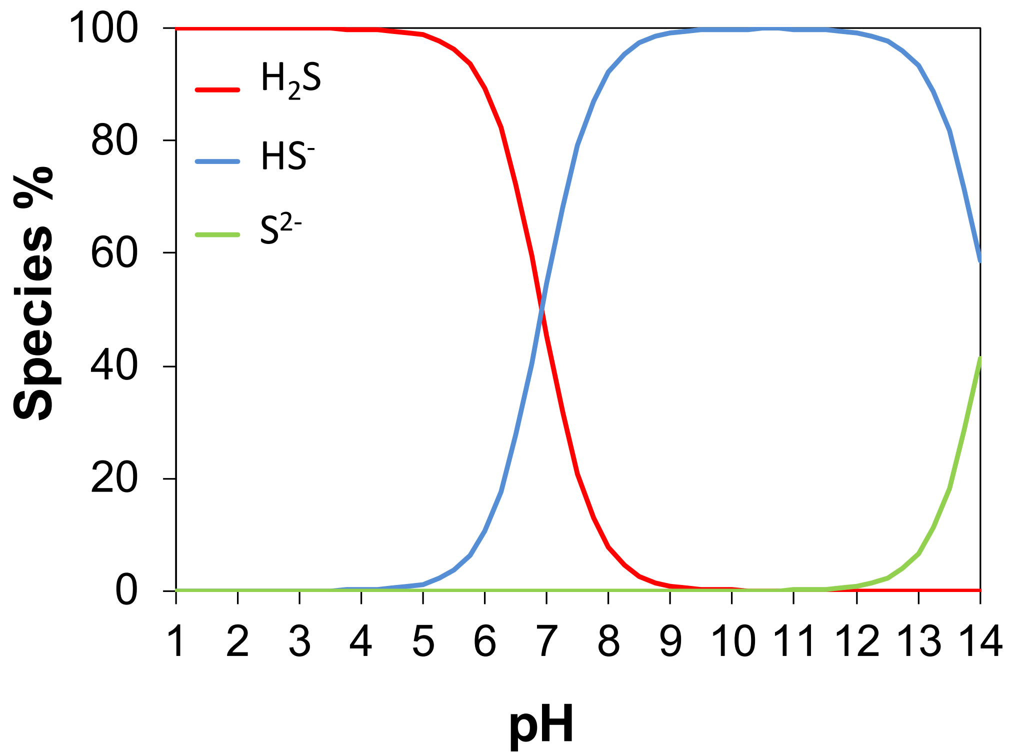
# Sulfide pH speciation

Distribution of sulfide species is pH dependent:

$H_2S \leftrightarrow HS^- + H^+ \leftrightarrow S^{2-} + 2H^+$

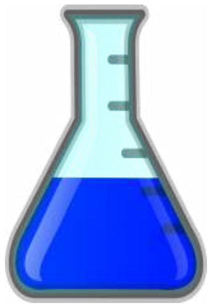



**Implications**

- Calibration

Stock solution: $Na_2S$ or $ZnS_2$

pH < 4 => > 99% in $H_2S$ form



- Total sulfide must be calculated

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# Calculating total sulfide

- Sensor measures only $H_2S$
- Total sulfide = $H_2S + HS^- + S^{2-}$
- Total sulfide can be calculated from $[H_2S]$ and pH

$$\left[S^{2-}{}_{tot}\right] = \left[H_2S\right] \cdot \left(1 + \frac{K_1}{\left[H_3O^+\right]} + \frac{K_1K_2}{\left[H_3O^+\right]^2}\right)$$

$K_1$ and $K_2$ are both temperature and salinity dependent and may be found in the literature!

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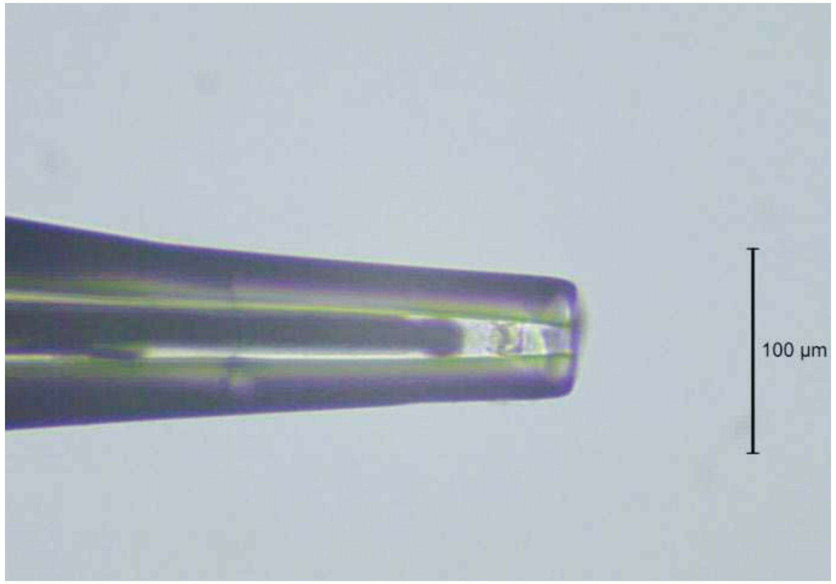
# $H_2S$ sensor – SULF-type

- Linear response (up to 300 µM)
- Wide range
  - Standard: 0-500 µM
  - High range up to 10 mM
  - Low range detection limit of 3 nM
- Life-time 6-12 months
- Stirring sensitivity < 2%
- Temperature sensitive (1-3% $°C^{-1}$)
- Needs pH to calculate total sulfide
- Response time <10 s




Versions:
10 µm, 25 µm, 50 µm, 100 µm, 500 µm, Needle, Micro Resp, flow cells, steel tube

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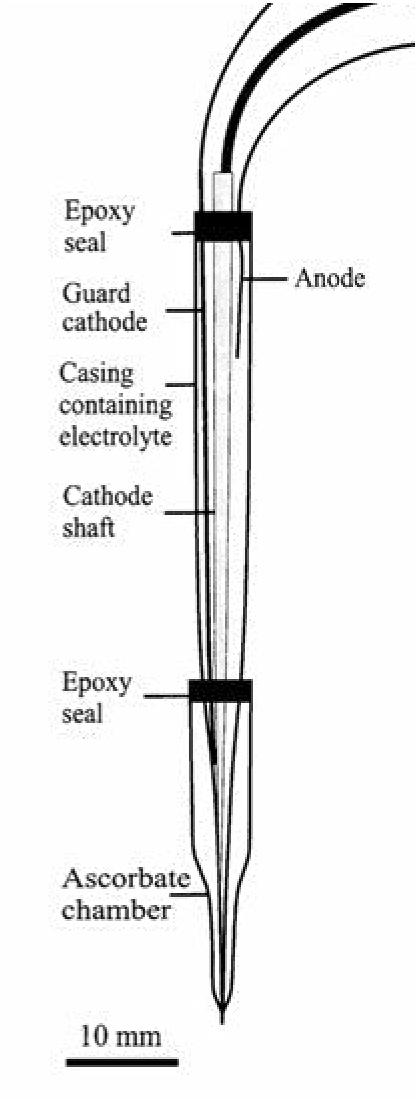
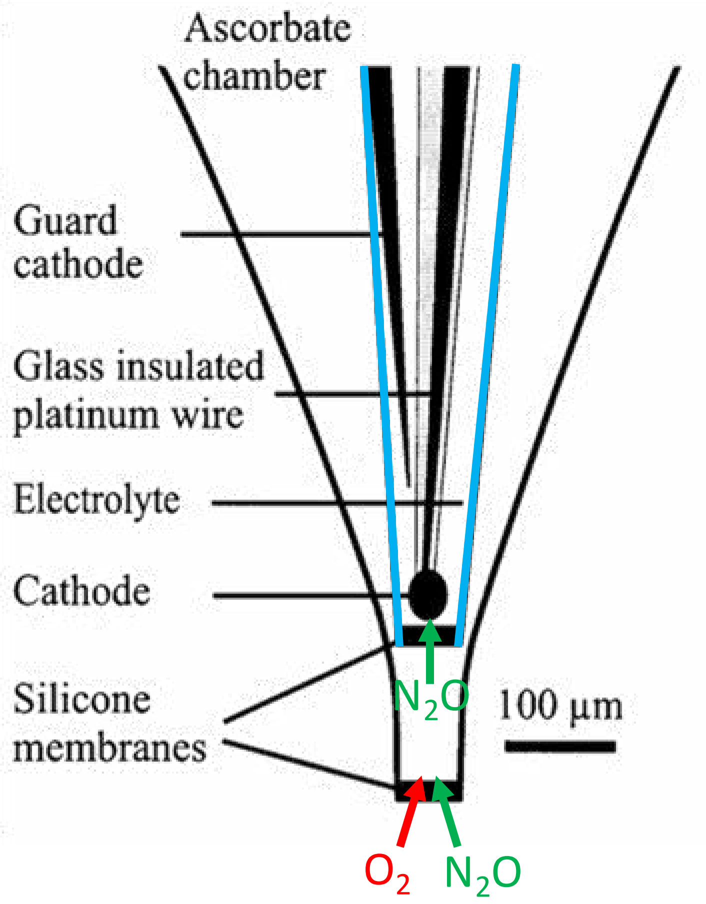
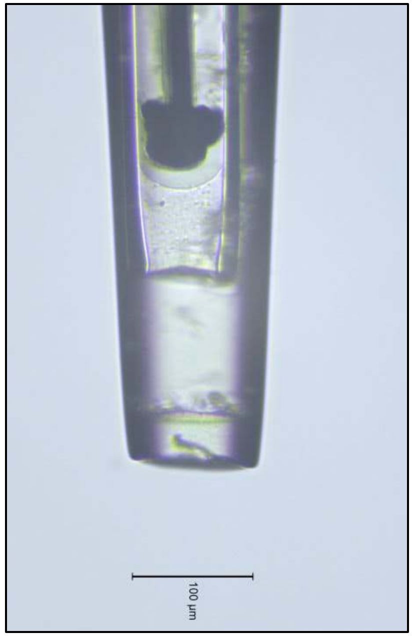
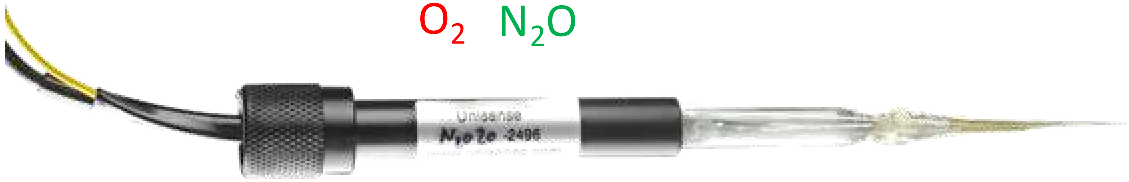
# $N_2O$ sensor



Tip $N_2O$-100



# Waste water $N_2O$ sensor

Tip design R-type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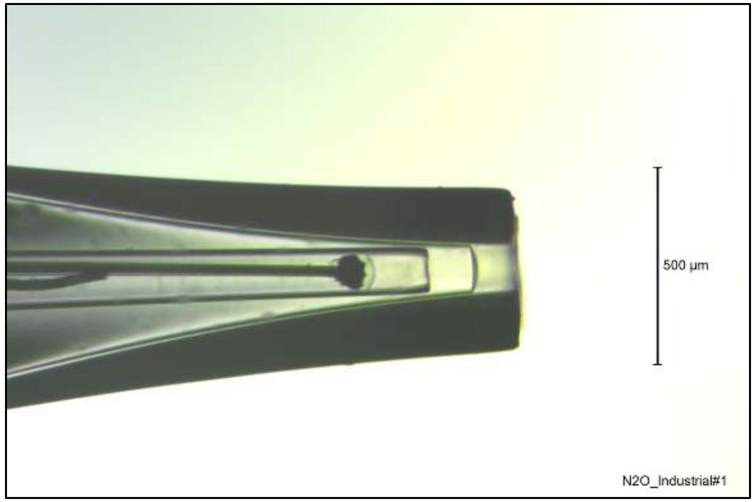
# $N_2O$ sensor

$N_2O$ sensor characteristics:

- Very stable sensor
- Wide range (0-500 µM, customized to 28 mM)
- Std. det. limit is 0.1 µM (Low Range 25 nM)
- Expected life-time 4-6 months
- Long pre-polarization to stabilize
- Long response time
- Linear response – easy calibration
- Quite specific, NO interferes
- Stirring insensitive
- Temperature sensitivity of 1-3% $°C^{-1}$




Versions:
25 µm, 50 µm, 100 µm, 500 µm, Micro Resp, needles, flow cells, steel tube, R (stable for waste water)

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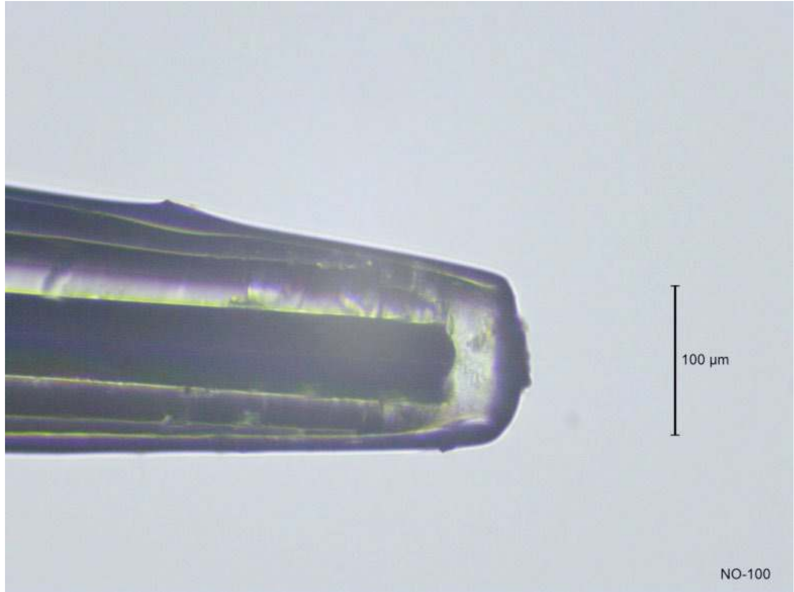
# Nitric oxide sensor

NO sensor characteristics:

- NO oxidation on carbon surface
- Detection limit 3 or 25 nM
- High temperature influence
- Short membrane – must be grounded
- Difficult calibration, NO is an unstable molecule
- Long polarization time to get stable base line
- Fast response time (1 or 10 sec)
- Linear response (0 - 3 µM)
- Some interferents
- Very sensitive to stirring




Versions:
15 µm, 50 µm, 100 µm, 500 µm, Needles, Micro Resp, steel tube, flow cells

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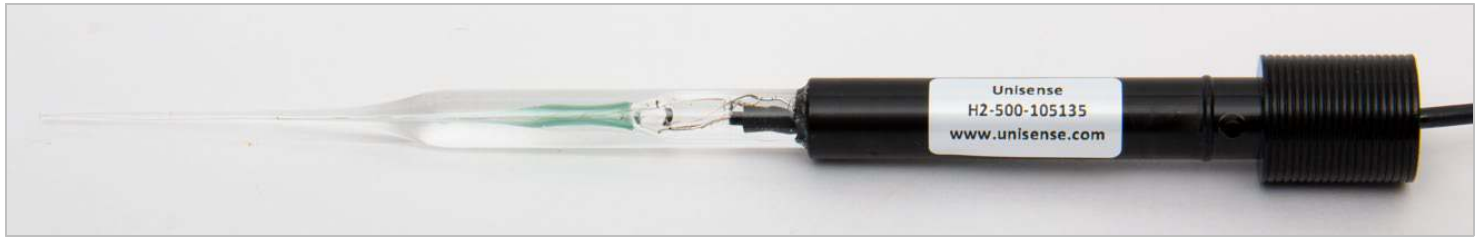
# Hydrogen sensor - Two versions

## $H_2$ sensor

- Sensitive to $H_2S$




Versions:

10 µm, 25 µm, 50 µm, 100 µm, 500 µm, Needles, Micro Resp, Flow cells, Steel tube

## $H_2$-X sensor

- In-sensitive to $H_2S$

50 µm, 100 µm, 500 µm, Needles, Micro Resp, Flow cells, Steel tube

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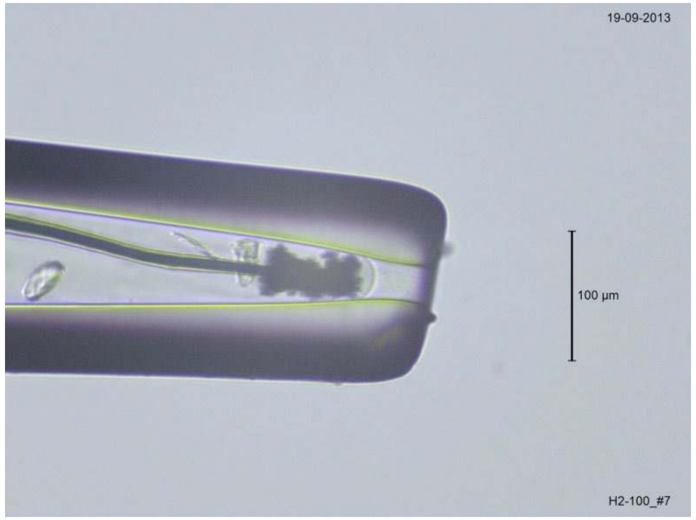
# Hydrogen sensor - Two versions

$H_2$-sensor characteristics:

- $H_2$-oxidation on metal surface
- Linear response (0 - 800 µM)
- Detection limits
  - 0.3 µM - standard
  - 0.05 µM - low range
- Temperature sensitivity of 1-3% $°C^{-1}$
- Expected life time > 12 months
- Response time < 10 sec standard
- $H_2S$ interference

$H_2X$-sensor characteristics:

- Same as $H_2$ version except:
  - Expected life time > 6 months
  - Response time < 20 sec standard
  - No $H_2S$ interference

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# Amperometric sensors - amplifiers


UniAmp amplifier - Multi ch.


Field Microsensor Multimeter

In situ UniAmp amplifier


UniAmp amplifier - Single ch.

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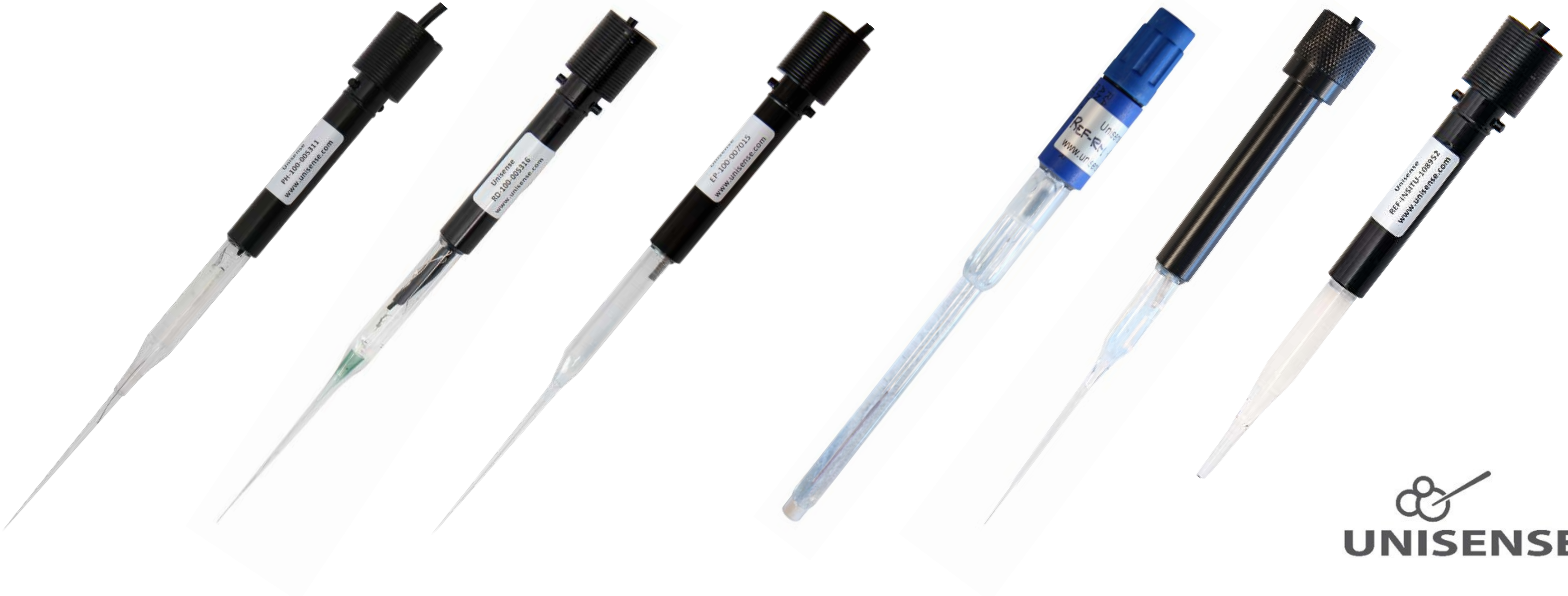
# Potentiometric Microelectrodes

## Microelectrodes

pH | Redox potential | Electrical potential

## Reference electrodes

Robust | Micro | In Situ



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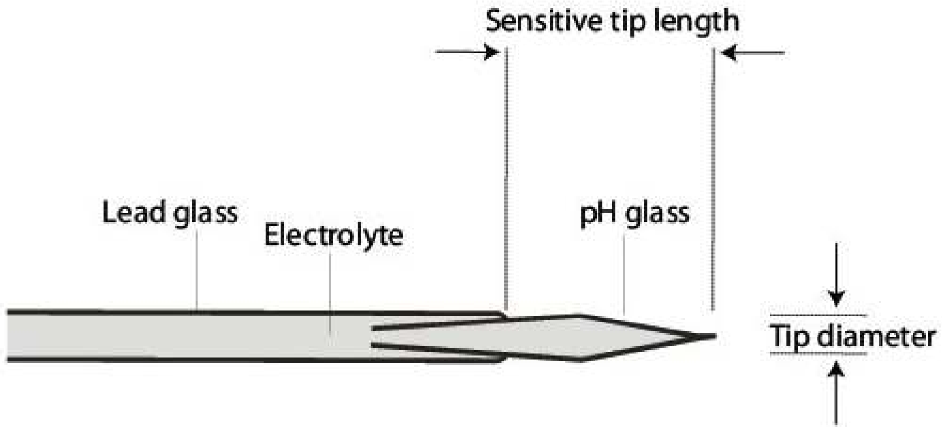
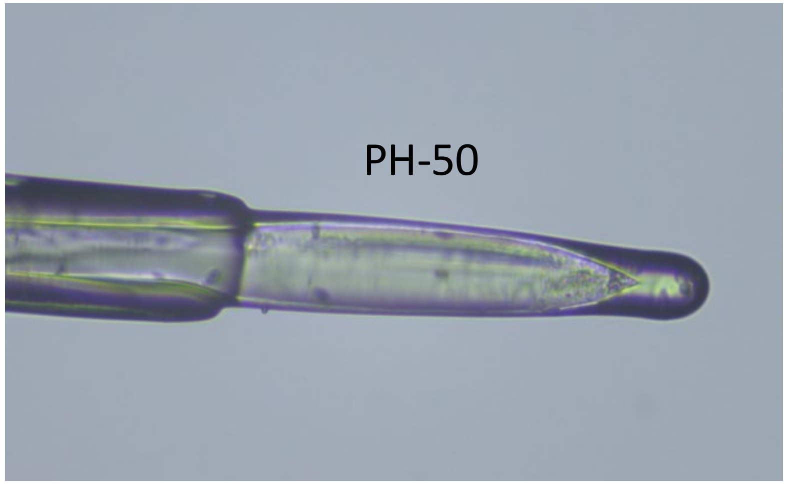
# pH electrode principle

- Microelectrode is a miniaturized conventional pH electrode
- The proton sensitive glass allows measurements of proton activity in a solution
- A high impedance mV meter is required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# pH electrode considerations

- Spatial resolution down to 50-100 µm
- External reference needed
  - Combination electrodes available
- Excellent for measurements in
  - Biofilms
  - Soft sediments
  - Tissues
- Needed for calculations of total sulphide!

Versions:
10 µm, 25 µm, 50 µm, 100 µm, 200 µm, 500 µm, Needles, Flow cells, Steel tube, Micro Resp
pH-500C - Combination


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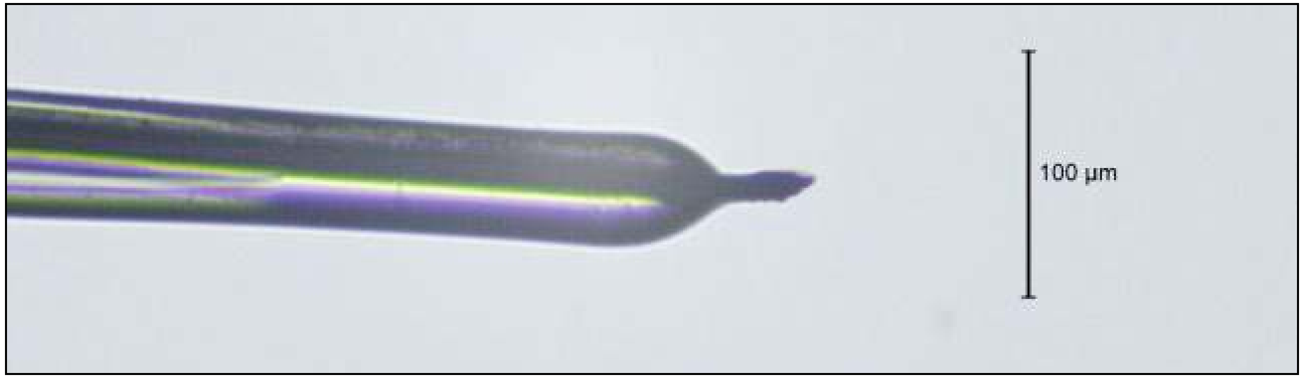
# Redox potential electrode

- The redox electrode is an exposed platinum (Pt) tip. Reference electrode needed
- Must be calibrated
- Limitations
  - Even a Pt surface can be contaminated
  - Problematic in reactive sediments
  - Always check calibration *after* measurements
  - Make multiple profiles

Versions:
10 µm, 25 µm, 50 µm, 100 µm, 200 µm, 500 µm, Needle, Flow cells, Steel tube, Micro Resp
RD-500C - Combination

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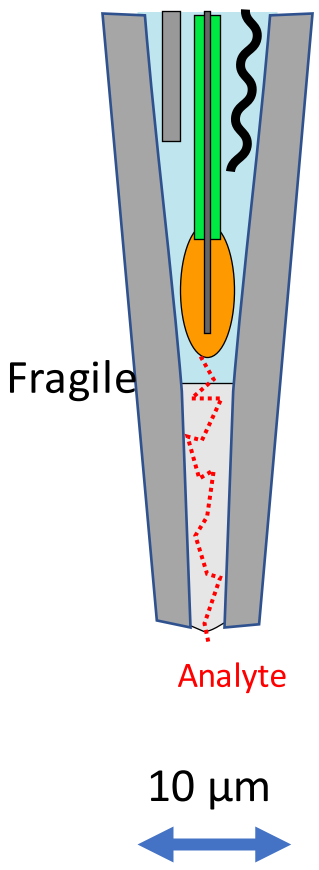
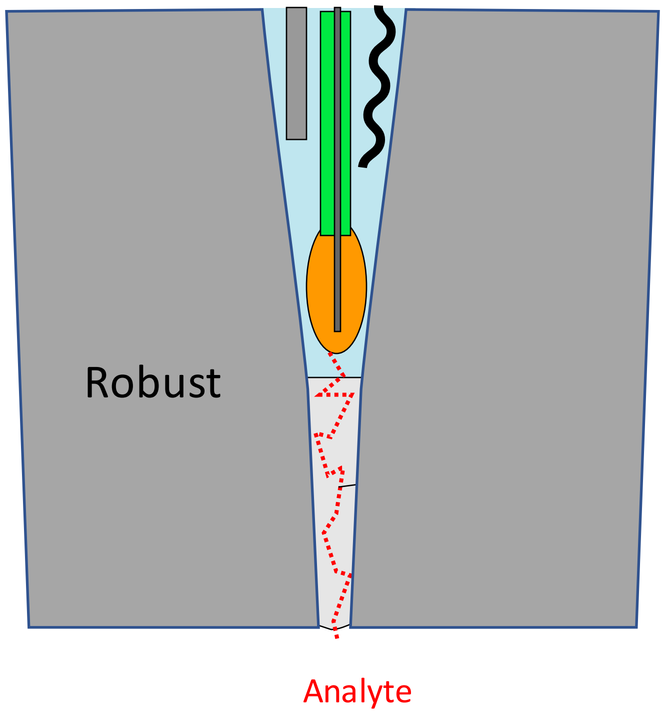
# Variations: Diameter

- All Unisense microsensors come in many sizes
- Clark type: Only thickness of glass wall changes




- Same internal design
- Same membrane size
- Same signal and consumption rate
- Glass walls thickened, by heat-collapsing the glass

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# MicroOptode System

**MicroOptode Sensor**

- Fluorescent $O_2$ sensitive dye
- Fast responding
- $E^2$PROM – ID and calibration
- Retractable needle design
- Tip sizes: 50, 430 and 3000 µm

**MicroOptode Meter**

- 1 or 4 channels - Optode only
- 1 channel - Combined with electrochemical sensors
- Integrate with Unisense software


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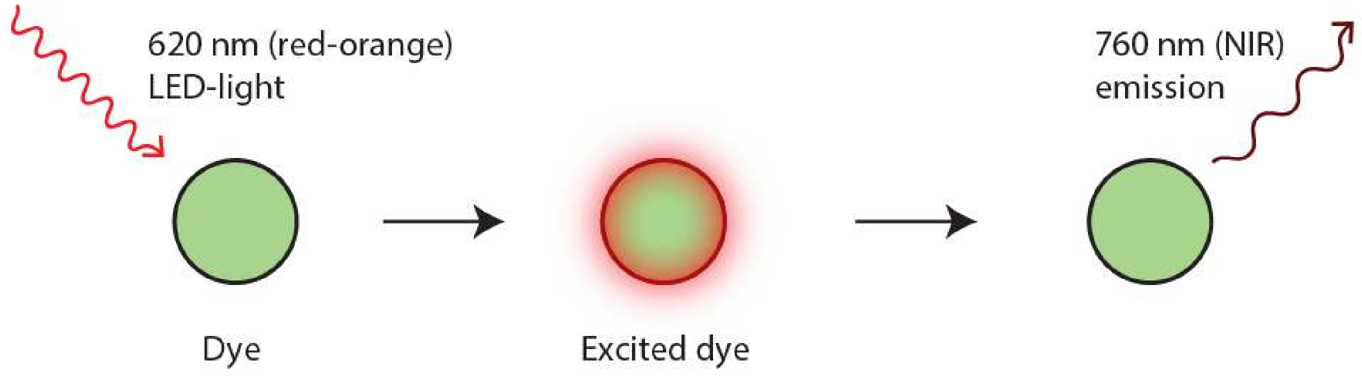
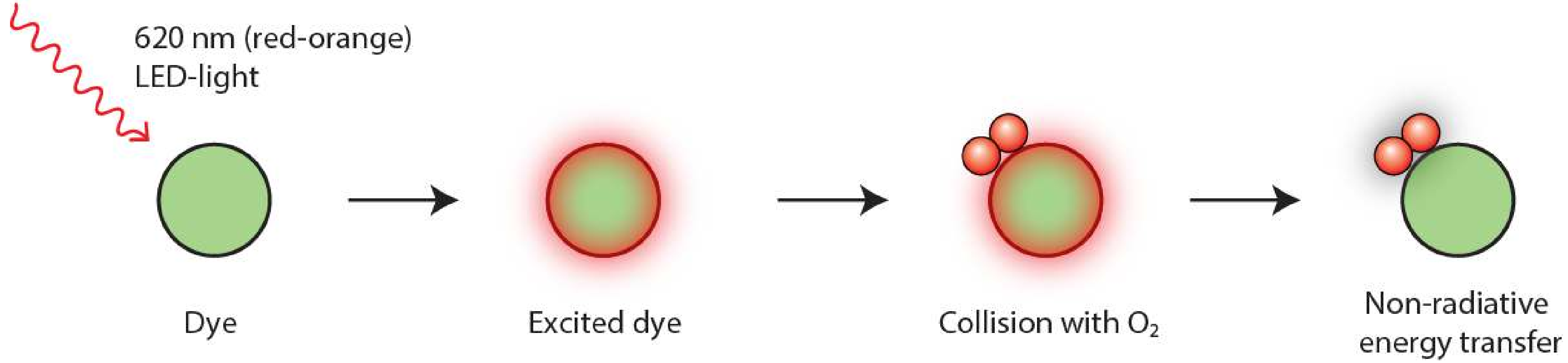
# Working principle of optodes

***Fluorescence in the absence of $O_2$:***




***Quenching in the presence of $O_2$:***

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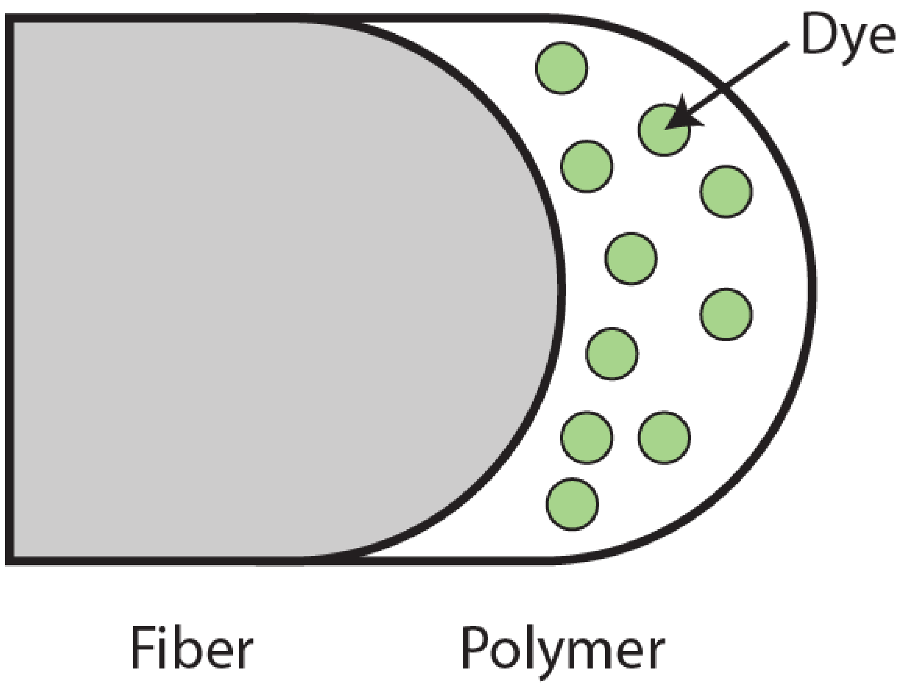
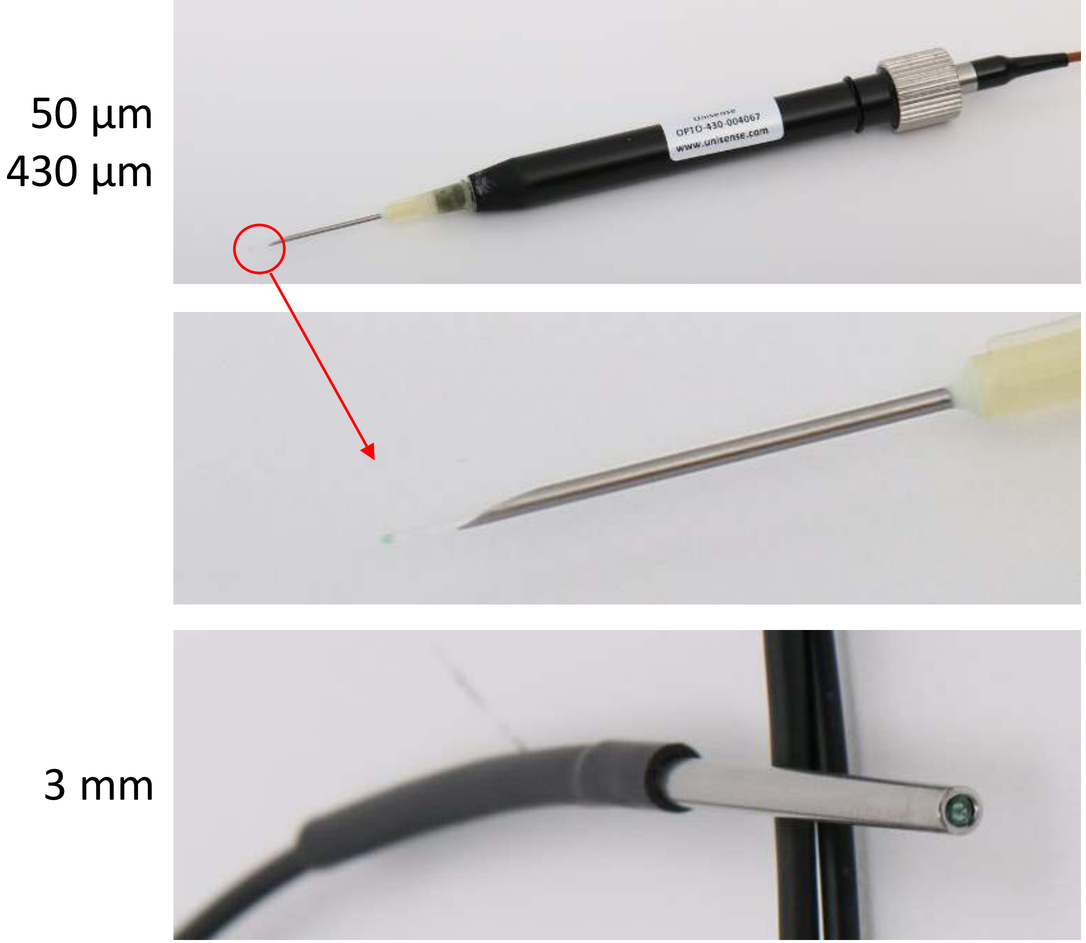
# Optical fibre optodes

## Tip of fibre

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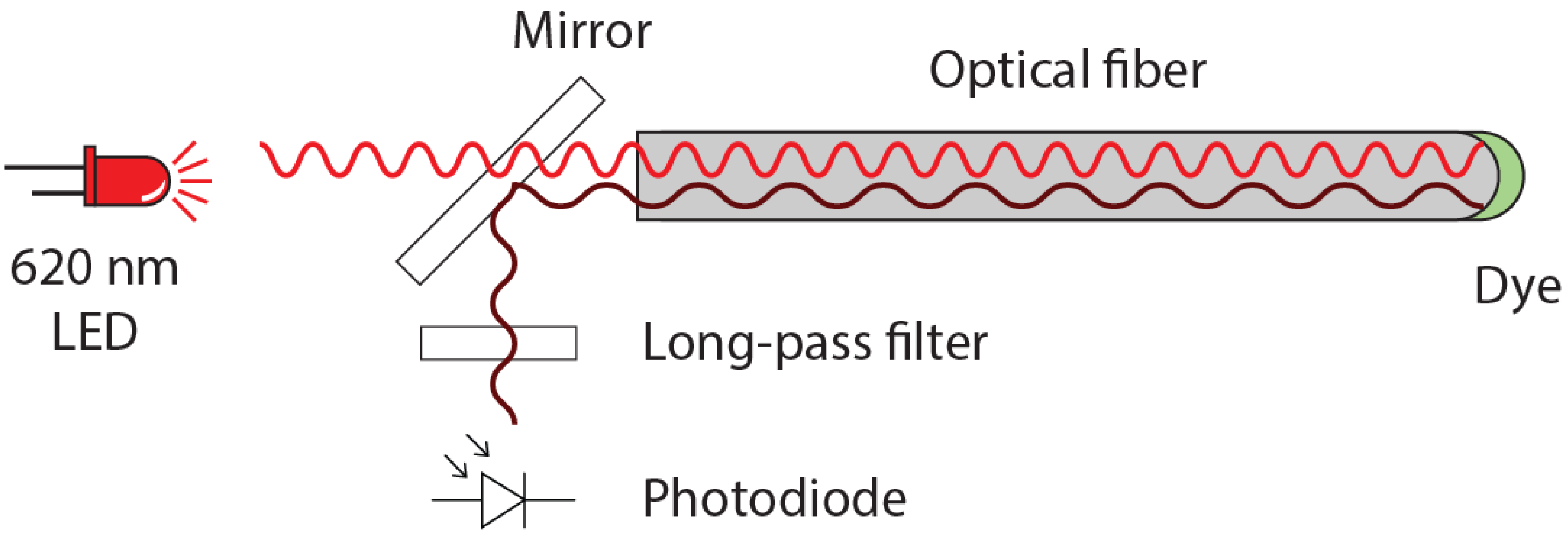
# Optical fibre optodes

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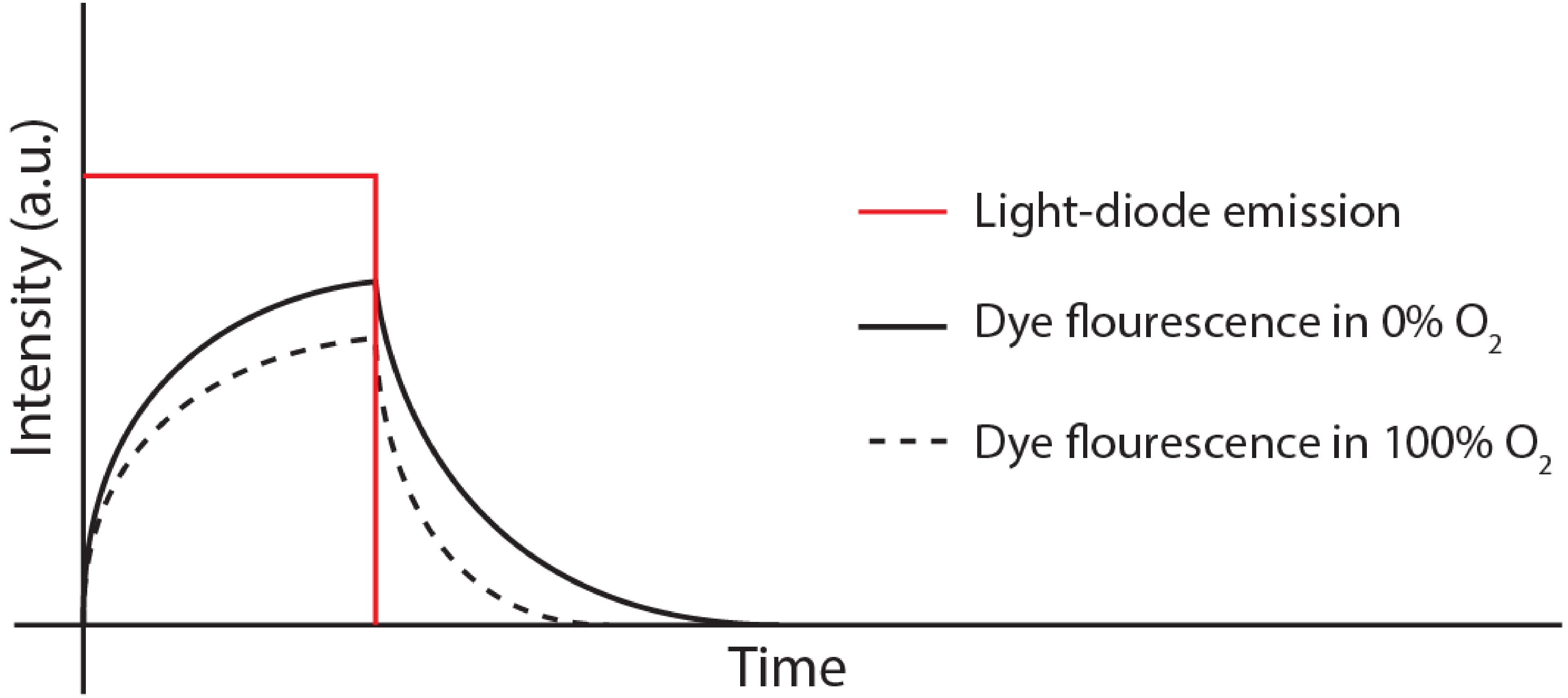
# Fluorescence lifetime

In the time-domain:

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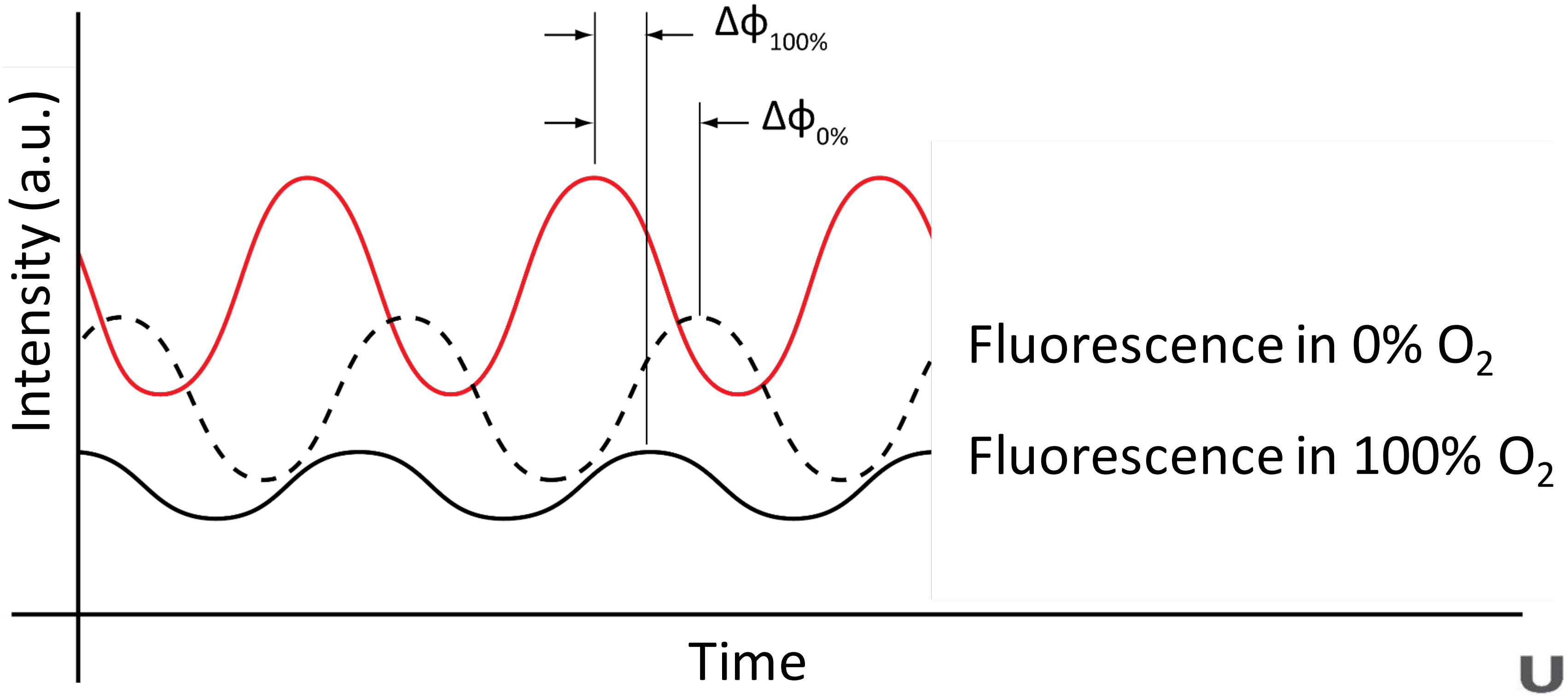
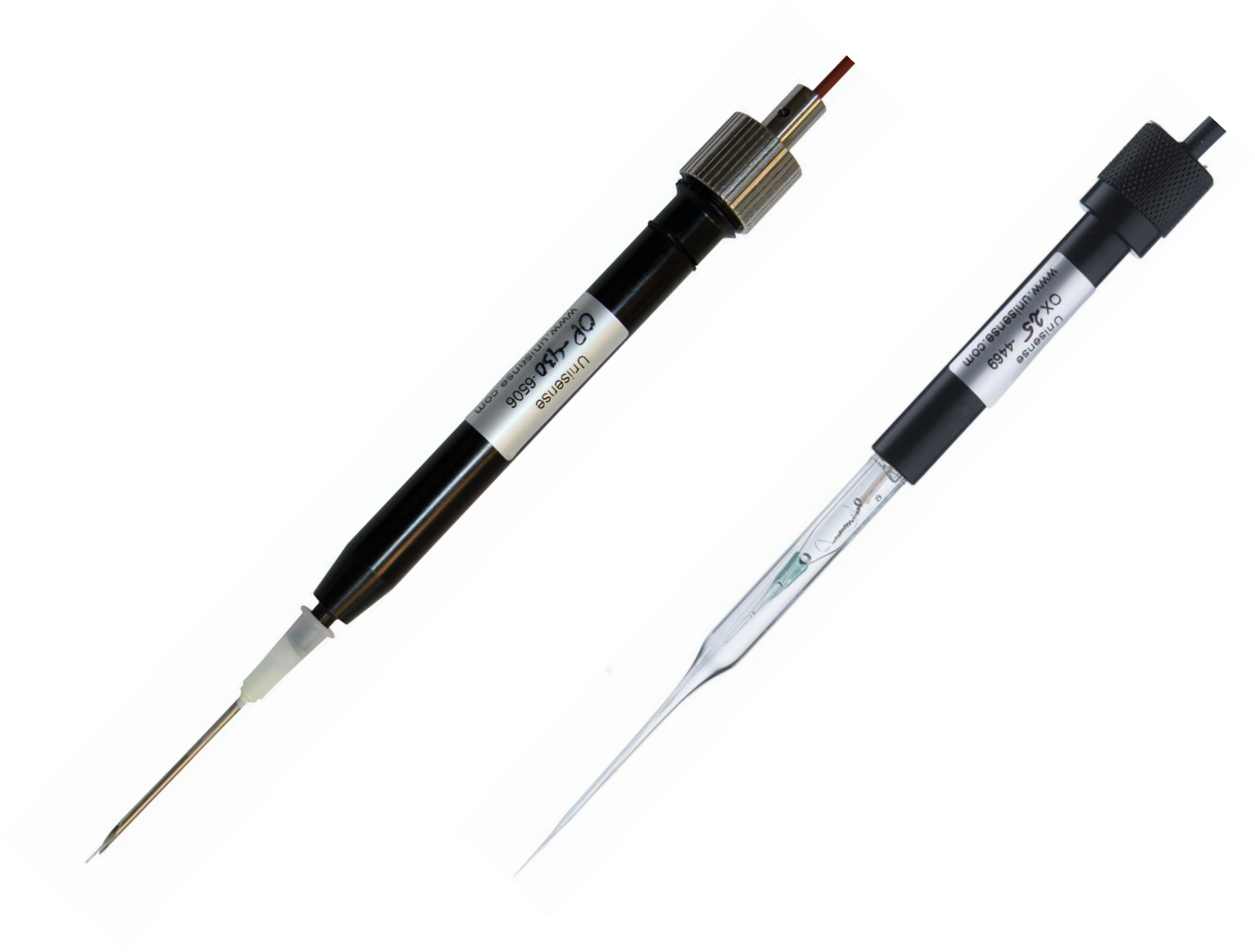
# Fluorescence life-time measurements

LED modulation frequency = 4 kHz
Light pulse 10 ms => 40 periods



# Choose the right $O_2$ sensor

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# Optodes and amperometric sensors - Advantages

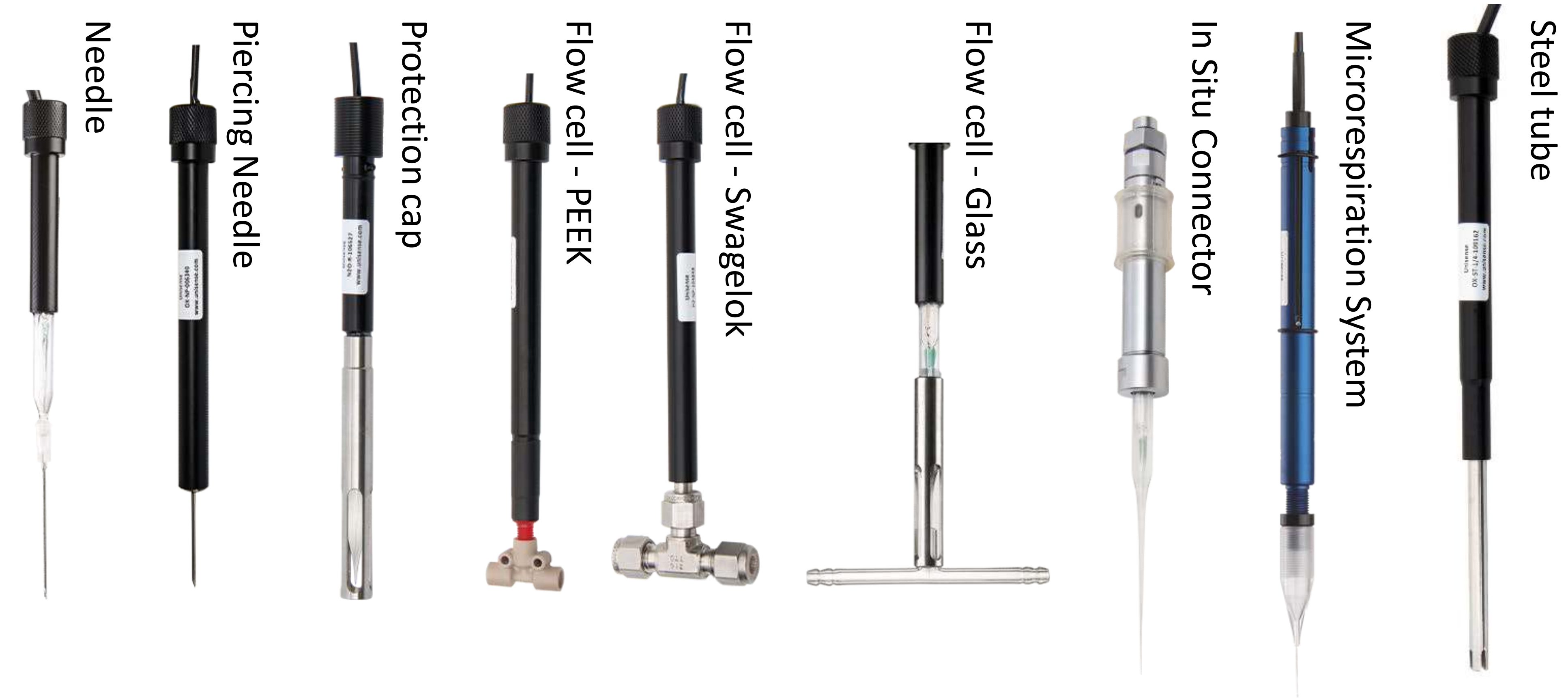
## $O_2$ Optode

- Long term stability
- Flexibility
- No $H_2S$ interference
- Retractable tip
- 3000 µm mini sensor

## Amperometric $O_2$ sensor

- Fast response
- Tip size (small)
- Rugged design for profiling
- Wide concentration range
- Large temperature range
- Not flexible
- Needle version

# Microsensors - adaptations

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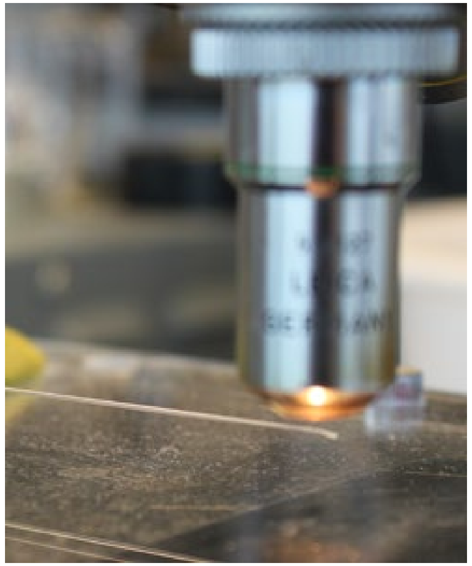
# Customizations

Physical:

- Length
- Diameter

Behaviour and response :

- Response time
- Sensitivity to stirring
- Concentration range (low range, high range)



# Effects of tip size

## Choosing the correct sensor size:

Small versus large tip diameter

- Higher profile resolution
- Less sample disturbance

# Sensor tip size

## Choosing the correct sensor size:

Small versus large tip diameter

- Higher profile resolution +
- Less sample disturbance +
- Faster response +
- More fragile –

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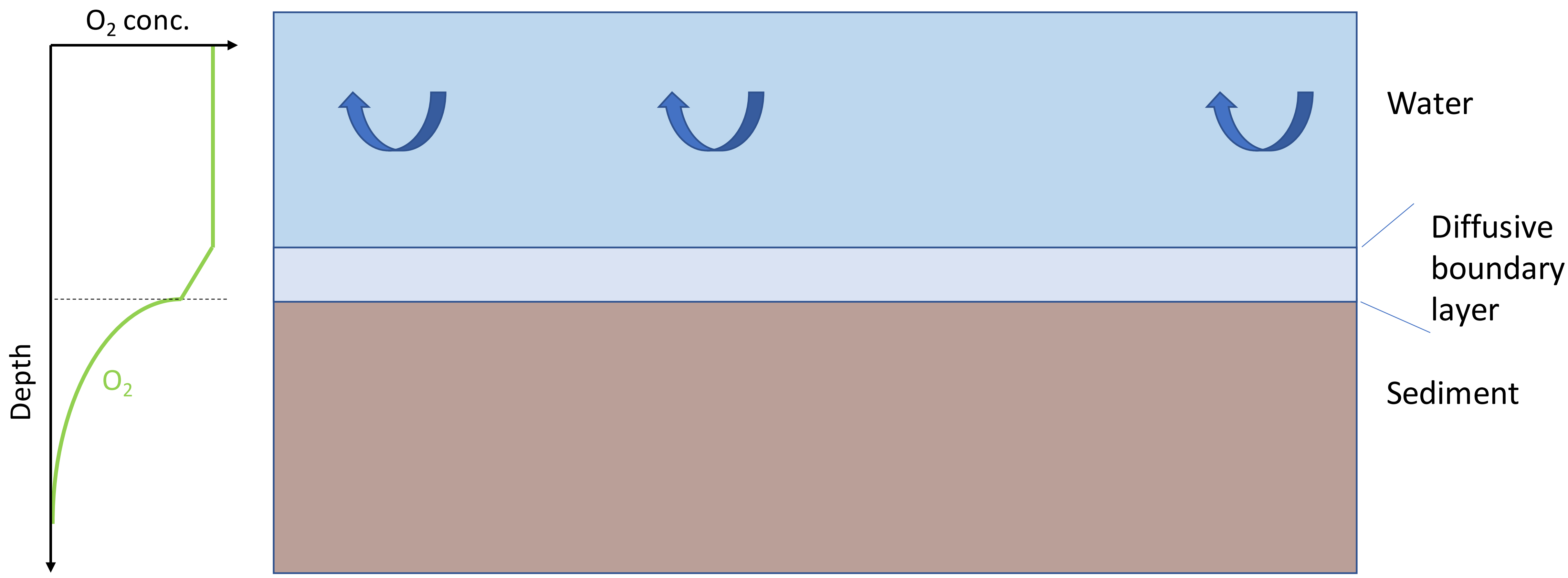
# Tip size - Effect on profile

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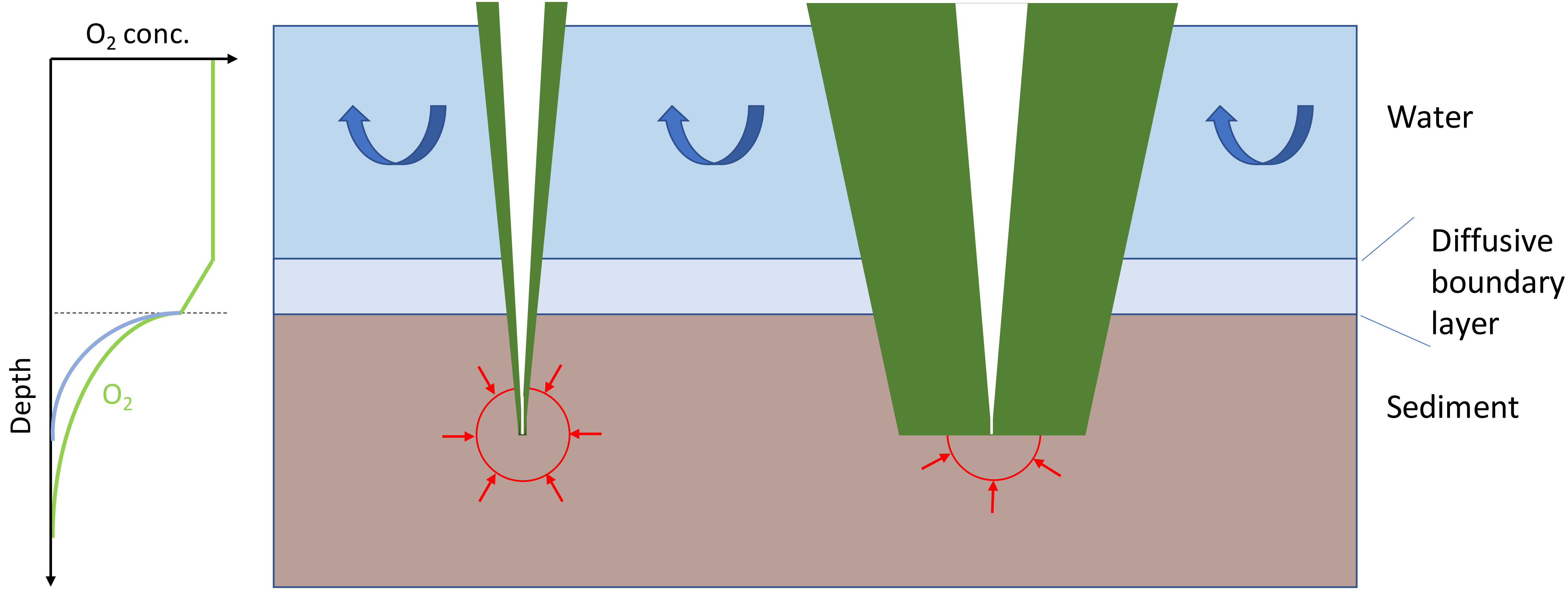
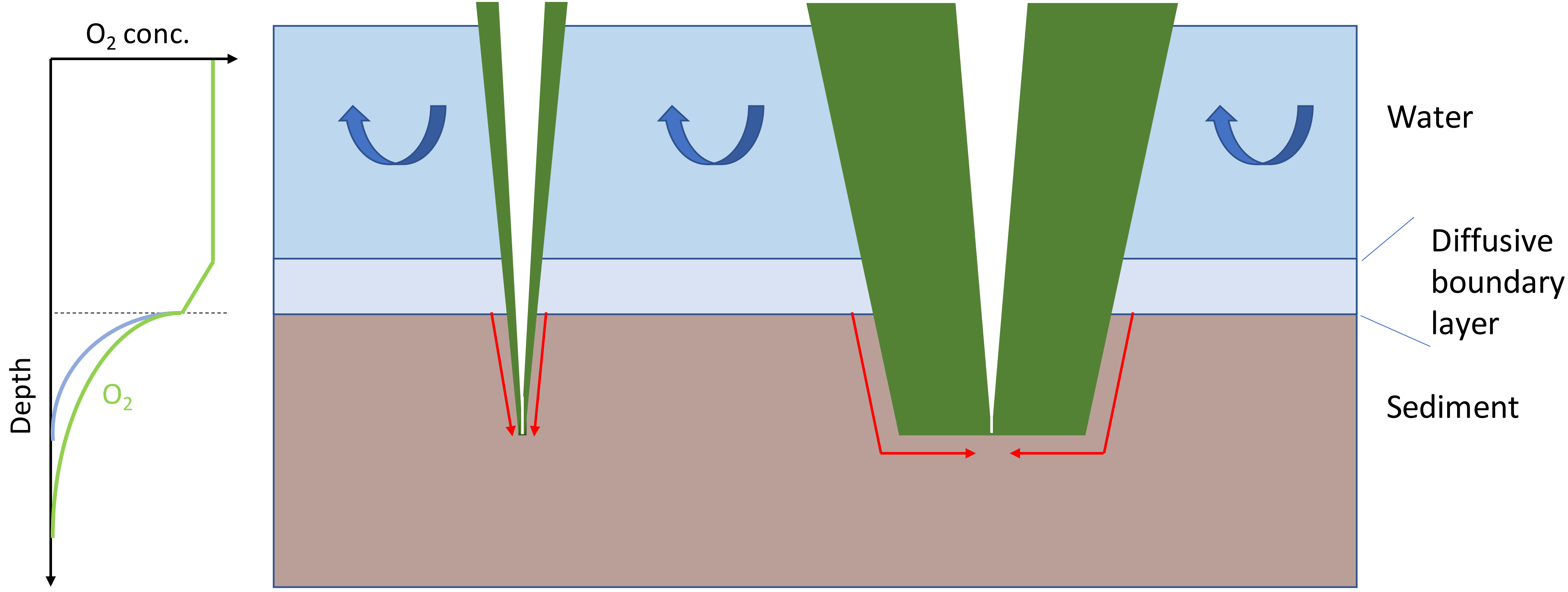
# Tip size - Effect on profile



# Tip size - Effect on profile

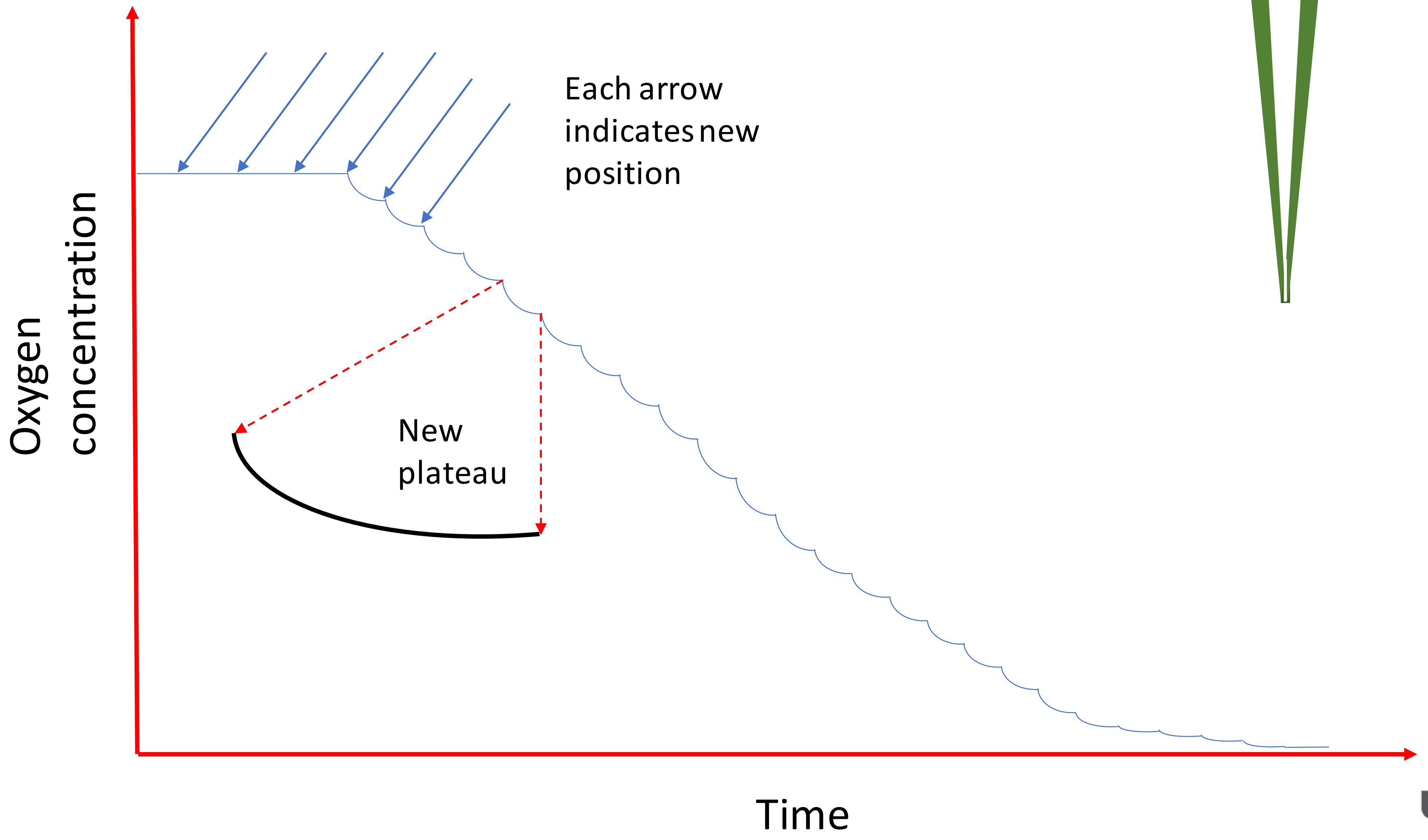



Effect of sensor:

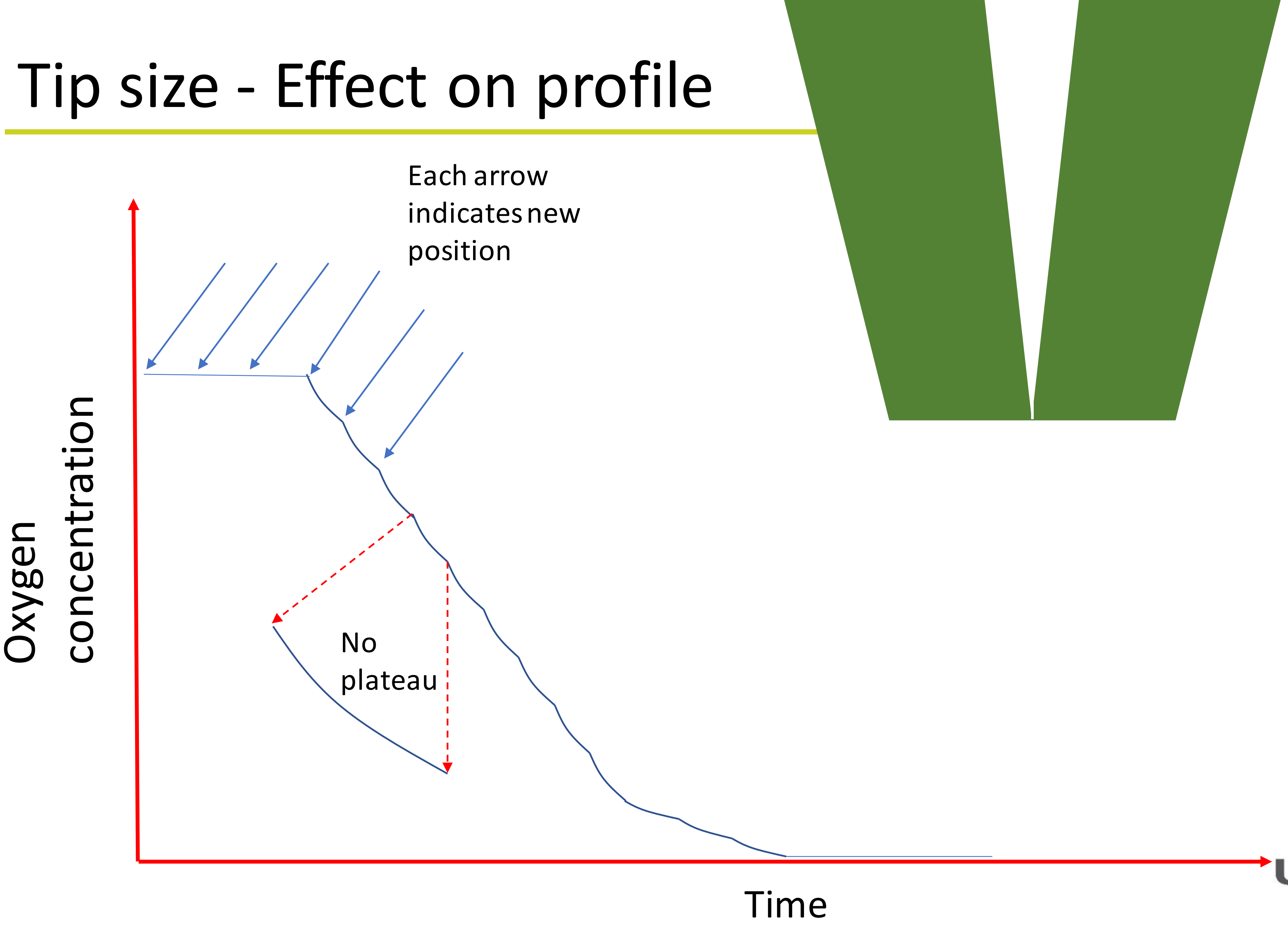
$O_2$ supply unaffected
Fast stabilization of signal

$O_2$ supply impeded
Drifting signal
Underestimation of $O_2$

# Tip size - Effect on profile



# Tip size - Effect on profile

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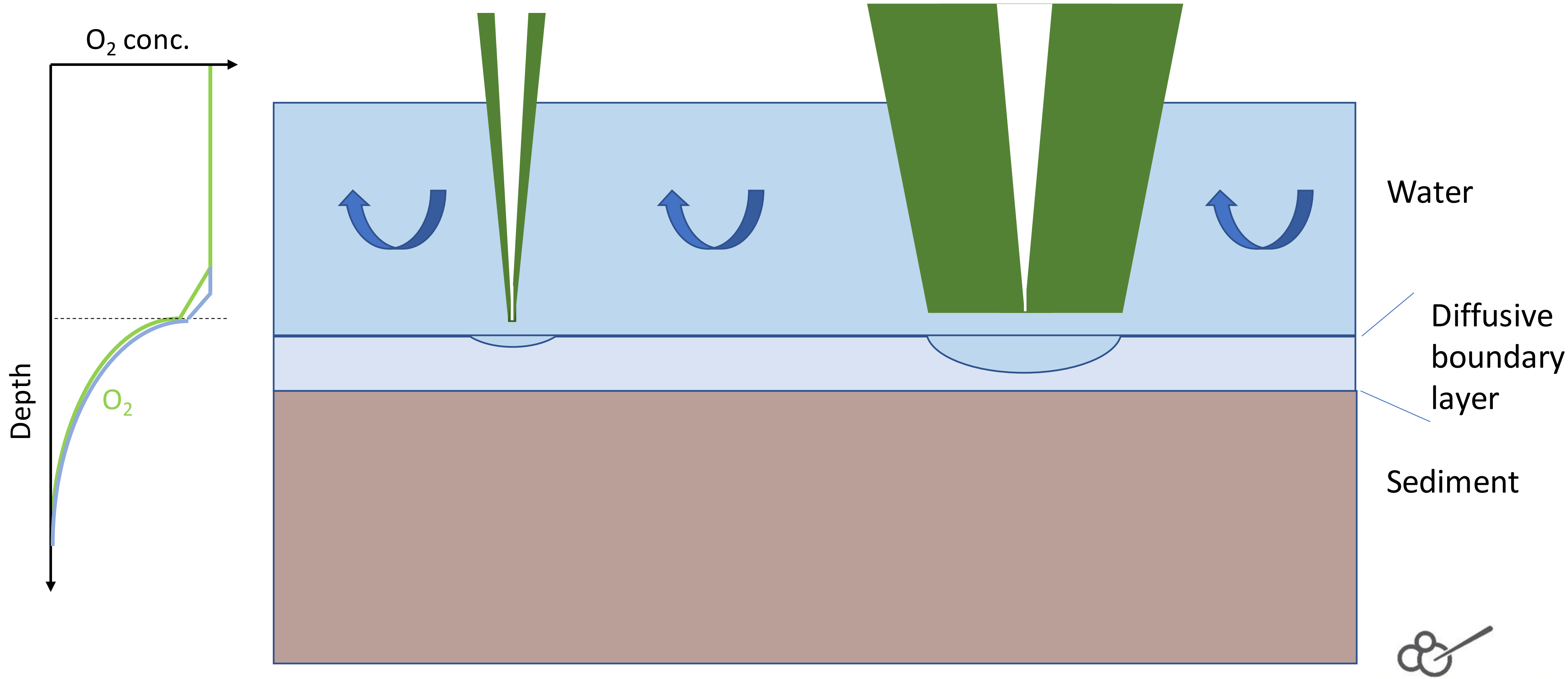
# Tip size - Effect on diffusive boundary layer

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# Interference

May affect the sensor signal

Examples:

- $O_2$ sensor: $H_2S$
- H2S-type sensor: Light
- SULF sensor: $H_2$
- $N_2O$ sensor: NO
- $H_2$ sensor: $H_2S$

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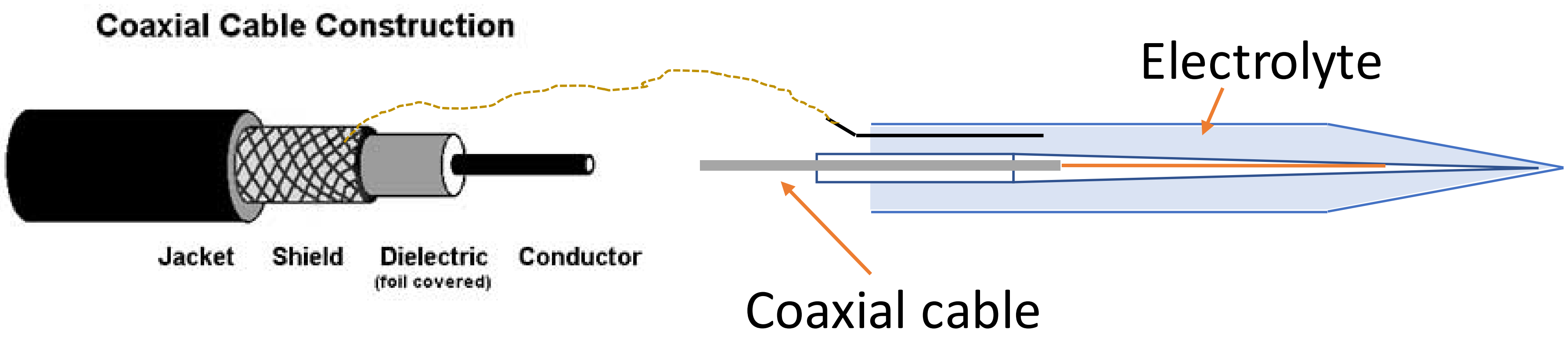
# Electrical noise

## Very low signals

- In the pA range ($10^{-12}$ A)
- Grounding important
  - Necessary for some sensors!
- Coaxial design of all sensors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# Sensor calibration

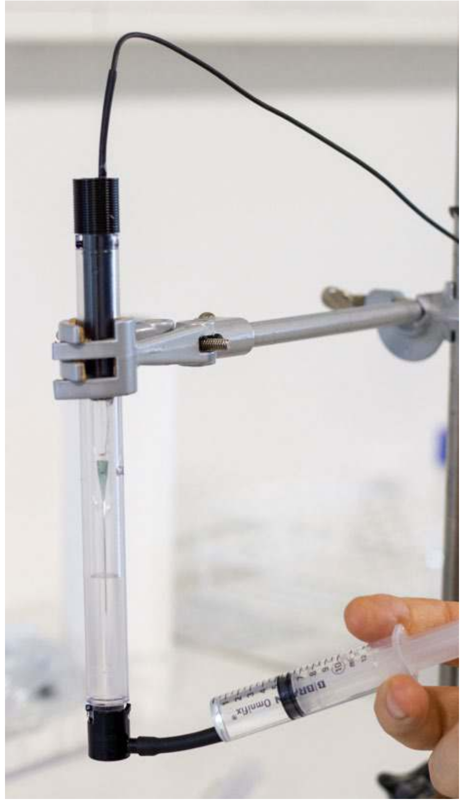
Use experimental/in-situ conditions for sensor calibration whenever possible

- Same temperature
- Same salinity

Sensors for gases are linear

- Two-point calibration
  - One known concentration
  - Zero

Calibration chamber



Calibration kit

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# Oxygen calibration

Two point calibration

- Anoxic - 0% $O_2$ (ascorbate, $N_2$ gas)
- Air saturated water (100% air, 20.9% $O_2$)
- Tabulated values for $O_2$ conc. at equilibrium between air and water as function of temperature and salinity.

Oxygen solubility at different temperatures and salinities of seawater

Units: µmol/l

| Salinity (‰) | Temperature (°C) 0.0 | 1.0 | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 | 7.0 | 8.0 | 9.0 | 10.0 | 11.0 | 12.0 | 13. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.0 | 456.6 | 444.0 | 431.9 | 420.4 | 409.4 | 398.9 | 388.8 | 379.2 | 369.9 | 361.1 | 352.6 | 344.4 | 336.6 | 329 |
| 1.0 | 453.5 | 441.0 | 429.0 | 417.6 | 406.7 | 396.3 | 386.3 | 376.7 | 367.6 | 358.8 | 350.4 | 342.3 | 334.5 | 327 |
| 2.0 | 450.4 | 438.0 | 426.1 | 414.8 | 404.0 | 393.6 | 383.7 | 374.3 | 365.2 | 356.5 | 348.1 | 340.1 | 332.4 | 325 |
| 3.0 | 447.3 | 435.0 | 423.2 | 412.0 | 401.3 | 391.0 | 381.2 | 371.8 | 362.8 | 354.2 | 345.9 | 338.0 | 330.4 | 323 |
| 4.0 | 444.2 | 432.0 | 420.4 | 409.2 | 398.6 | 388.5 | 378.7 | 369.4 | 360.5 | 351.9 | 343.7 | 335.9 | 328.3 | 321 |
| 5.0 | 441.1 | 429.1 | 417.5 | 406.5 | 396.0 | 385.9 | 376.3 | 367.0 | 358.2 | 349.7 | 341.6 | 333.7 | 326.2 | 319 |
| 6.0 | 438.1 | 426.1 | 414.7 | 403.8 | 393.3 | 383.3 | 373.8 | 364.6 | 355.9 | 347.5 | 339.4 | 331.6 | 324.2 | 317 |
| 7.0 | 435.1 | 423.2 | 411.9 | 401.1 | 390.7 | 380.8 | 371.3 | 362.3 | 353.6 | 345.2 | 337.2 | 329.6 | 322.2 | 315 |
| 8.0 | 432.1 | 420.3 | 409.1 | 398.4 | 388.1 | 378.3 | 368.9 | 359.9 | 351.3 | 343.0 | 335.1 | 327.5 | 320.2 | 313 |
| 9.0 | 429.1 | 417.5 | 406.3 | 395.7 | 385.5 | 375.8 | 366.5 | 357.6 | 349.0 | 340.8 | 333.0 | 325.4 | 318.2 | 311 |
| 10.0 | 426.1 | 414.6 | 403.6 | 393.0 | 383.0 | 373.3 | 364.1 | 355.2 | 346.8 | 338.6 | 330.8 | 323.4 | 316.2 | 309 |
| 11.0 | 423.2 | 411.8 | 400.8 | 390.4 | 380.4 | 370.8 | 361.7 | 352.9 | 344.5 | 336.5 | 328.7 | 321.3 | 314.2 | 307 |
| 12.0 | 420.3 | 409.0 | 398.1 | 387.8 | 377.9 | 368.4 | 359.3 | 350.6 | 342.3 | 334.3 | 326.7 | 319.3 | 312.2 | 305 |
| 13.0 | 417.4 | 406.2 | 395.4 | 385.2 | 375.3 | 366.0 | 357.0 | 348.3 | 340.1 | 332.2 | 324.6 | 317.3 | 310.3 | 303 |

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# Sensor calibration

Gas sensors respond to <u>partial pressure</u> not concentration

<u>Henrys law:</u>

- Concentration = Solubility × Partial pressure
- Partial pressure = Concentration/solubility

If solubility is constant

- Δ Concentration = Δ Partial pressure

If concentration is constant

- Δ Solubility = Δ Partial pressure

Partial pressure is affected by temperature and salinity

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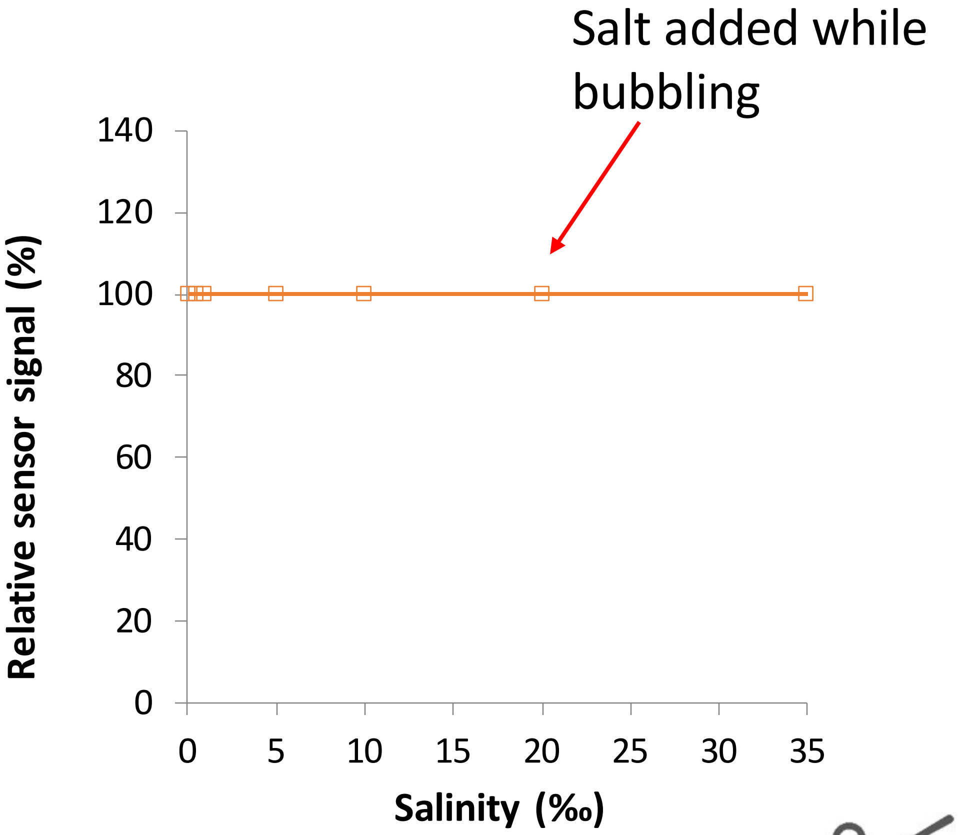
# Sensor calibration

Salinity effect – Gas sensors: i.e. $O_2$, $H_2$, $N_2O$, $H_2S$, NO

Bubbling with gas, add salt to increase salinity
=> constant partial pressure




The partial pressure of the gas is **not** affected by salinity at continuous bubbling

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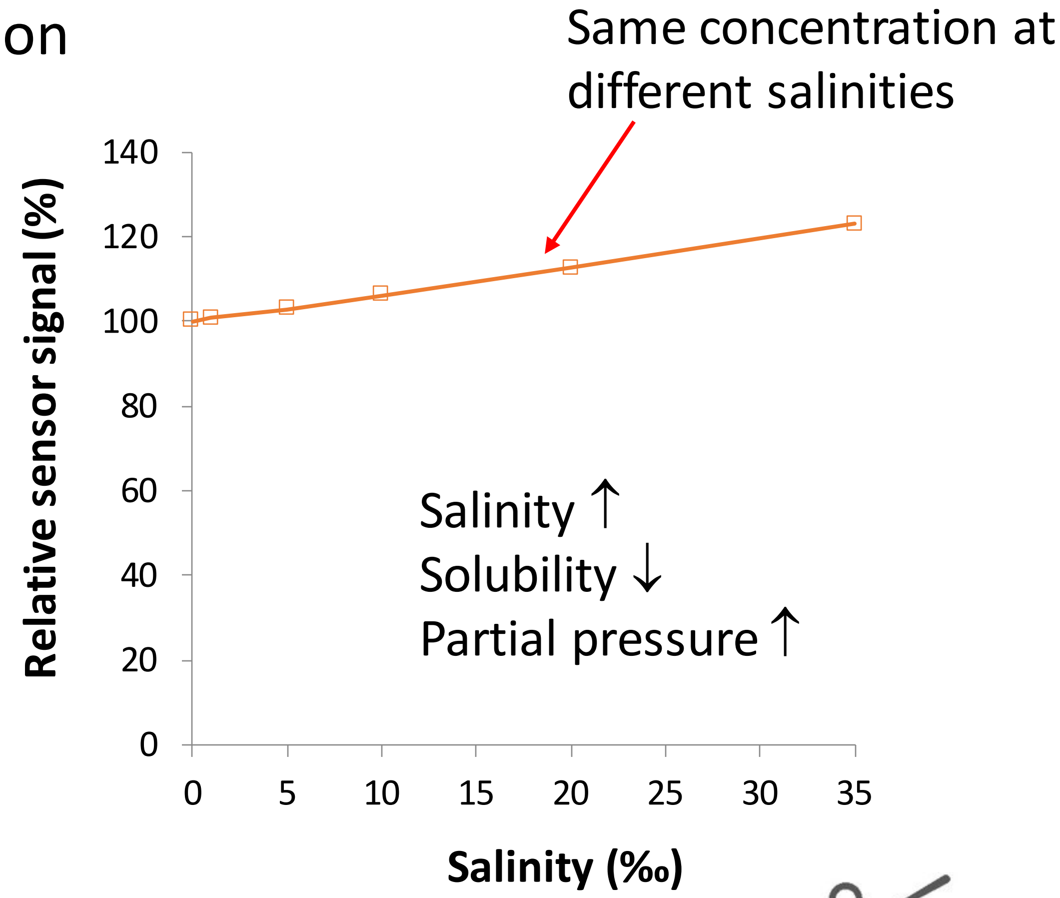
# Sensor calib

**Henrys law:**

Partial pressure = Concentration/solubility

## Salinity effect – Gas sensors: i.e. $O_2$, $H_2$, $N_2O$, $H_2S$, NO

Dilution of stock solution to a given concentration at different salinities




At fixed concentration:

The partial pressure of the gas is affected by salinity

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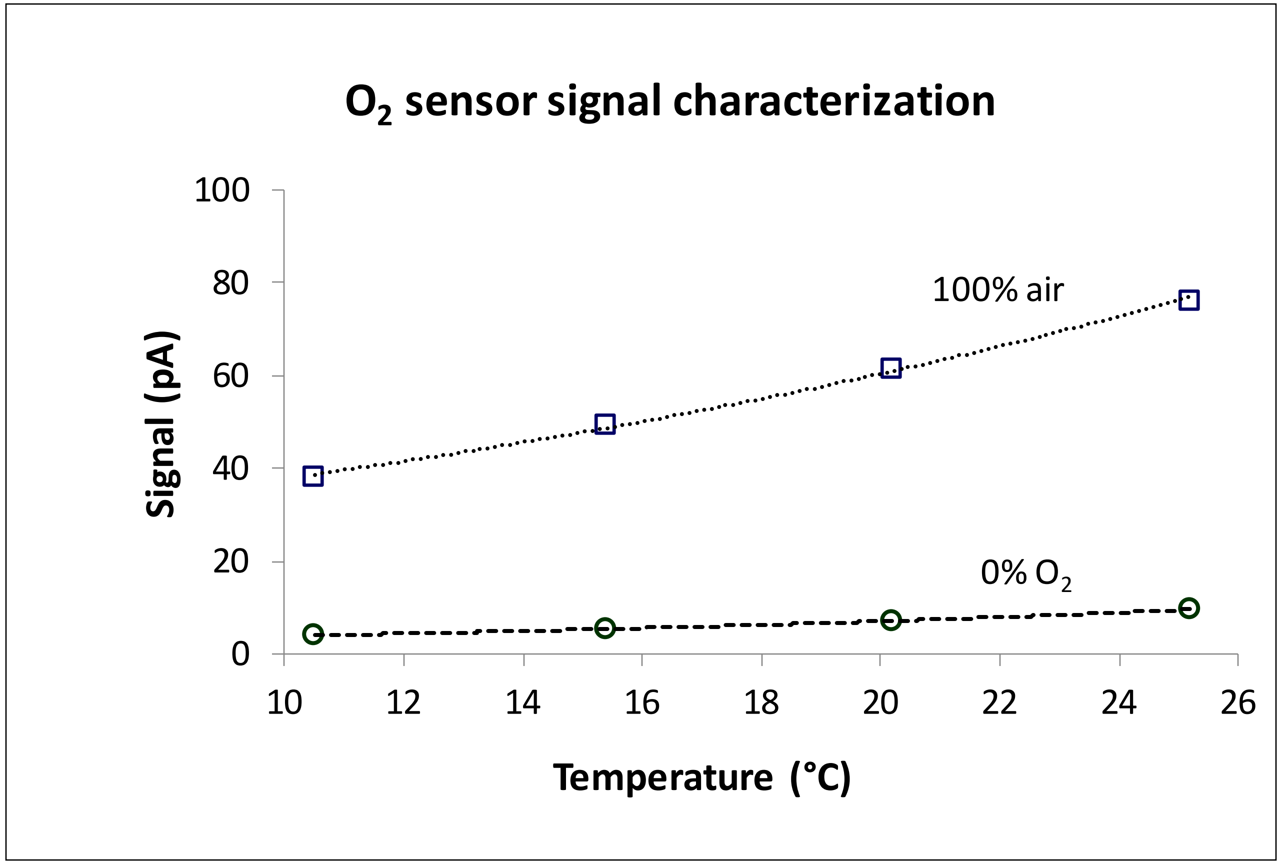
# Temperature effect on measurement

## Temperature effect – example $O_2$ sensor





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# Temperature affects measurements

The Lab UniAmp instruments may automatically correct for this

- Calculation of concentration from sensor signal is a two-step process:
    1. Sensor signal (mV) → Partial pressure (mmHg)
    2. Partial pressure (mmHg) → Concentration (µM)
- Temperature affects both 1 and 2
    1. The internal *Sensor response*
    2. The *Solubility effect*


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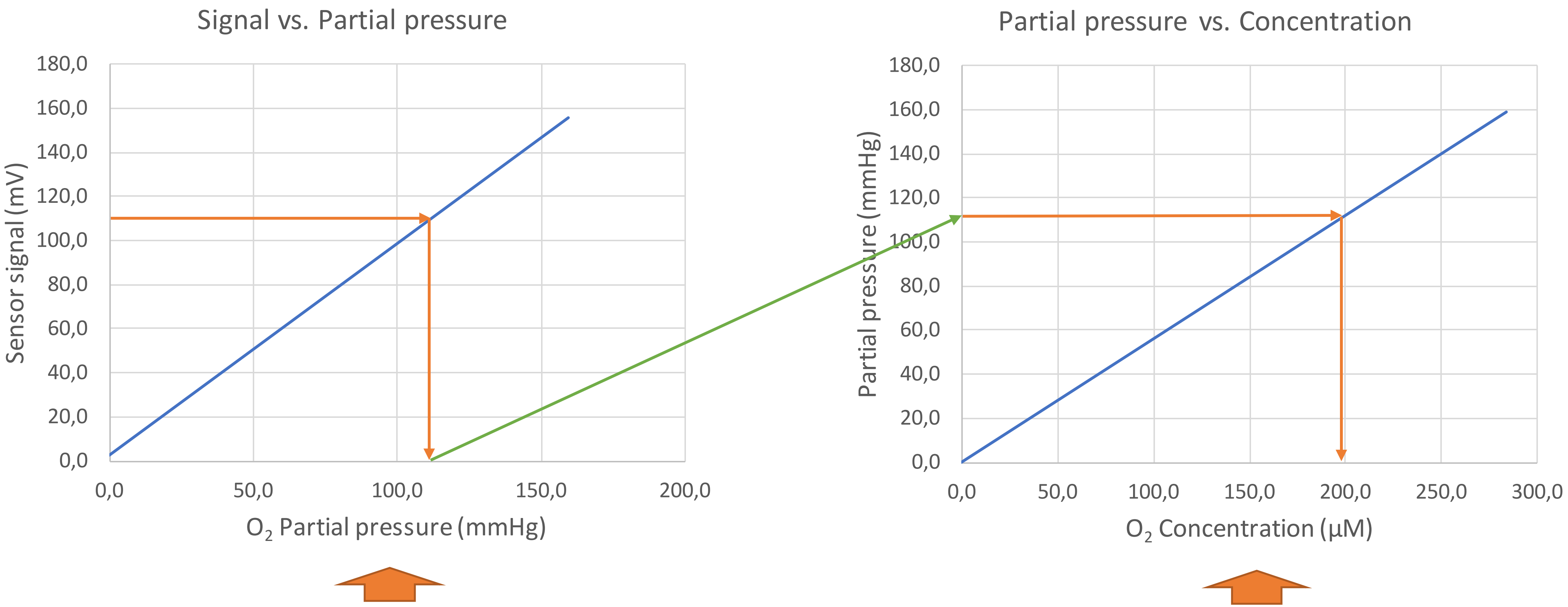
# Conversion of signal to concentration




*Sensor response*
*Relation established by calibration*

*Solubility effect*
*Knowledge from the literature*

These two relations are affected by temperature

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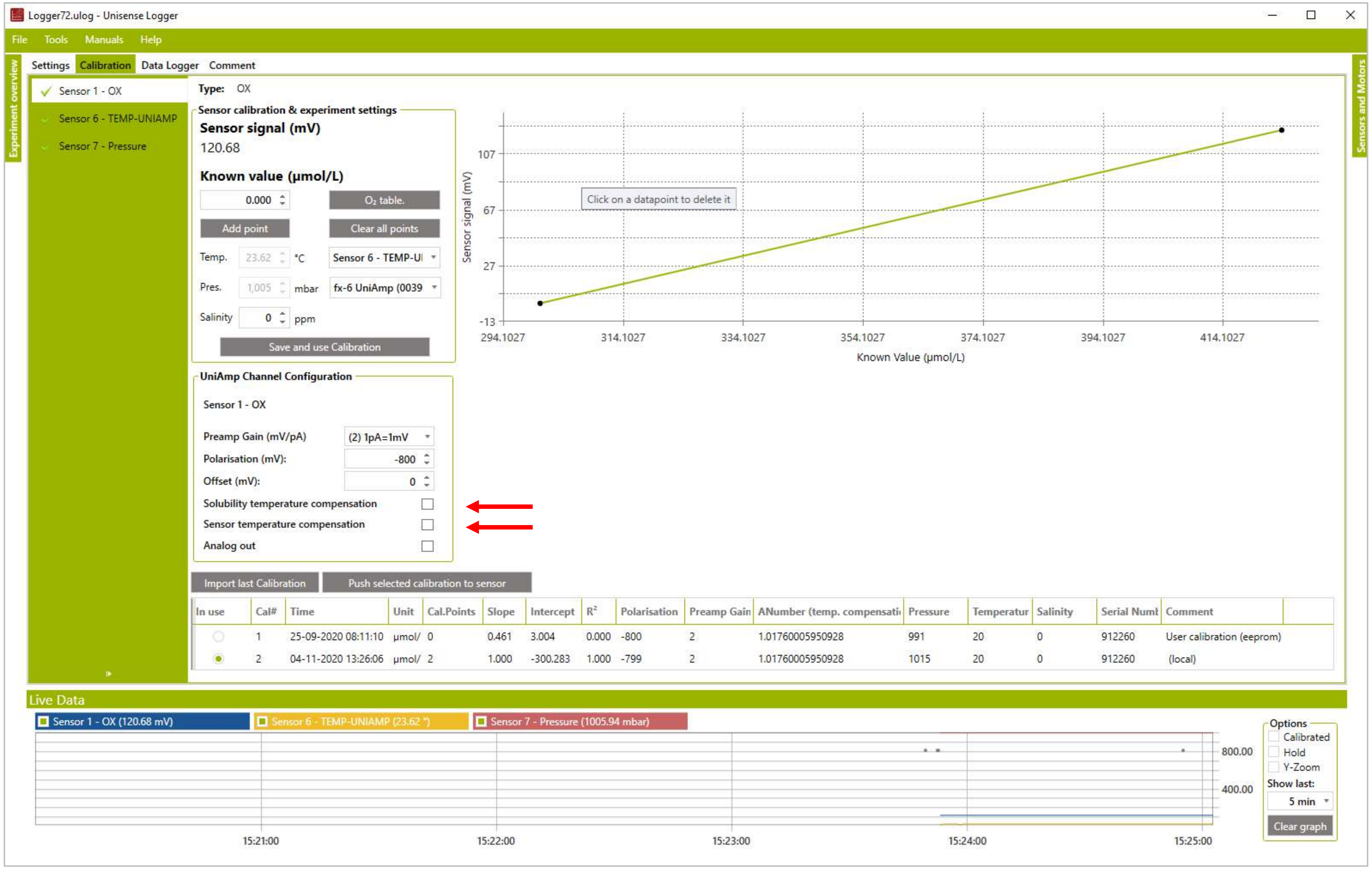
# SensorTrace – Temperature compensation

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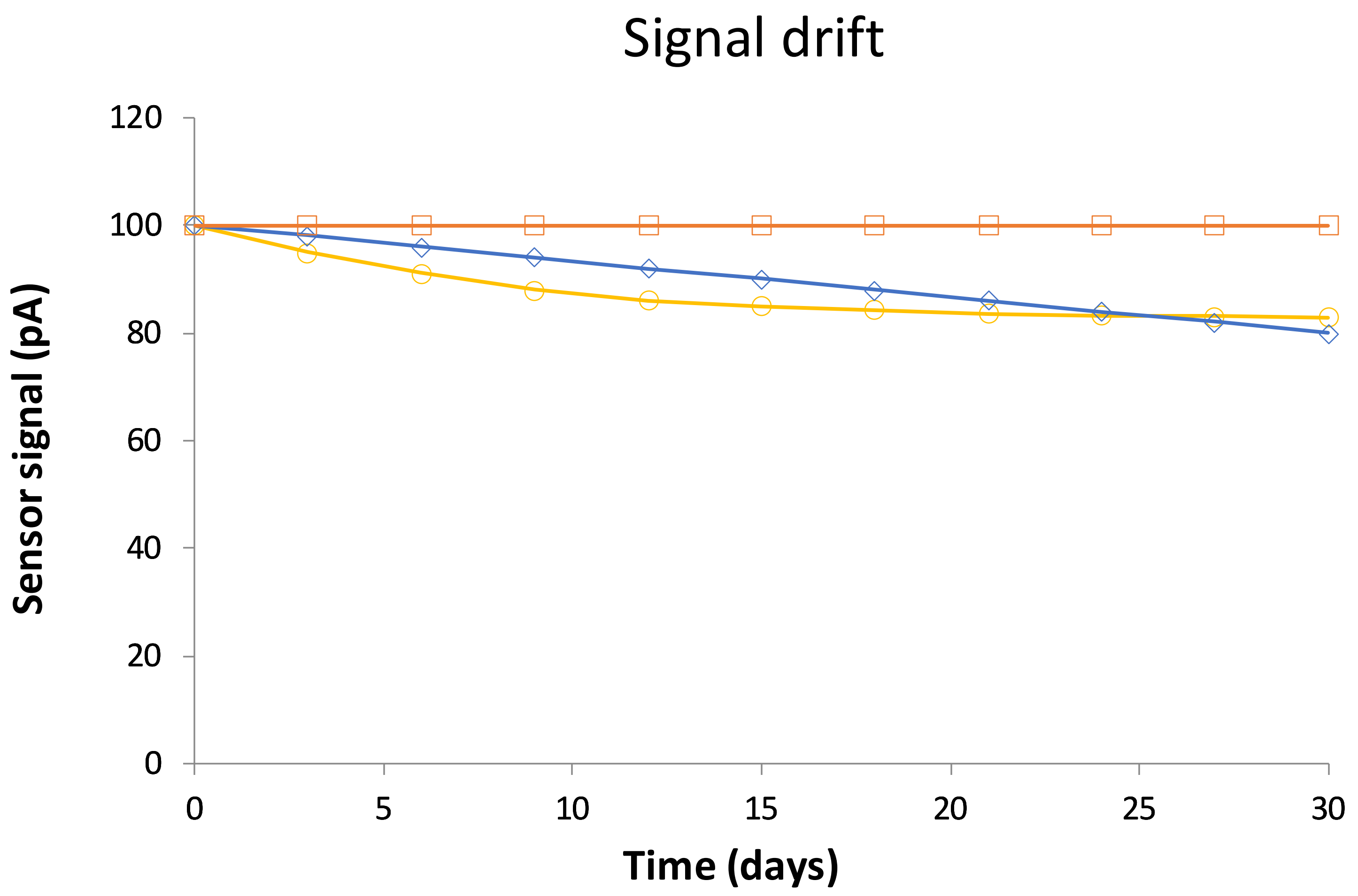
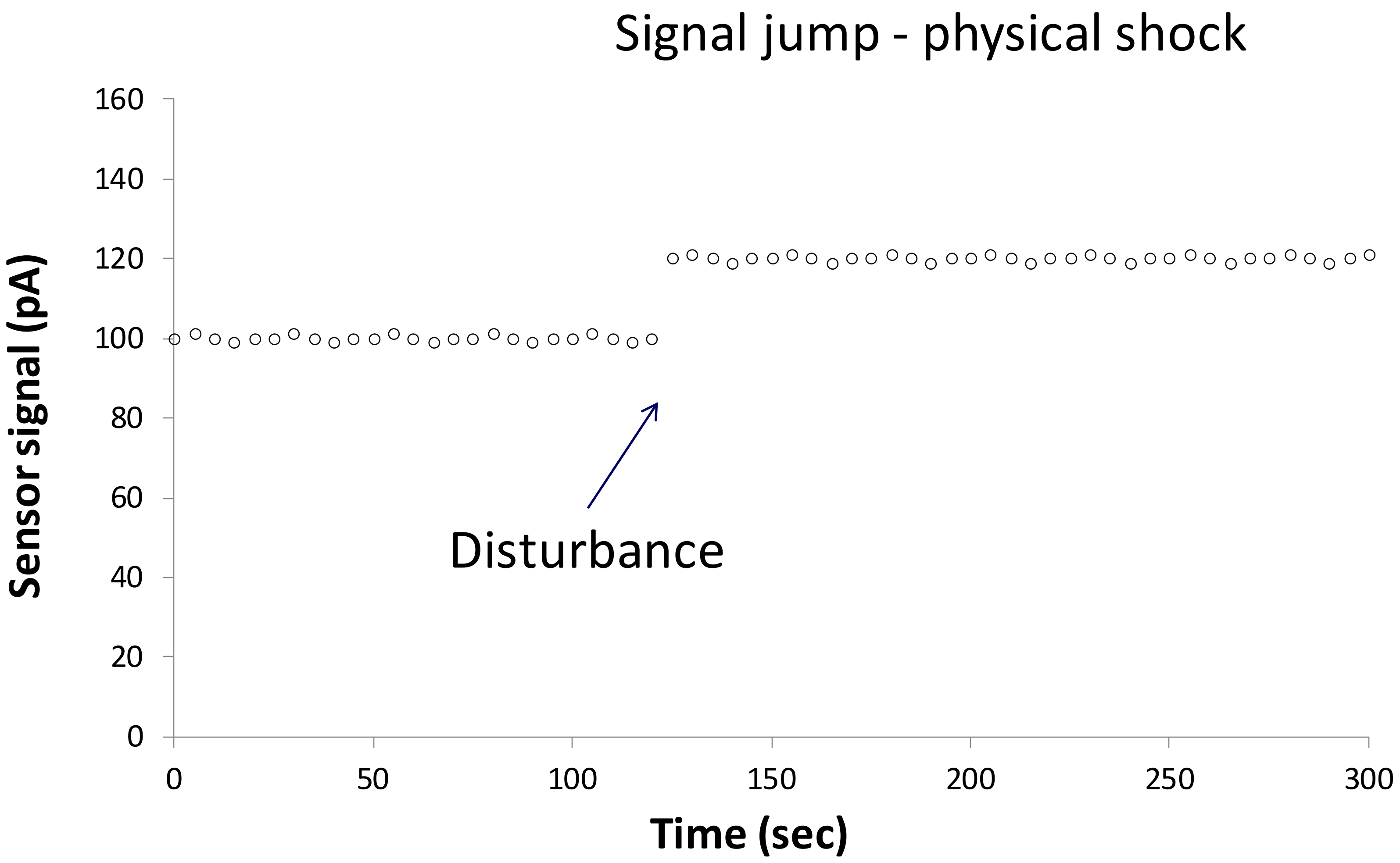
# Sensor calibration - Potential challenges

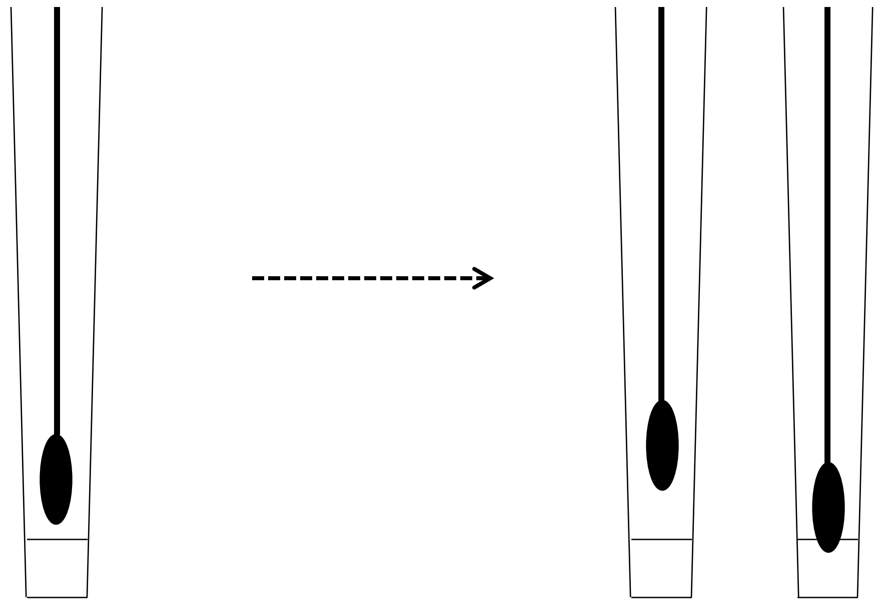


# Sensor calibration - Potential challenges



# Sensor calibration - Potential challenges

Displacement of working electrode!



Before

After

# Sensor calibration - Recommendations

- Use experimental/in-situ conditions for sensor calibration
  - Temperature
  - Salinity
- Be careful with baseline (zero) definition
- Always calibrate the sensor at the beginning of the experimental work
  - Know the sensor works well
  - Get results even when breaking a sensor
- Calibrate often
  - Signal drift
  - Signal jump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# Time for questions !

Unisense Microsensor Academy:
https://www.unisense.com/support/knowledge

Contact us: sales@unisense.com