Winkler Analysis
(Ifremer metrology lab)

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O2 Meeting – Paris/LOCEAN, 09/28/15

1 – Winkler analysis

2 – Inter Laboratory Comparisons

Winkler Analysis

Standard:

- NF EN 25813 / ISO 5813 Standard

Oceanographic recognized references:

- World Ocean Circulation Experiment recommendations (1990-1998)

- French reference literature: “Hydrologie des écosystèmes marin. Paramètres et analyses” - Editions Ifremer

Sampling description

Winkler Analysis

- **Winkler analysis : Reagents**

- KIO_3 solution: $Na_2S_2O_3$ calibration

- $Na_2S_2O_3$ solution: I_2 titration

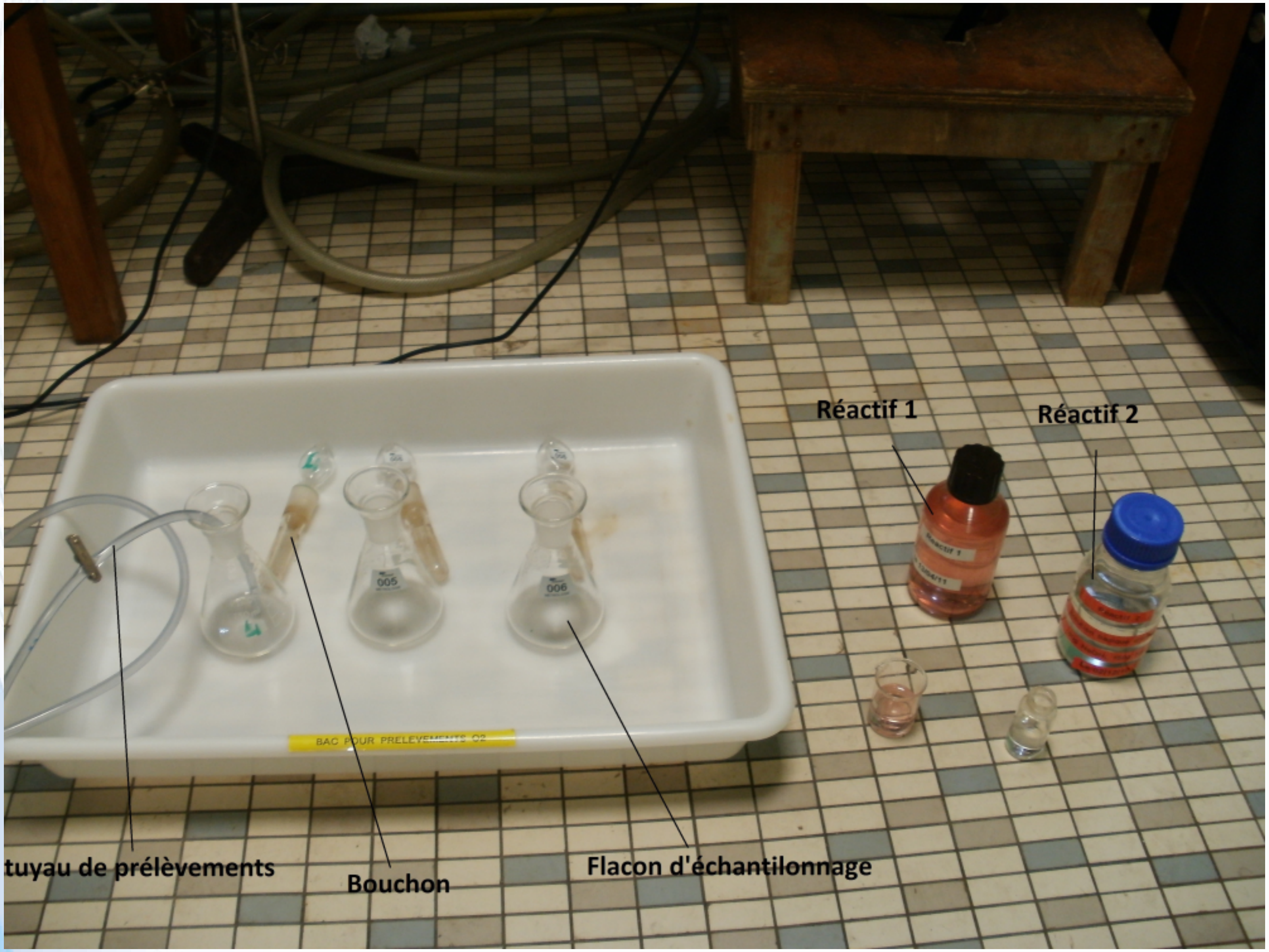
- Reagent 1: $MnCl_2$

- Reagent 2: NaOH / NaI

- Reagent 3: H_2SO_4

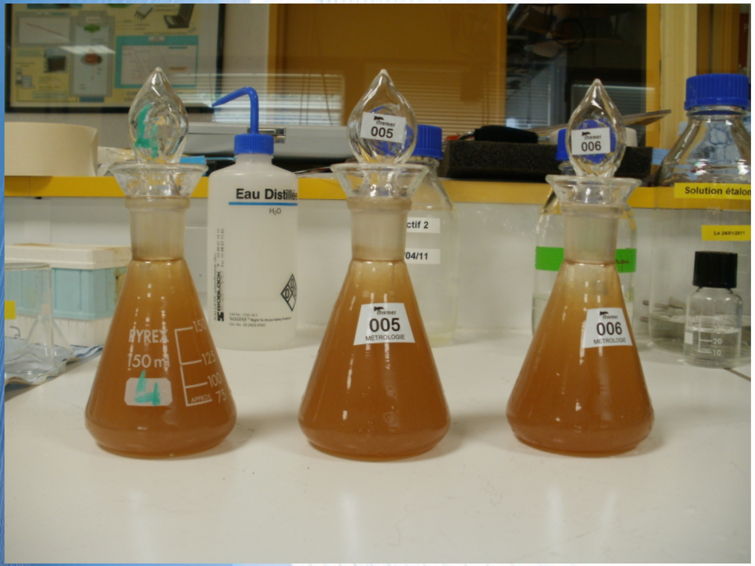
Winkler Analysis

- **Winkler analysis: Sampling**



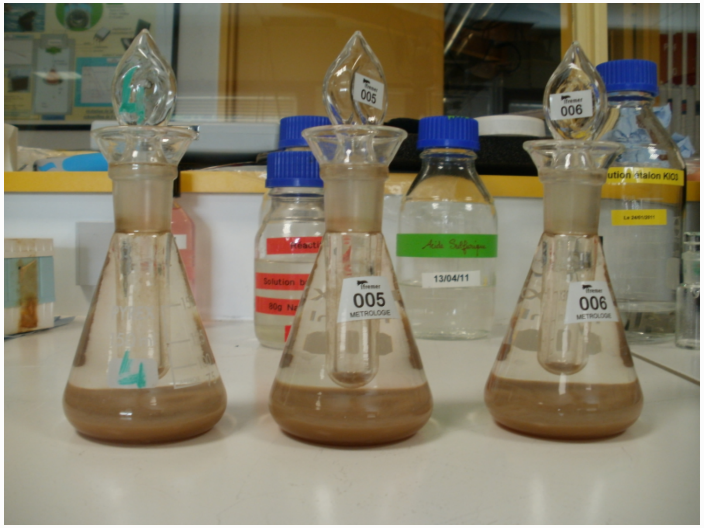
Winkler Analysis

- **Winkler analysis: Sampling**



Just after sampling …

… a few minutes later



Winkler Analysis

- **Winkler analysis: Waiting and storage…**

… Still many practices

- Waiting time:

4 hours before analysis

immediate analysis

…

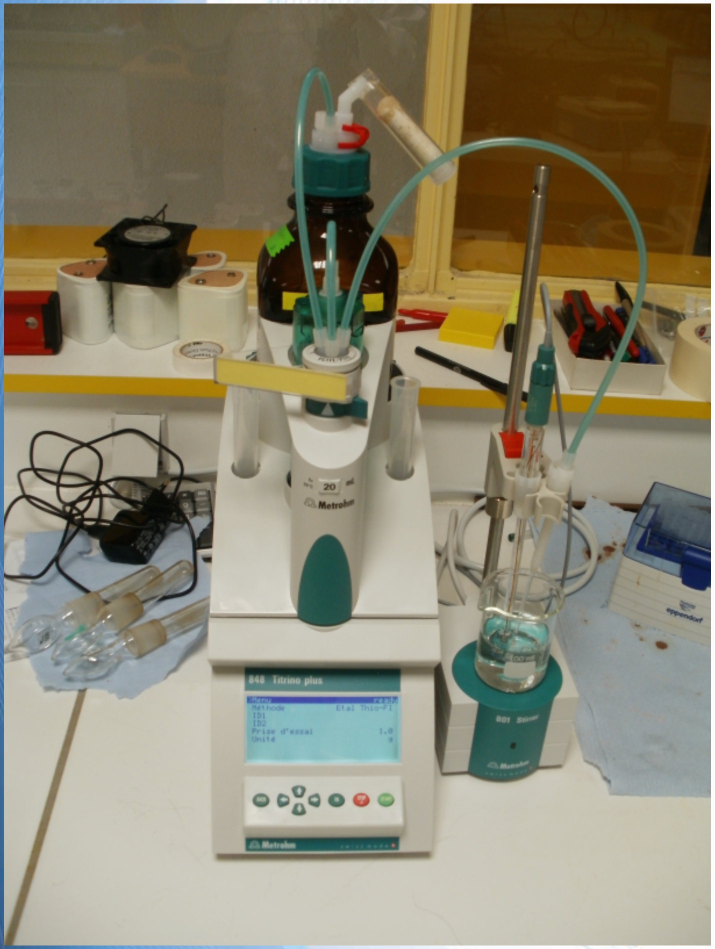
- Storage:

condition: distilled water around the stopper

time: only one day / several days

Winkler Analysis

- **Winkler analysis**



Metrohm titrino plus 848

Gilson pipetman pipette

Blanks !

Winkler Analysis

- **Winkler analysis**

$$[O_2]\mu mol/l = \left[\frac{n_i V_i}{(VT_{étal} - B_{étal})} \times \frac{(VT_{éch} - B_{éch})}{1000} \times \frac{1000000}{4} \times \frac{1000}{V_{éch}} \times \frac{V_{flac}}{(V_{flac} - V_{réac})}\right] - 38 \times \frac{V_{réac}}{V_{flac}}$$

Vflac : volume du flacon d'échantillonnage,

Vréac : volume total des réactifs introduits dans le flacon*,

Véch : volume d'échantillon titré (≤Vflac),

VTéch : volume de thiosulfate utilisé pour titrer l'échantillon,

VTétal : volume de thiosulfate utilisé pour l'étalonnage,

Béch : volume de thiosulfate correspondant au blanc des réactifs pour l'échantillon,

Bétal : volume de thiosulfate correspondant au blanc des réactifs pour l'étalonnage,

Vi : volume d'iodate ayant servi à l'étalonnage,

ni : normalité de l'iodate,

O2réac : oxygène ajouté par les réactifs 1 et 2.

Winkler Analysis

- **Winkler analysis: results**

CALCULS POUR LES DOSAGES WINKLER

D'après l'ouvrage hydrologie des écosystèmes marins paramètres et analyses d'Alain Aminot et Roger Kérouel édition de 2004

Acquisition du thiosulfate	
Etalonnage du thio ml	
1	4,963
2	4,961
3	4,962
4	4,968
5	4,953
Moyenne	4,961

Volume dosage thiosulfate

Numéros flacons permeettant de connaître le volume exact des flacons

Acquisition du thio échantillonnage		Acquisition temp, salinité	
N° Flacon	Vthio echant. (ml)	T°	Sal
4	8,685	20,0	0
5	8,747	20,0	0
6	8,930	20,0	0

Volume dosage flacons

Paramètres influençant la mesure

Résultats thio échantillonnage			Résultats de t et S		Résultats complémentaires			
Flacon	O2mg/l	O2 ml/l	température °C	Salinité	O2 Sat ml/l	O2 Sat mg/l	O2 Sat µmol/l	% sat
flacon 4	9,00	6,30	20,0	0,0	6,36	9,09	284,15	98,9
flacon 5	9,00	6,30	20,0	0,0	6,36	9,09	284,15	99,0
flacon 6	9,00	6,30	20,0	0,0	6,36	9,09	284,15	99,0

Résultats concentration flacons

Concentration de saturation en plusieurs unités

Résultats moyennés								
	O2mg/l	O2 ml/l	température °C	Salinité	O2 Sat ml/l	O2 Sat mg/l	O2 Sat µmol/l	% sat
moyenne	9,00	6,30	20,00	0,00	6,36	9,09	284,15	98,97

Constantes en ml		
V thio étalonnage	4,96	ml
Volume retiré	0,00	ml
Volume de 1 réac	1,00	ml
Blanc d'étalonnage	0,04	ml
Blanc d'échantillon	0,04	ml

Blancs chimiques

statistiques acquisition du thiosulfate	
moyenne	4,961
ecart type	0,005
mini	4,953
maxi	4,968
maxi-mini	0,015

statistiques acquisition O2 (mg/l et ml/l)	
moyenne O2 (mg/l)	9,00
ecart type O2 (mg/l)	0,003
moyenne O2 (ml/l)	6,30
ecart type O2 (ml/l)	0,002

Winkler Analysis

Ifremer

• Winkler analysis: uncertainty (GUM)

Fiche de synthèse de l'incertitude sur la mesure Winkler

Equipement de référence

N° Flacon :	14	Volume du flacon (mL) :	159.326059	V Thio échantillon (mL) :	9.980

Détermination de U(O2 par Winkler) en µmol/L

Détermination de U(Normalité du KIO3)

Y = f(X_i) : modelisation du processus de mesure		Incertitudede la composante $U(X_i)$		Provenance et infos sur la composante	Traitement de la composante	Coeff. diviseur de $U(X_i)$ k_i	Incertitude-type de la composante $u_i = U(X_i)/k_i$		Coeff. de sensibilité $\partial Y/\partial X_i$		Incertitude-type en "mesure du pont (X)" $u_j=(\partial Y/\partial X_i) \times u_i$ (sans dim)	Variance en "(mesure du pont)^2 (X^2)" u_j^2 (sans dim)
KIO3												
Puretée		1%		Puretée Produit	Loi normale	2	0.005	-	0.1		5.00E-04	2.50E-07
Balance de pesée												
Precision balance		1.00E-02	mg	Donnée constructeur	1 sigma	1	1.00E-02	mg	2.804E-05		2.80E-07	7.86E-14
Linearité balance	(de ... à ...°C)	1.00E-01	mg	Donnée constructeur	1 sigma	1	1.00E-01	mg	2.804E-05		2.80E-06	7.86E-12
Repetabilité balance		3.00E-02	mg	Donnée constructeur	1 sigma	1	3.00E-02	mg	2.804E-05		8.41E-07	7.07E-13
Etalonnage balance		Sans objet	-	Donnée d'étalonnage	Loi normale	2	-	-	-	-	-	-
Dilution KIO3												
Classe fiole		4.00E-04	L	Classe A	Loi rectangle	2	2.31E-04	L	0.1		2.31E-05	5.33E-10

Incertitude élargie calculée U(Normalité du KIO3) (= k u_c) **U = +/- 1.00E-03** avec **k = 2** ($u_c^2=\Sigma(u_j^2)$)

k = facteur d'élargissement

Arrondi de U(Normalité KIO3) : -

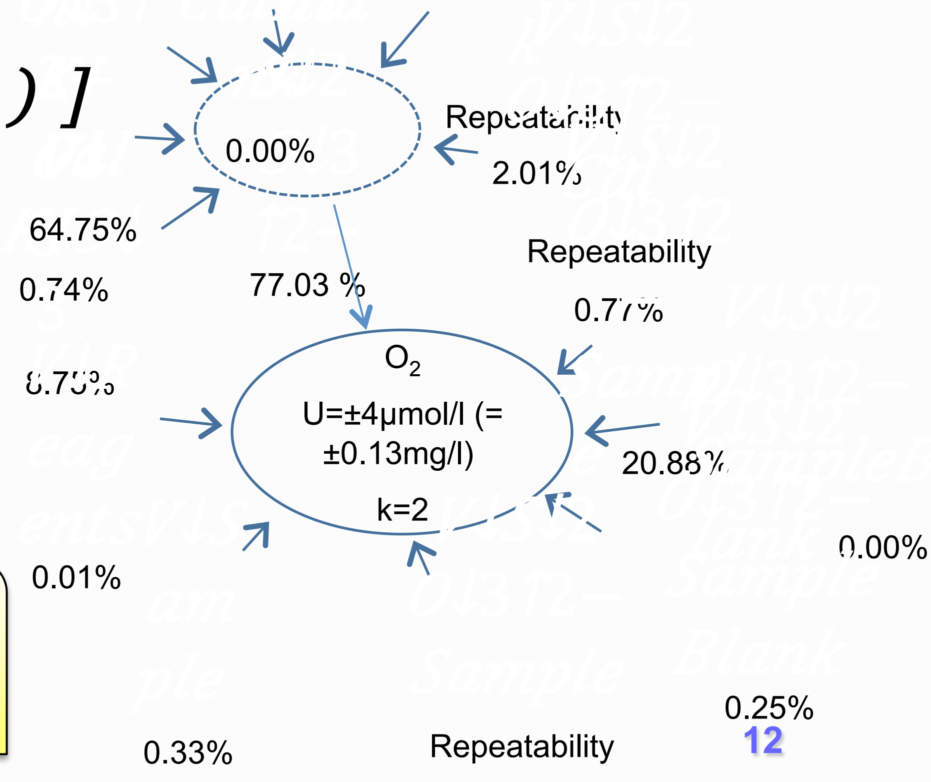
U = +/- 3.70 µmol/L

Winkler Analysis

- **Winkler analysis: uncertainty**

U (Winkler) = 4 µmol/l at 440 µmol/l (0.13mg/l at 14 mg/l)

[O↓2] µmol/l=[■n↓KIO↓3 V↓KIO↓3 /
(V↓S↓2 O↓3↑2− Cal −V↓S↓2 O↓3↑2−
CalBlank)×(V↓S↓2 O↓3↑2− Sample −
V↓S↓2 O↓3↑2− SampleBlank)/4 ×
1000000/(V↓Sample V↓Reagents)]
−38×V↓Rea... ...mple



Gravimetric Winkler: U/2
I.Helm, L.Jalukse, I. Leito, Anal. Chim. Acta. 741 (2012) 21-31.

Dissolved oxygen calibration:

Issues still to be solved:

- **Substance matrix: seawater**

(Winkler's method overestimates dissolved oxygen in seawater: Iodate interference and its oceanographic implication. George T.F. Wong and Kuo-Yuan Li, Marine Chemistry, 2009, vol.115, n°1-2, pp.86,91)

- **Lack of understanding of optical sensors (optodes) behaviour (interferences, corrosion issues, interactions with some materials, …)**

1 – Winkler Analysis

2 – Inter Laboratory Comparisons

Inter Laboratory Comparisons

Aim:

- ✓ Control Winkler sampling protocols on boat
- ✓ Control Winkler analysis methods

Free ILC:

- Annual and local ILC: Ifremer (2 laboratories), IRD, CNRS

! Unformal ILC (no ILC accreditation, uncertainty, statistical analysis, …)

Metrology European projects (EMPIR):

- 1 ILC in the framework of the ENV05 project

Inter Laboratory Comparisons

Free ILC:

- **Annual and local ILC: Ifremer (2 laboratories), IRD, CNRS**

Inter Laboratory Comparisons

Free ILC:

Labo Analyse : LPO				[O2]	Labo Analyse : METROLOGIE				[O2]
Niskin	Temp Echant	N° Flacon	Prélevé par :		Niskin	Temp Echant	N° Flacon	Prélevé par :	
Niskin 1	9.4	321	LPO	6.328	Niskin 1		16	Métrologie	6.299
Niskin 1	9.4	324	LPO	6.357	Niskin 1		17	Métrologie	6.295
Niskin 1	9.4	325	LPO	6.361	Niskin 1		18	Métrologie	6.305
				6.349					6.300
				0.018					0.005
Niskin 2		283	SBR	6.362	Niskin 3		287	SBR	6.332
Niskin 2		285	SBR	6.111	Niskin 3		288	SBR	6.326
Niskin 2		286	SBR	6.069	Niskin 3		289	SBR	6.33
				6.181					6.329
				0.158					0.003
Niskin 3		2	Métrologie	6.329	Niskin 3	9.4	339	LPO	6.345
Niskin 3		3	Métrologie	6.332	Niskin 3	9.4	340	LPO	6.339
Niskin 3		10	Métrologie	6.331	Niskin 3	9.4	346	LPO	6.354
				6.331					6.346
				0.002					0.008
Niskin 3	9.5	181	IRD	6.344	Niskin 4	9.5	184	IRD	6.295
Niskin 3	9.5	182	IRD	6.338	Niskin 4	9.5	185	IRD	6.31
Niskin 3	9.5	183	IRD	6.541	Niskin 4	9.5	186	IRD	6.302
				6.408					6.302
				0.116					0.008

Inter Laboratory Comparisons

EMRP ILC: (ENV05 0cean project: Metrology for ocean salinity and acidity)

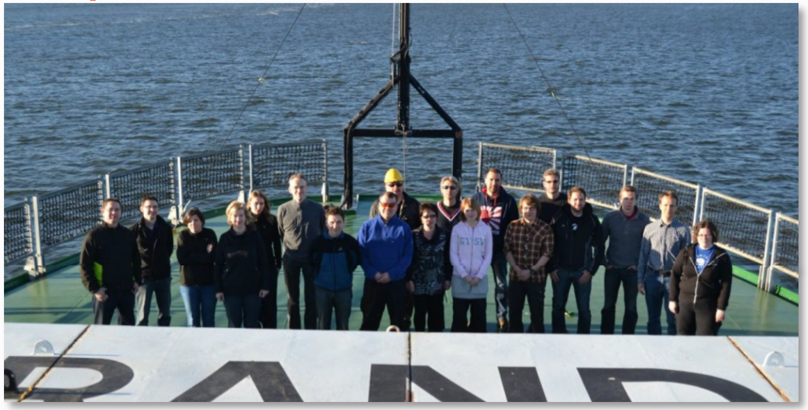
- **Held the 23/04/2014 by SYKE (finnish NMI) and University of Tartu (Estonia).**
- **21 participants from 10 institutes:**
  - **5 winkler (with 1 analyzed in lab) + reference winkler**
  - **18 dissolved oxygen sensors**

Field measurements of dissolved oxygen concentration

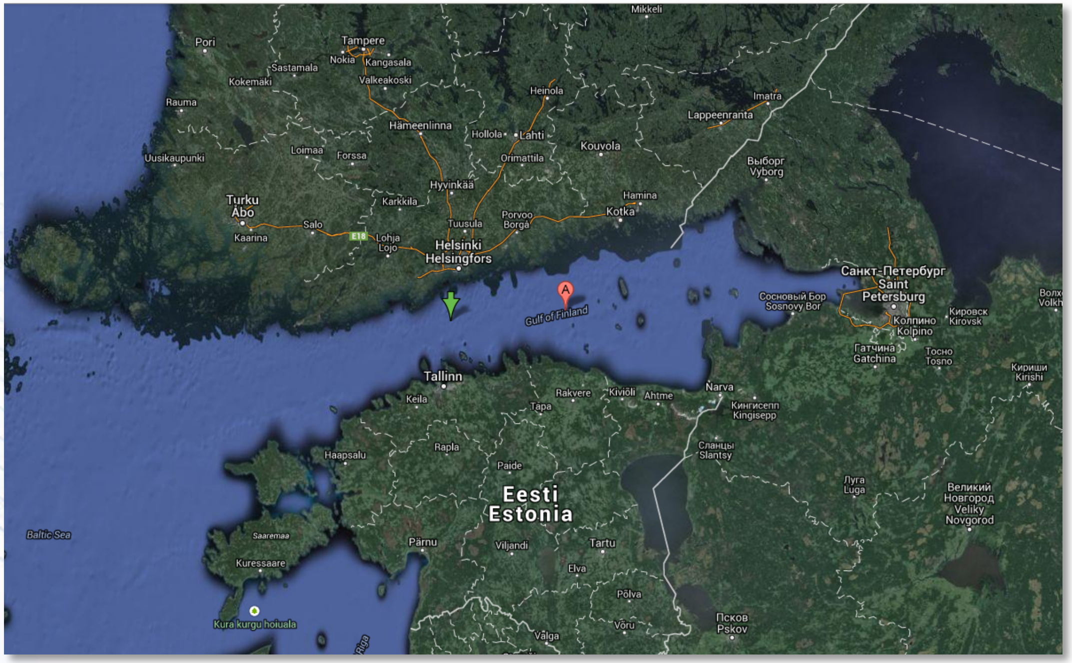
Mirja Leivuori, Teemu Näykki, Ivo Leito, Irja Helm, Lauri Jalukse, Lari Kaukonen, Panu Hänninen and Markku Ilmakunnas

Inter Laboratory Comparisons

EMRP ILC: (ENV05 Ocean project)



The Aranda (1989, 59m)



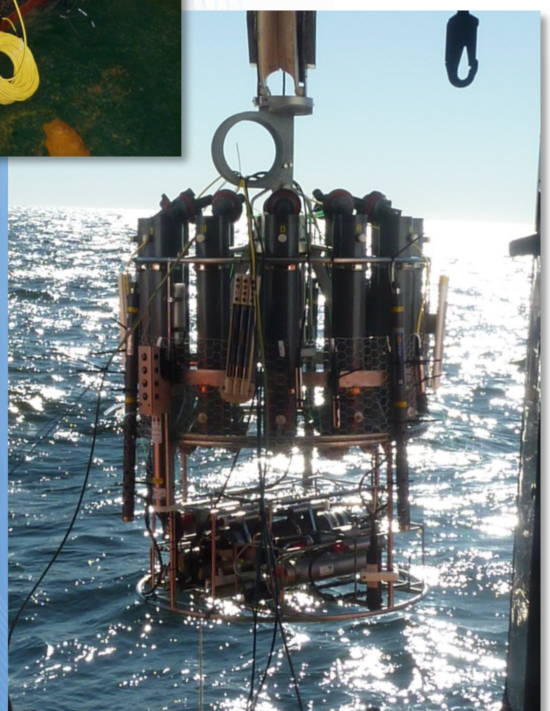
3 depth:

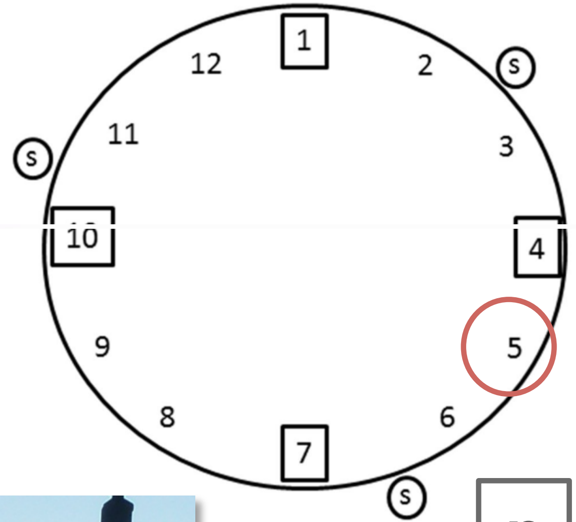
- 5m
- 23m
- 40m

Inter Laboratory Comparisons

Estonie	Estonian Marine Institute University of Tartu
Finlande	EHP-Tekniikka HSY Käyttölaboratorio Pitkäkoski Hyxo Oy SYKE Laboratory of Hakuninmaa, Helsinki SYKE Marine Research Centre, Helsinki SYKE Freshwater Centre, Oulu
France	IFREMER
Allemagne	Federal Maritime and Hydrographic Agency Germany
Suède	Sweden Stockholm University, Department of Ecology Environment and Plant Sciences Umeå Marine Sciences Centre

Inter Laboratory Comparisons





n Reference Winkler

s Sensors for homogeneity

3 winkler samples for each lab and depth

Inter Laboratory Comparisons

Swedish Winkler

Finnish Winkler

German Winkler

Ifremer Winkler

Inter Laboratory Comparisons

Reference Winkler

Depth (m)	Rosette no	C_{O2_Wink} [mg/l]	u_c (C_{O2_Wink}) [mg/l]	Number of values obtained	u_c ($C_{O2\_Wink\_averaged}$) [mg/l]	*u* (between vessels) [mg/l]	$u_c(C_{O2})$ [mg/l]	Effective degrees of freedom	Assigned value, C_{O2} [mg/l]	*k* (95% coverage probability)	$U(C_{O2})$ [mg/l]
5	all averaged	14.932		17	0.051	0.101	0.11	4.7	14.93	2.78	0.31
	1	14.875	0.047	4							
	4	14.966	0.040	4							
	7	14.843	0.075	4							
	10	15.067	0.039	5							
23	all averaged	13.794		16	0.045	0.033	0.06	16.9	13.79	2.12	0.12
	1	13.825	0.038	4							
	4	13.748	0.049	4							
	7	13.793	0.039	4							
	10	13.811	0.054	4							
40	all averaged	13.631		14	0.054	0.063	0.08	8.4	13.63	2.31	0.19
	1	13.533	0.089	3							
	4	13.679	0.038	4							
	7	13.648	0.044	3							
	10	13.640	0.043	4							

Inter Laboratory Comparisons

Ifremer Winkler

Laboratory 20												
Analyte	Unit	Sample	-3 0 3	z score	Assigned value	2^*s_p, %	Lab's result	Md	Mean	SD	SD%	n (stat)
O_2	mg/l	D1_05		-0.276	14.93 ± 0.31	8	14.77	14.90	14.90	0.3	2.1	22
	mg/l	D2_23		-0.027	13.79 ± 0.12	8	13.78	13.70	13.79	0.5	3.3	20
	mg/l	D3_40		0.073	13.63 ± 0.19	8	13.67	13.63	13.70	0.5	3.6	17

Other labs Winkler

Laboratory 11												
Analyte	Unit	Sample	-3 0 3	z score	Assigned value	2^*s_p, %	Lab's result	Md	Mean	SD	SD%	n (stat)
O_2	mg/l	D1_05		0.368	14.93	8	15.15	14.90	14.90	0.3	2.1	22
	mg/l	D2_23		0.208	13.79	8	13.91	13.70	13.79	0.5	3.3	20
	mg/l	D3_40		0.495	13.63	8	13.90	13.63	13.70	0.5	3.6	17

Laboratory 14												
Analyte	Unit	Sample	-3 0 3	z score	Assigned value	2^*s_p, %	Lab's result	Md	Mean	SD	SD%	n (stat)
O_2	mg/l	D1_05		0.100	14.93	8	14.99	14.90	14.90	0.3	2.1	22
	mg/l	D2_23		0.354	13.79	8	13.99	13.70	13.79	0.5	3.3	20
	mg/l	D3_40		0.293	13.63	8	13.79	13.63	13.70	0.5	3.6	17

Laboratory 24												
Analyte	Unit	Sample	-3 0 3	z score	Assigned value	2^*s_p, %	Lab's result	Md	Mean	SD	SD%	n (stat)
O_2	mg/l	D1_05		-0.469	14.93	8	14.65	14.90	14.90	0.3	2.1	22
	mg/l	D2_23		-0.411	13.79	8	13.56	13.70	13.79	0.5	3.3	20
	mg/l	D3_40		-0.267	13.63	8	13.48	13.63	13.70	0.5	3.6	17

Thanks for your attention

« Cofrac - Calibration » Accreditation:

(since 1996)

Quality part:

- Quality system: ISO/IEC 17025

Technical part:

- T°: Range: -10°C to +60°C (14°F to 140°F)
  with U= +/- 4m°C to 13m°C

Fresh water or seawater bath

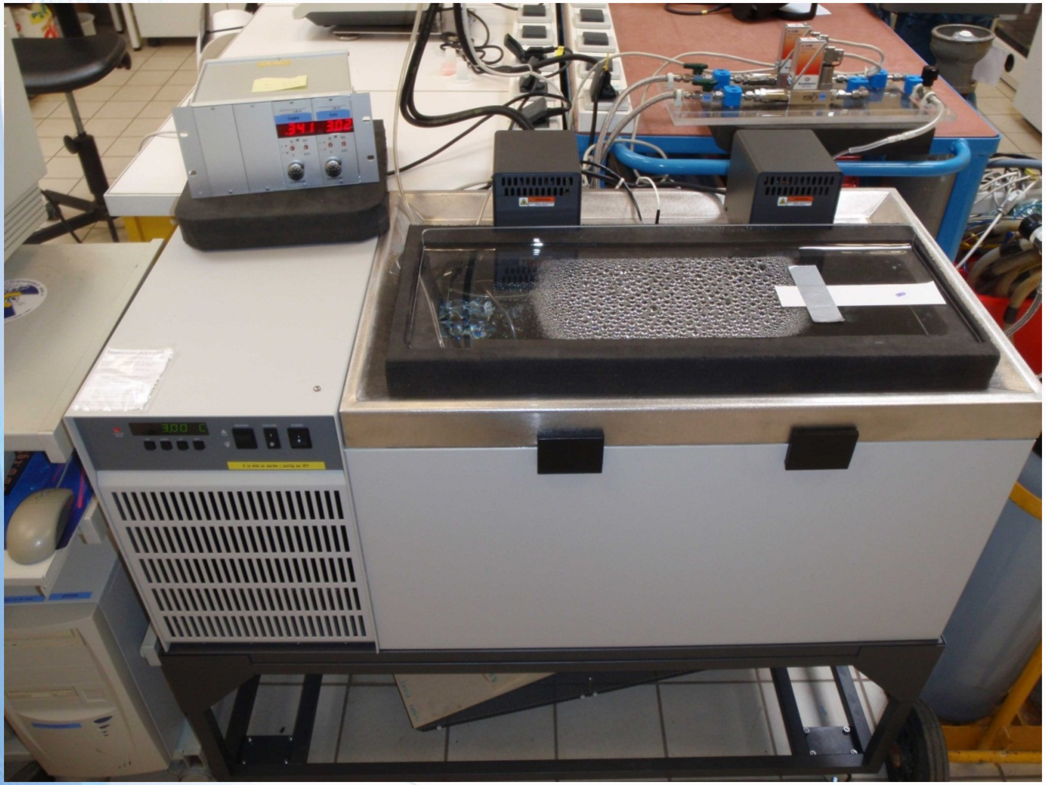
- Relative P: Range: 0.1 MPa to 80MPa
  with U # $1.10^{-4} \times$ Pr

➠ **Calibration activity: 250 sensors / year**

Pressure balance

Ifremer

Oxygen multi level calibration bench: description



Thermoregulated bath:

Fresh water or seawater

Temperature

Homogeneity: 0.005°C

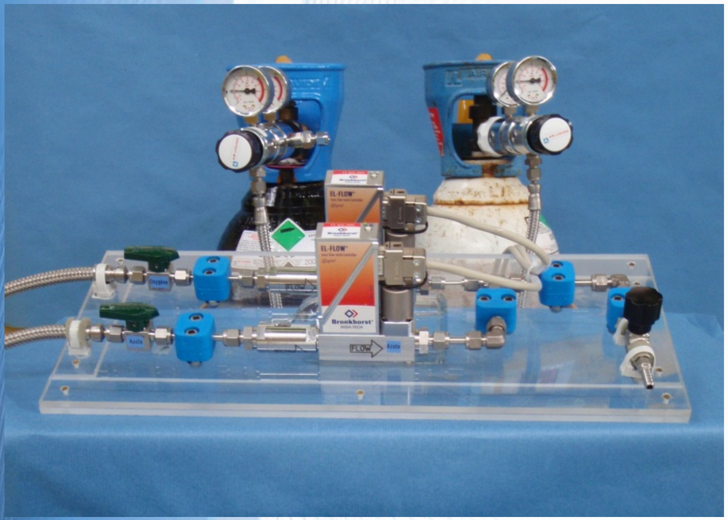
Stability: 0.001°C

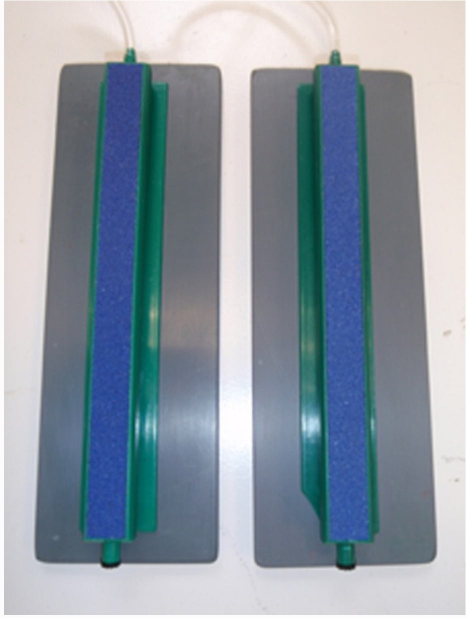
- work volume: 65 l

(H x W x D = 70 x 28 x 33 cm (27.5 x 11 x 13 in))

- initial range: from -10°C to 110°C
- using range: from -1.5°C to 40°C

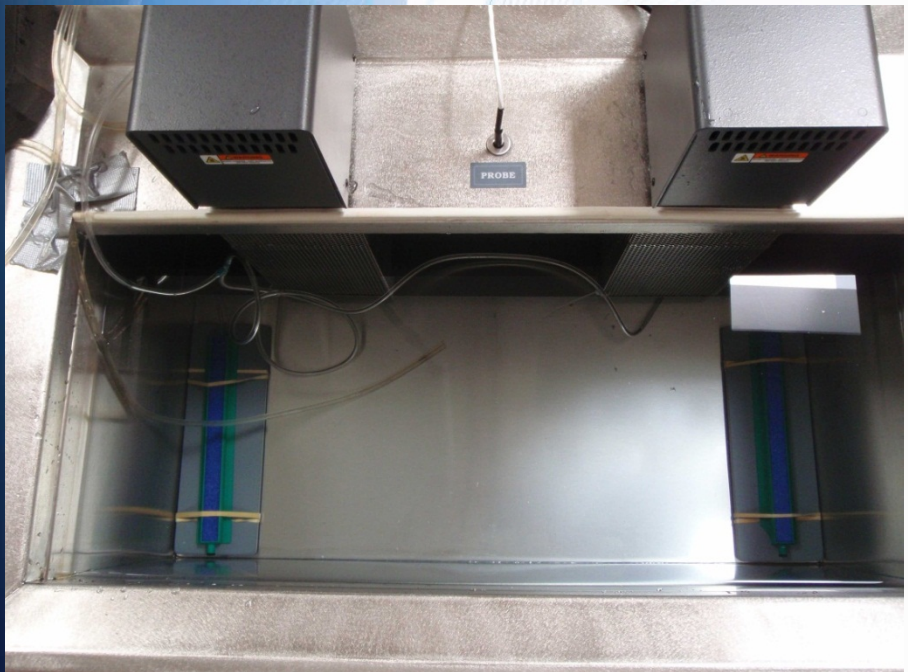
Bubbling system:

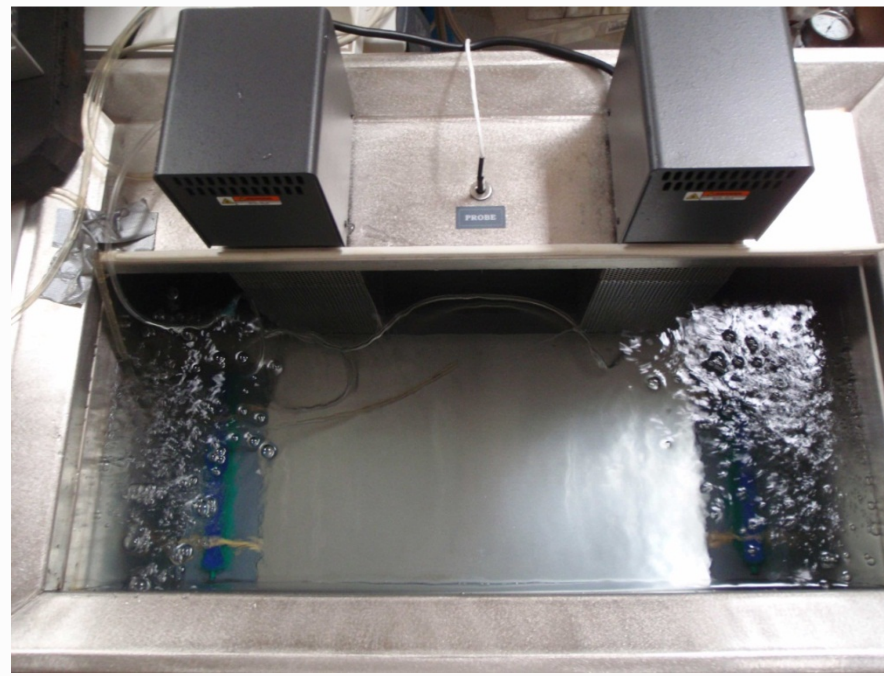




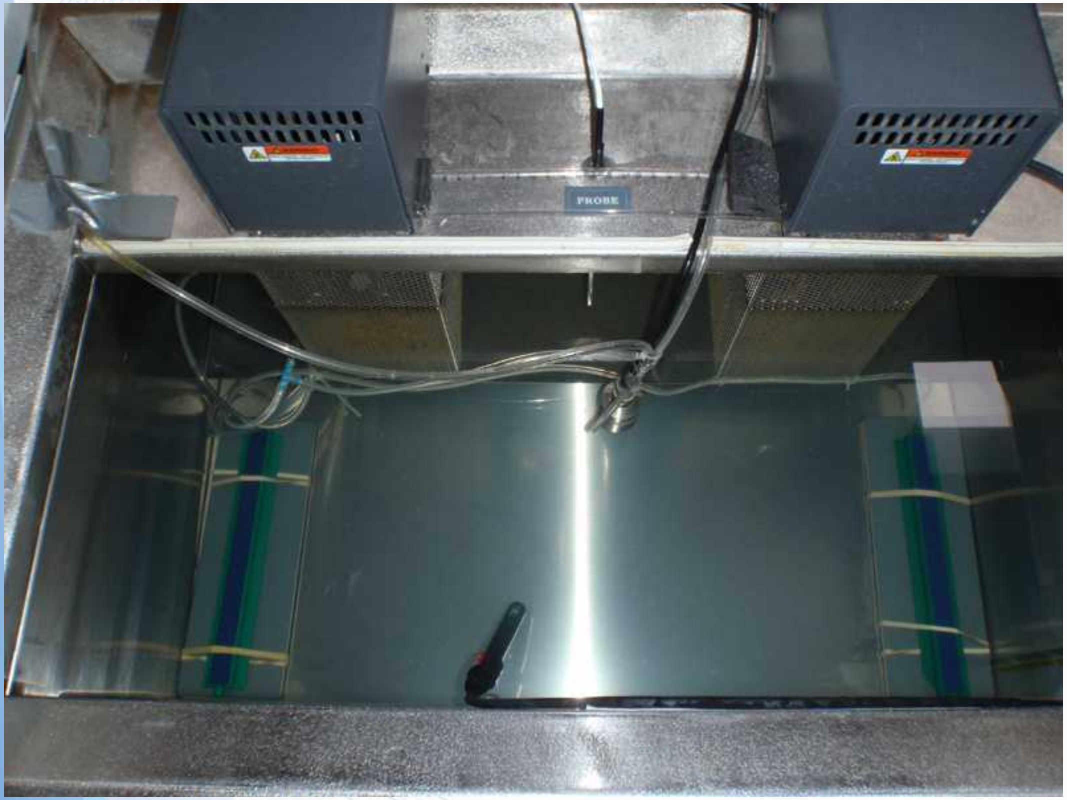
- O_2 and N_2 mass flow meter/controller
- aquarium air stones

Bubbling system:





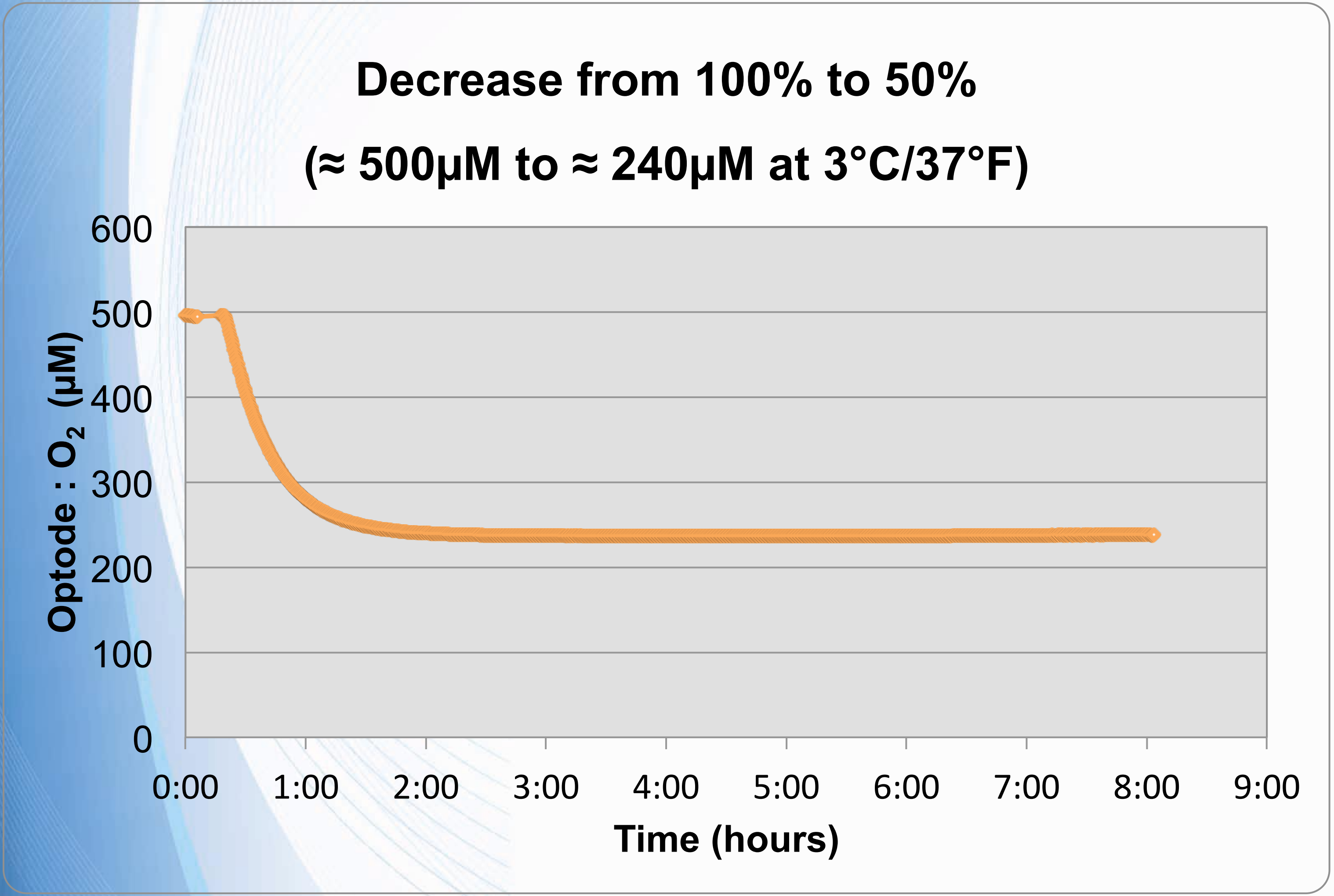
Bench characterization



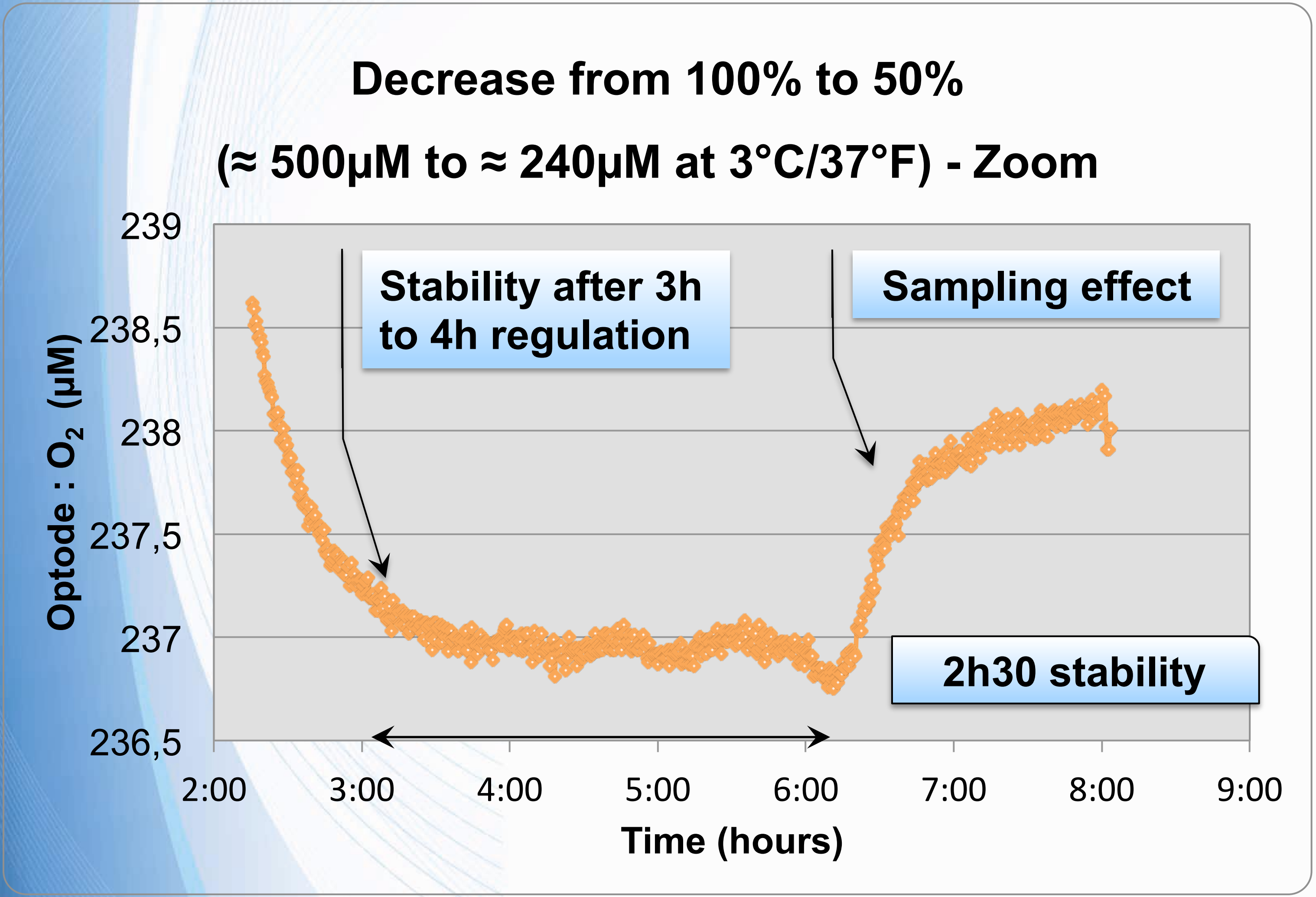
Bench characterization

- Decrease and stability analysis

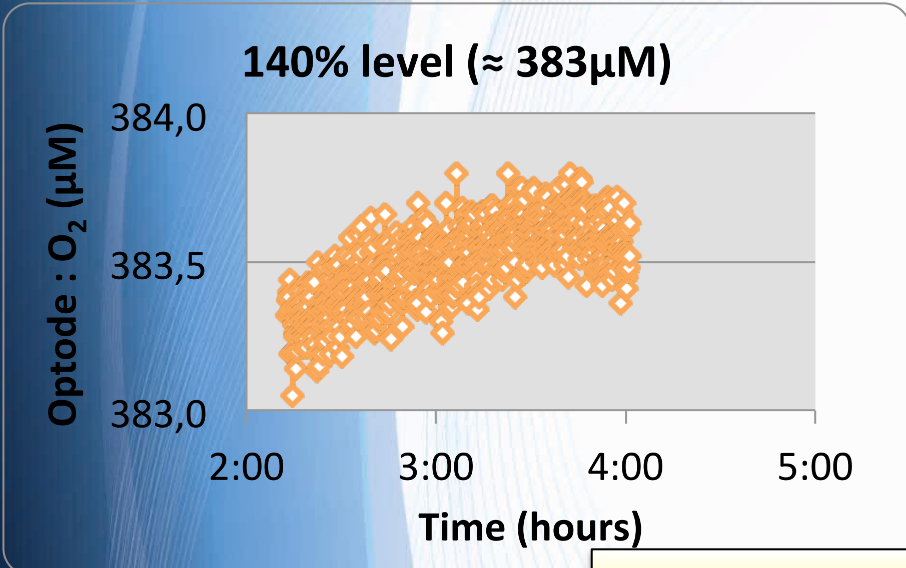
100% to 50% level (≈ 500µM to ≈ 240µM)

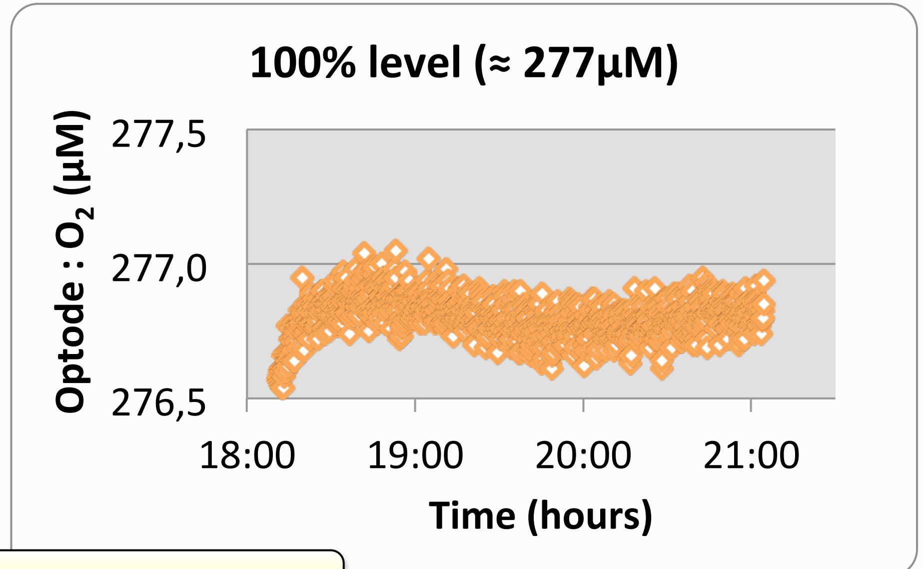


100% to 50% level (≈ 500µM to ≈ 240µM)

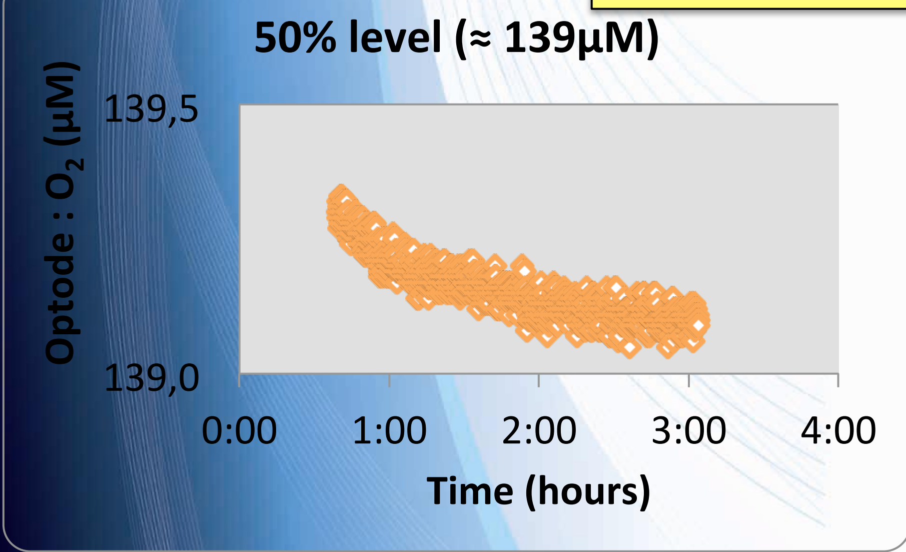


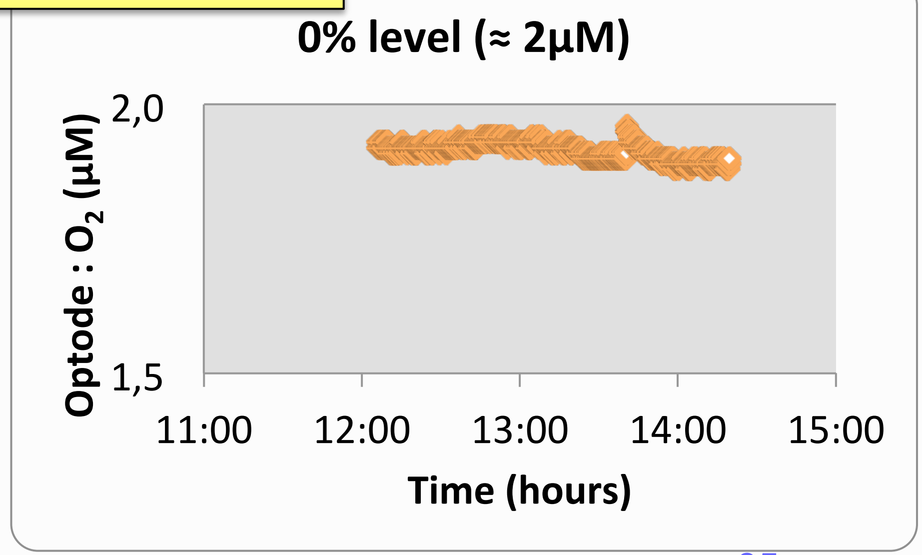
Stability overview





Stability < 0.5 µM within 1h

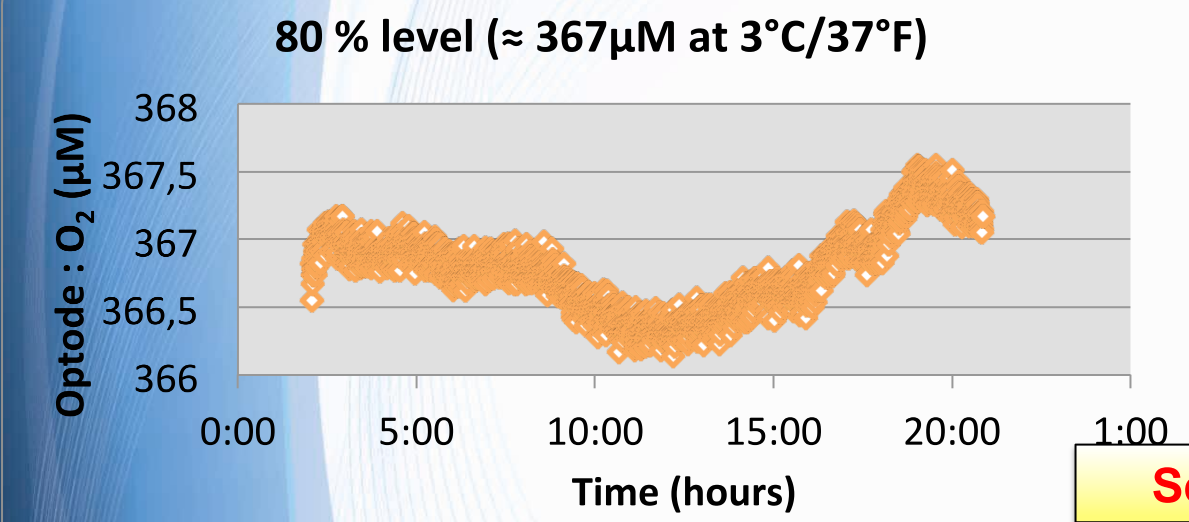




Bench characterization

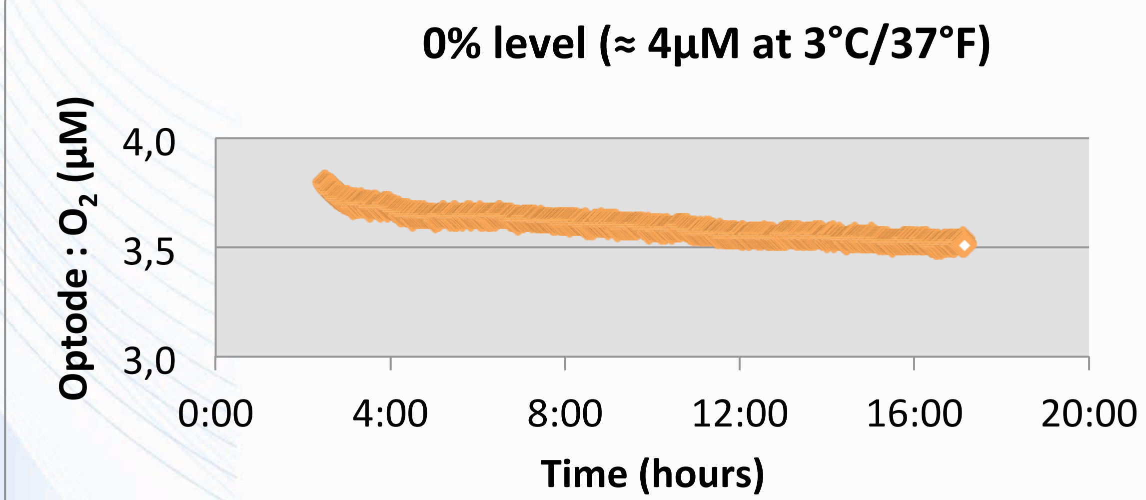
- Decrease and stability analysis
- Stability length

Lasting stability



19h00 stability

Several hours stability

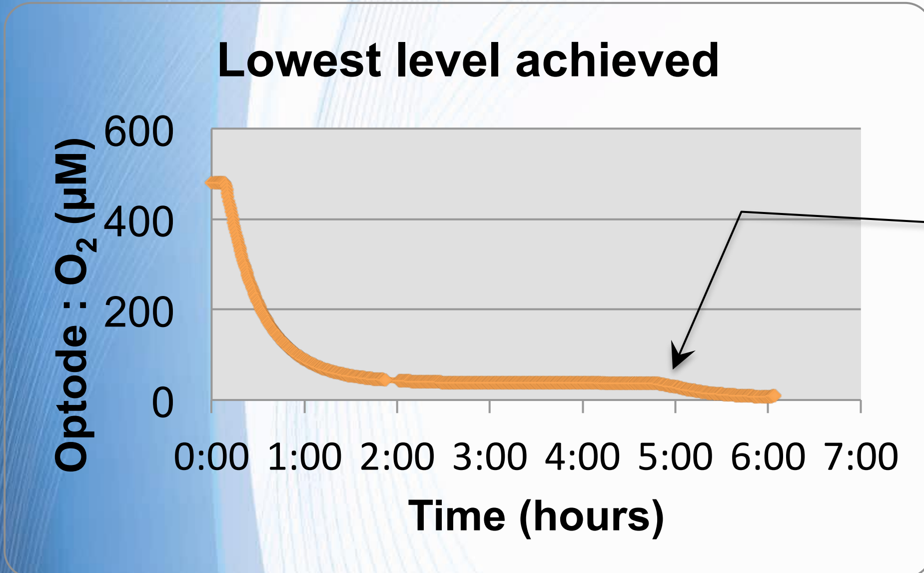


14h00 stability

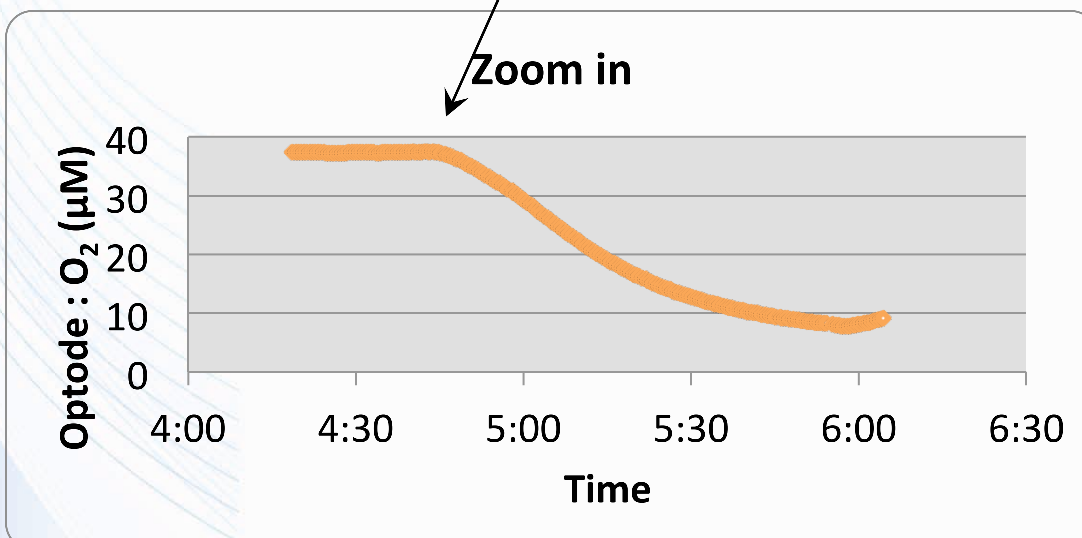
Bench characterization

- Decrease and stability analysis
- Stability length
- Lowest level

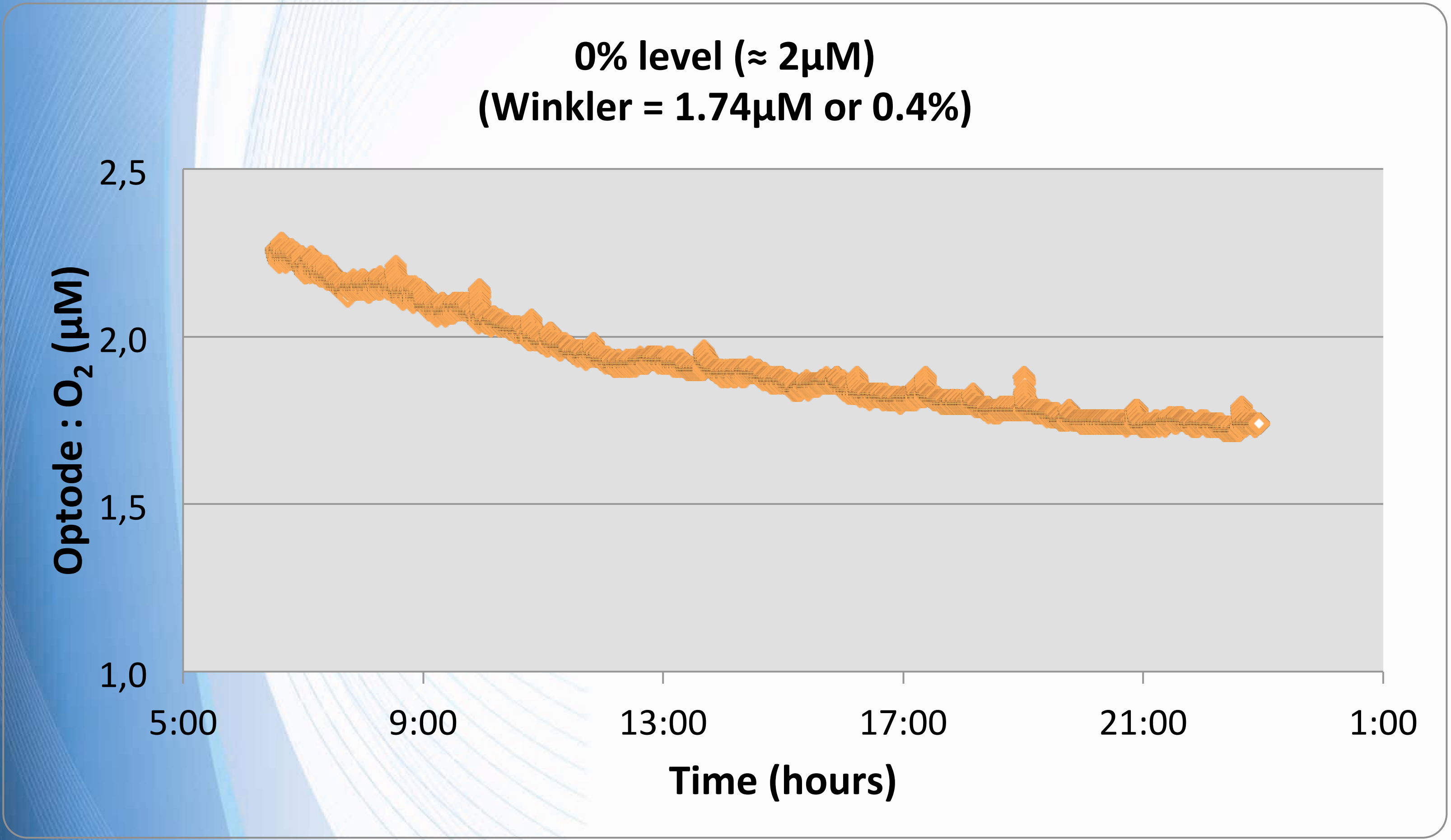
Lowest level



Airtightness improvement



Lowest level

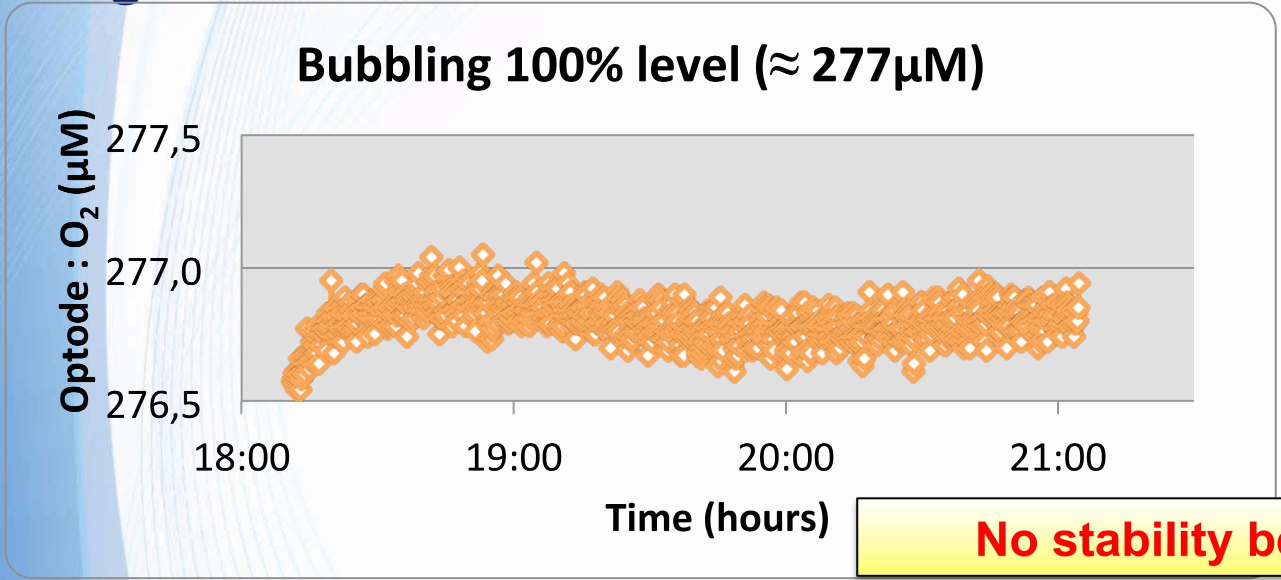


Low level ≈ 0µM or 0%

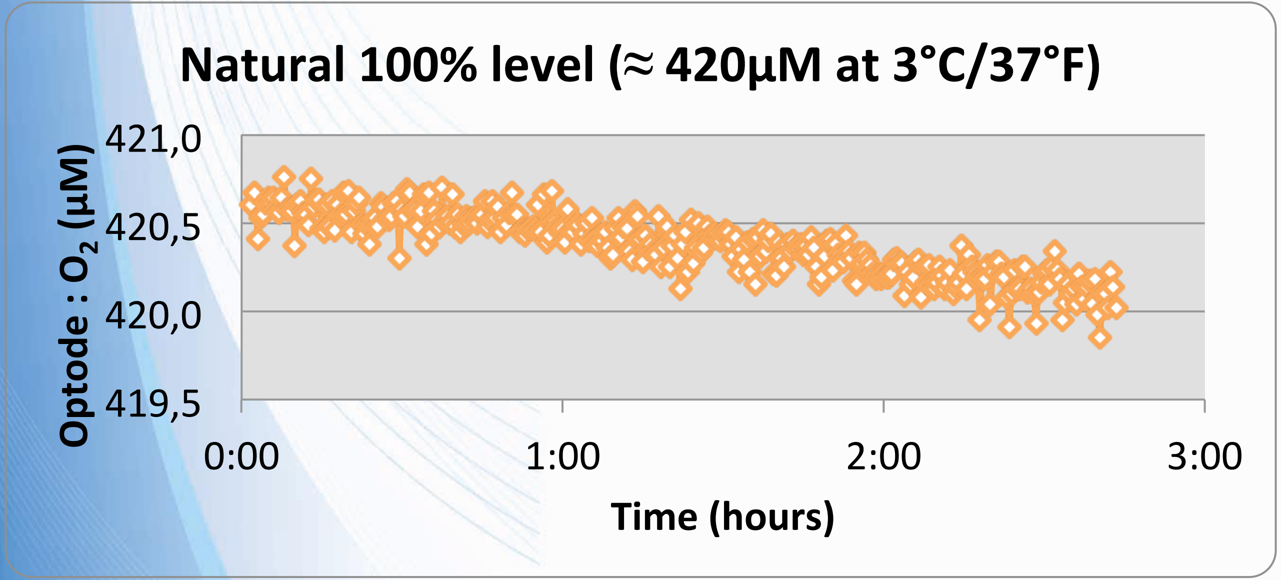
Bench characterization

- Decrease and stability analysis
- Stability length
- Lowest level
- Bubbling vs natural 100%

Bubbling vs natural 100%



No stability benefit

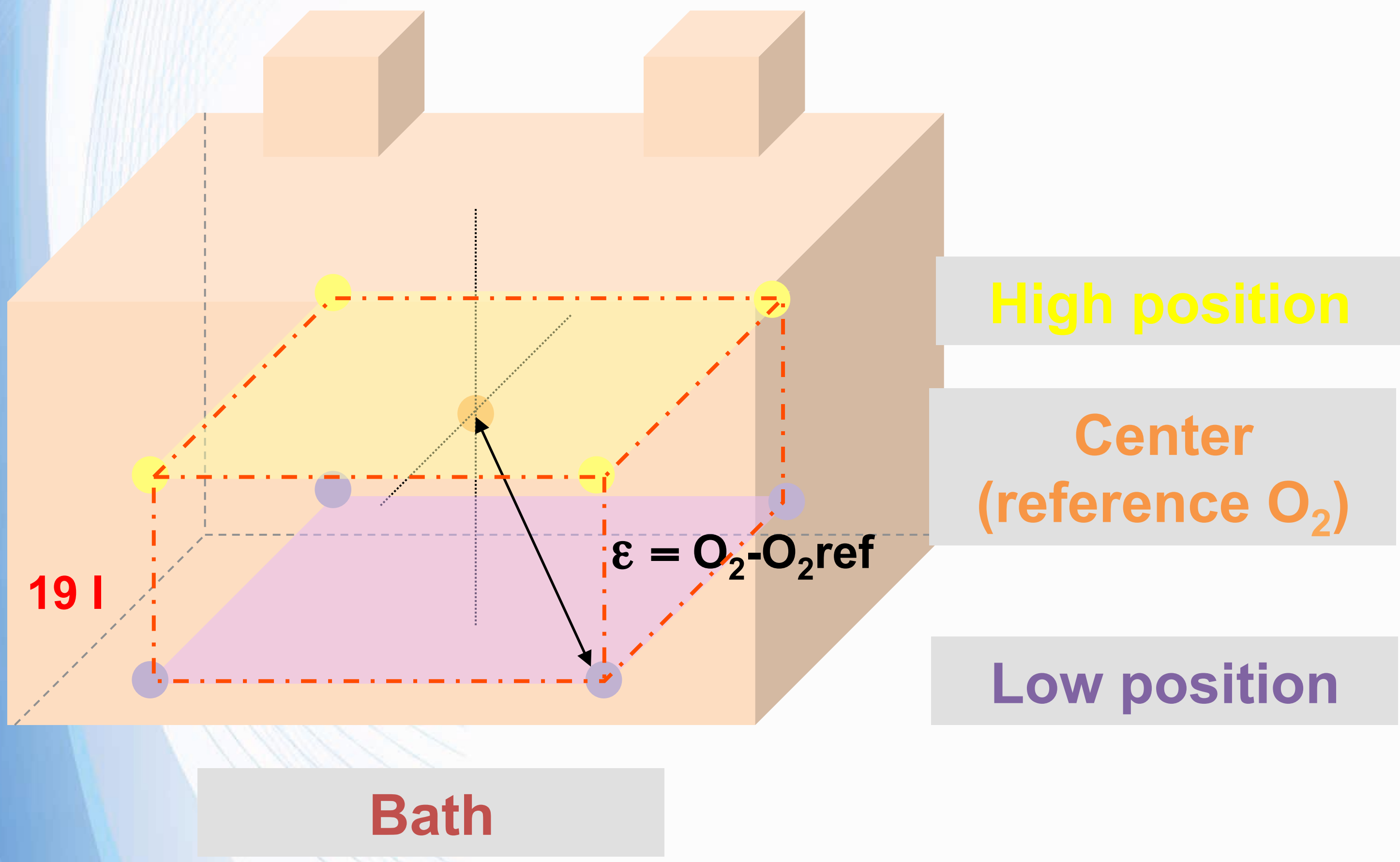


Bench characterization

- Decrease and stability analysis
- Stability length
- Lowest level
- Bubbling vs natural 100%
- 0_2 homogeneity

Protocol:

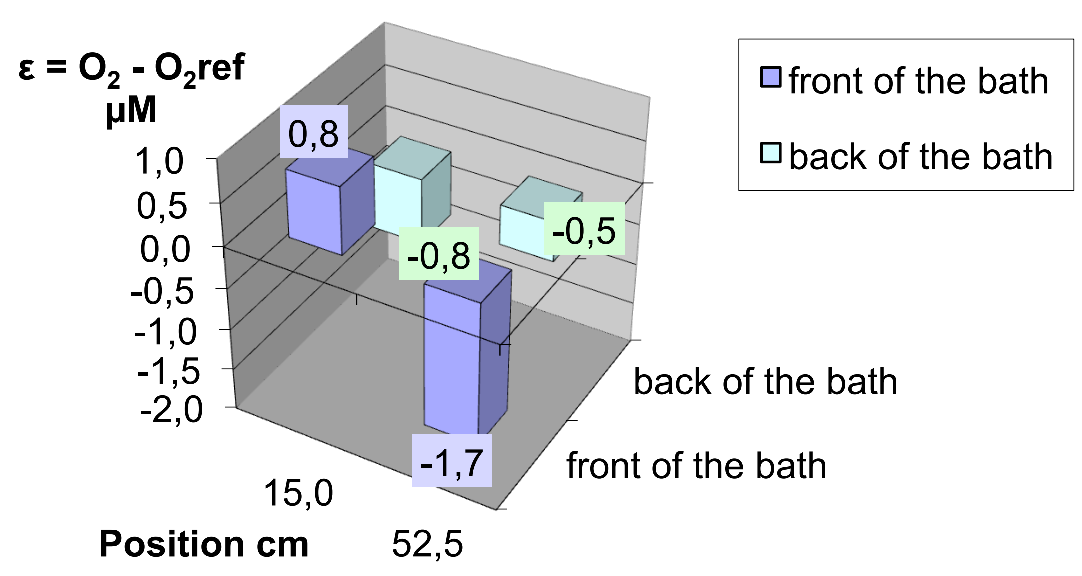
Winkler samples ○



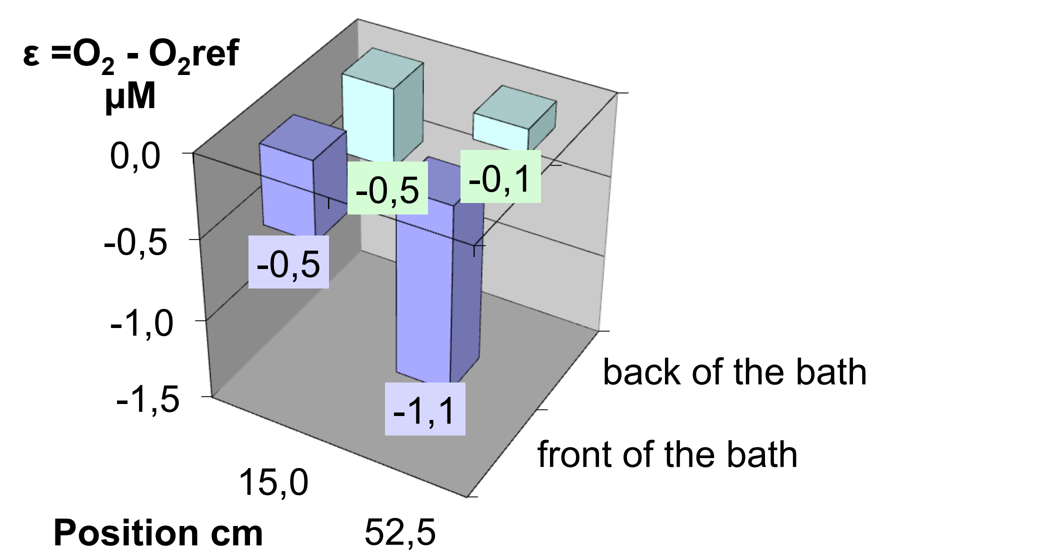
Results

10% level

High



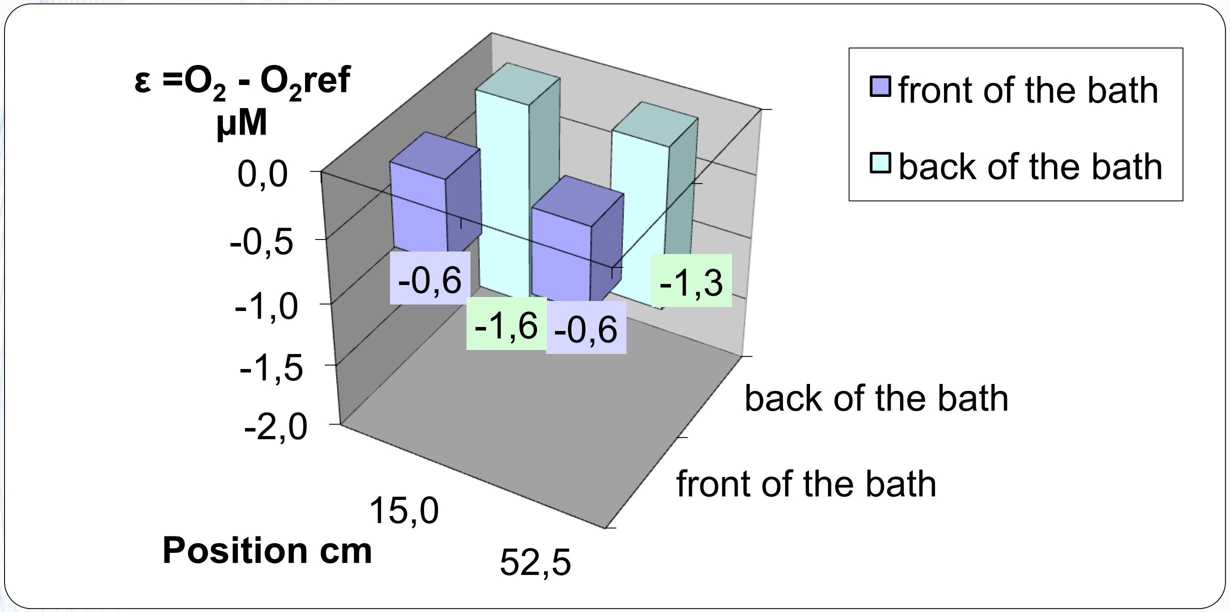
Low



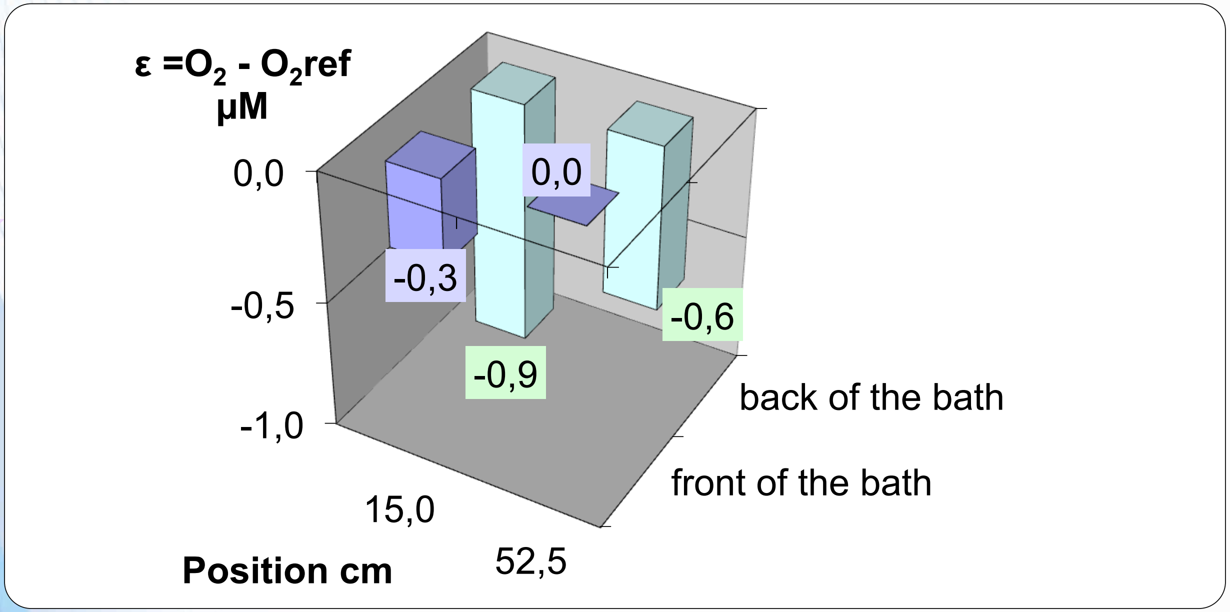
Results

50% level

High



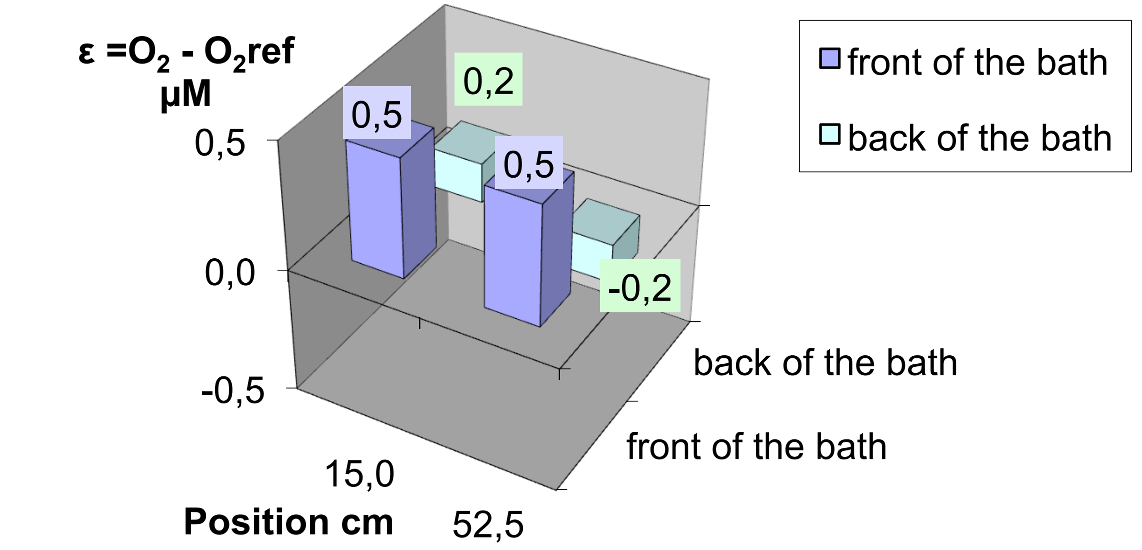
Low



Results

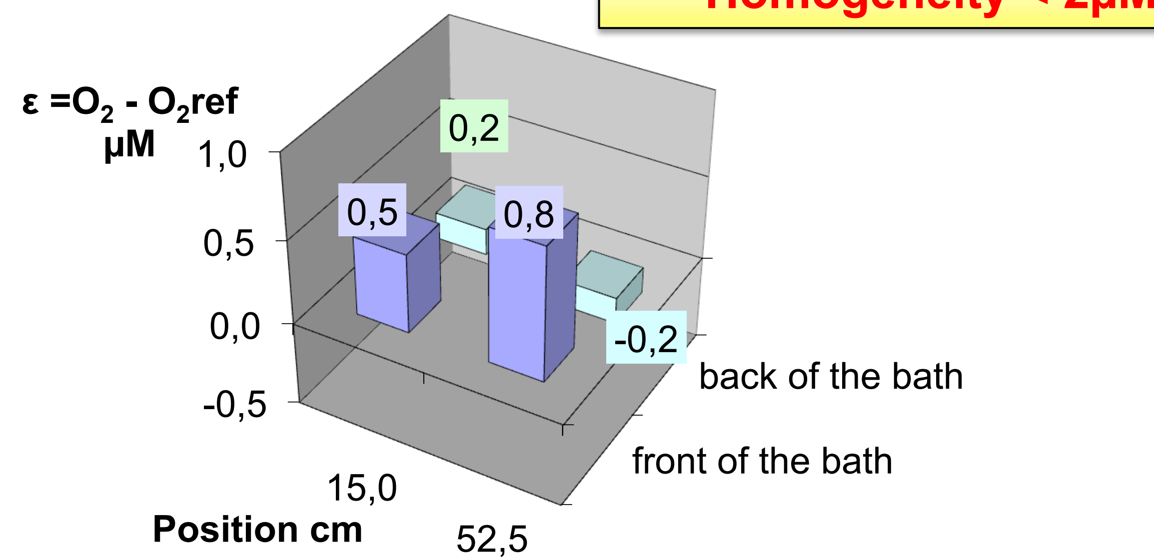
100% level

High



Homogeneity < 2µM

Low



Bench characterization

Conclusions

- Stability:
  - < 0.5 µM within 1 hour
  - long stability levels
- Lowest level: nearly 0%
- 0_2 homogeneity: < 2µM (! first results)
- Importance of airtightness

Bench characterization

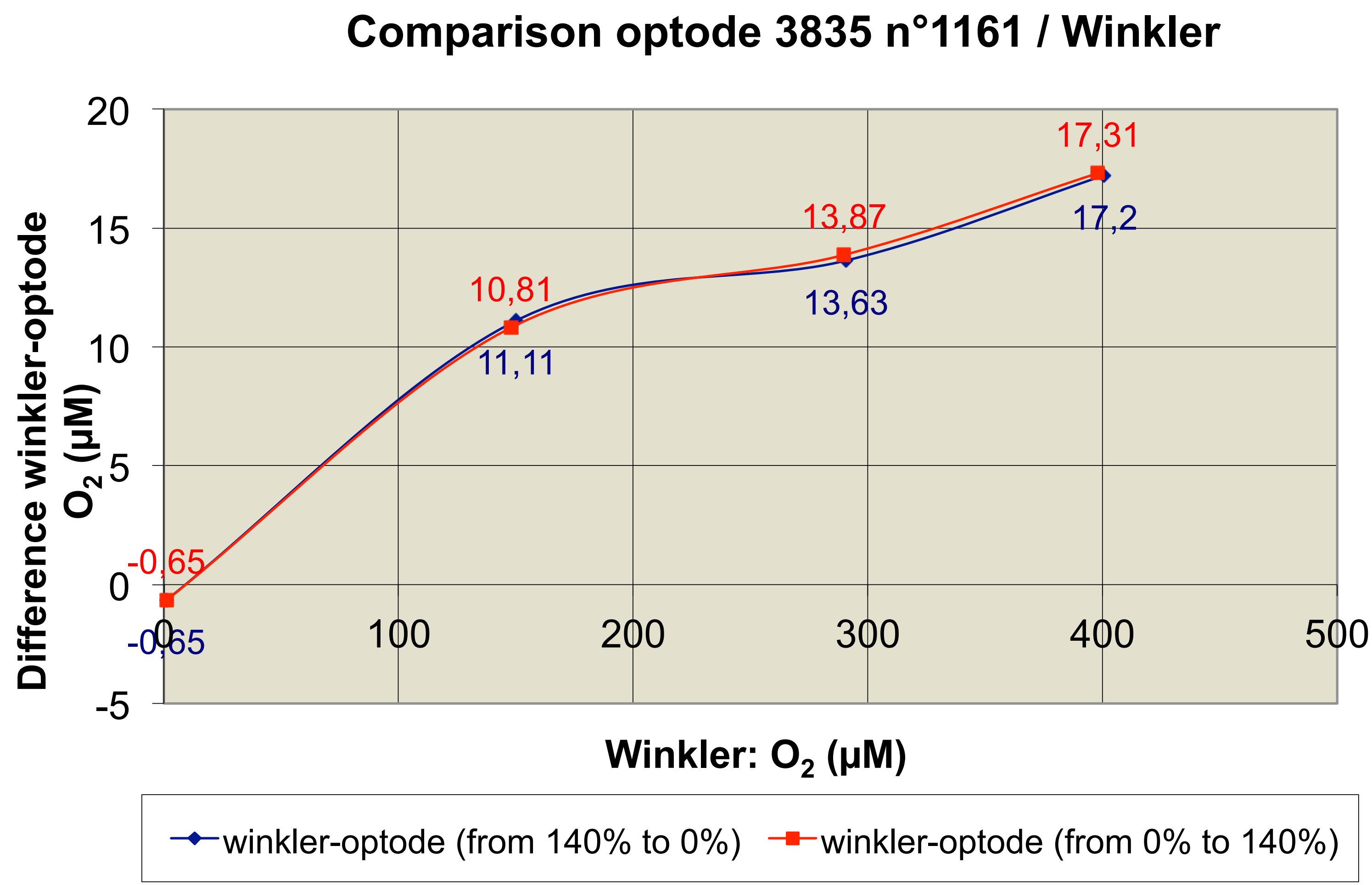
Perspectives

- Complete 0_2 homogeneity testing
- Complete testing of temperature and salinity influence
- Improve sampling method
- Build uncertainty budgets

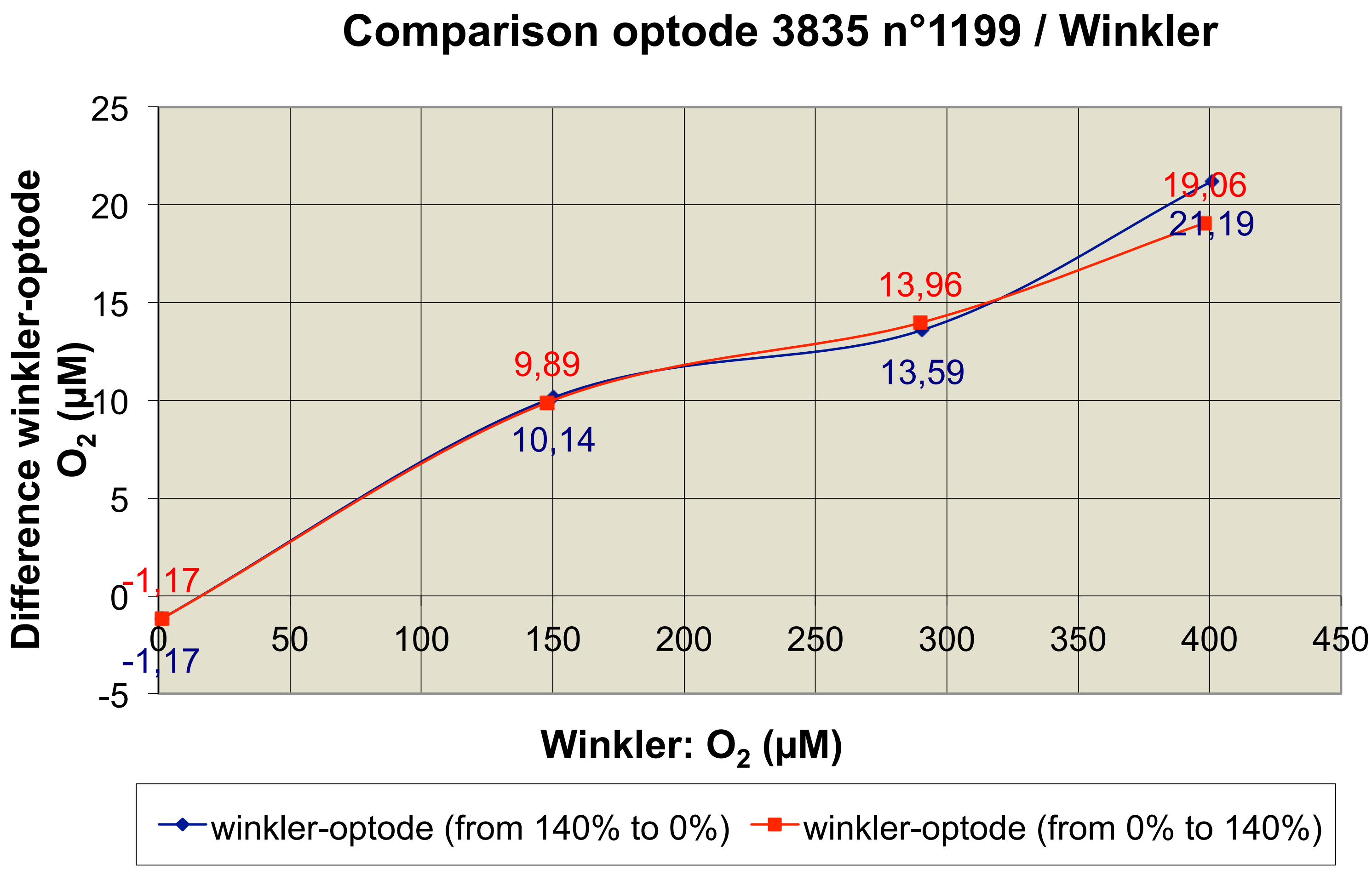
Optode calibration

First results

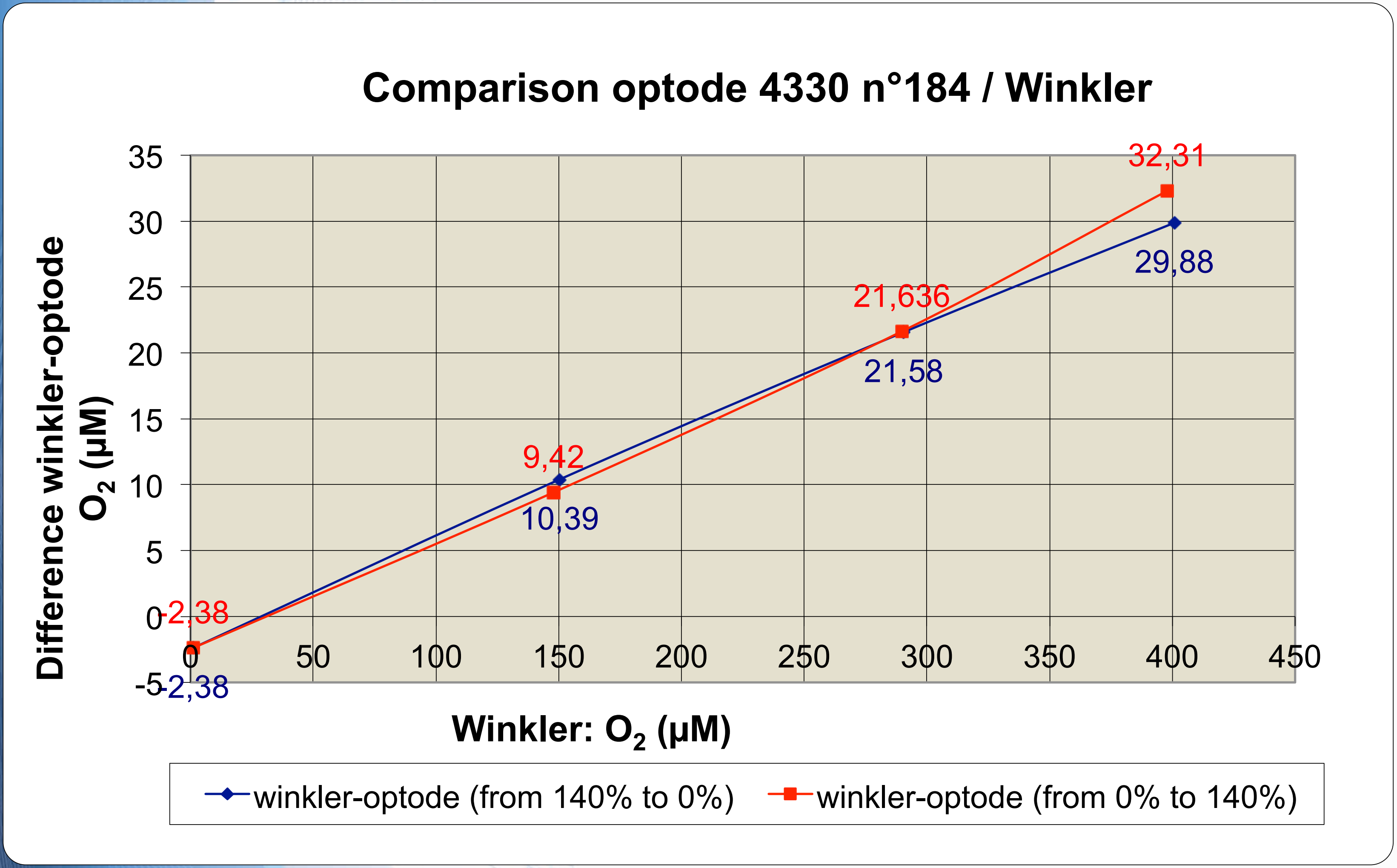
Optode 3835 n°1161



Optode 3835 n°1199



Optode 4330 n°184



Optode calibration

Conclusions and perspectives

- Check for optode conditions of use
- Perform calibration repeatability
- Build uncertainty budgets
- Look for ILC

- Plan O_2 membrane sensor calibration

Thanks for your attention