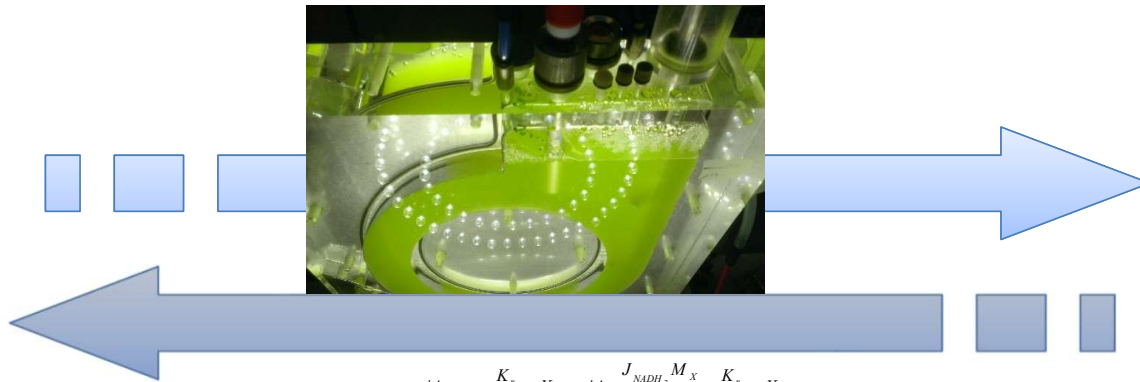
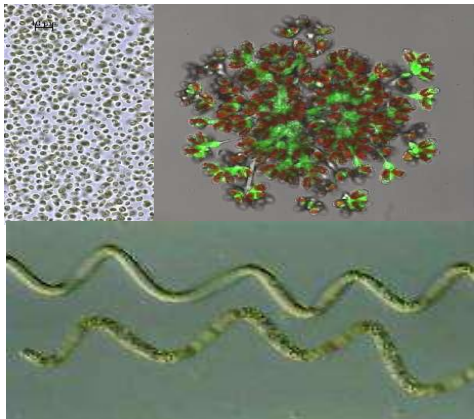


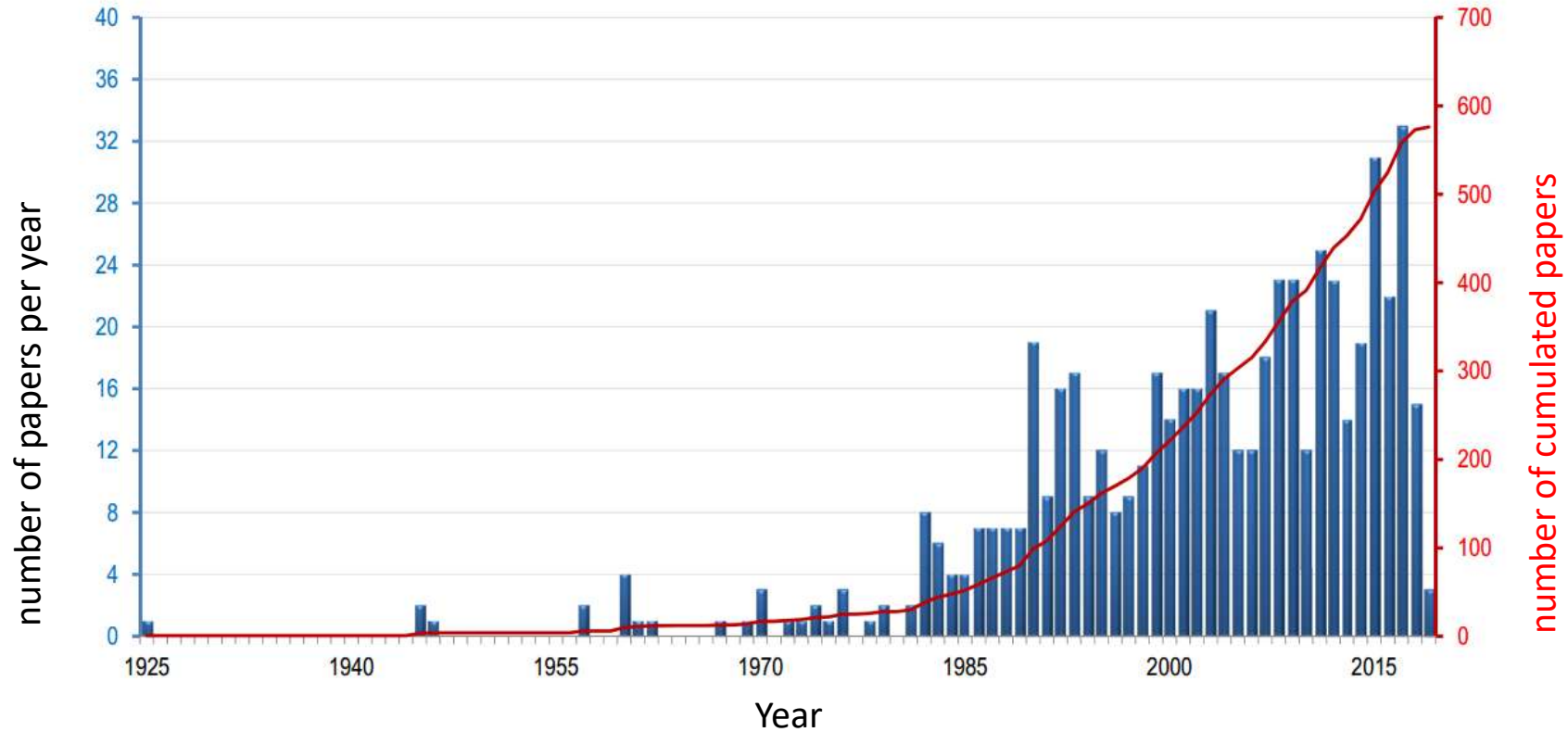
The assessment of the physiology and the biochemistry of microalgae thru non-invasive approaches offers new perspectives for their monitoring in PBRs



$$r_x = \rho\phi A - \mu S \frac{K_r}{K_r + G} X = \rho\phi A - \frac{J_{NADH_2} M_x}{v_{NADH_2-X}} \frac{K_r}{K_r + G} X$$

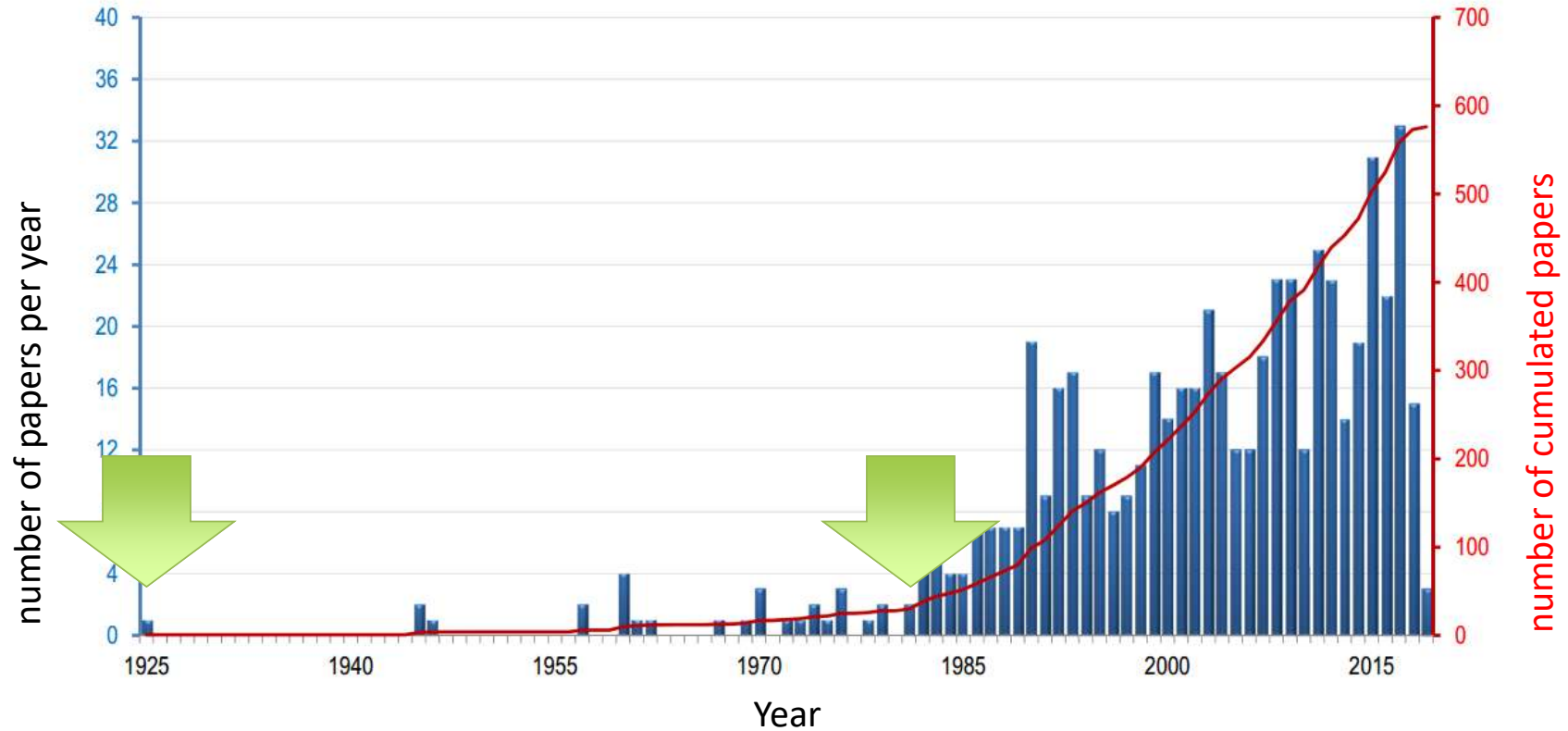


State of the art about bioprocesses monitoring



- pubmed from 1925 to 2020
- key words : “monitoring **or** control” **and** “bioprocess **or** fermentation”
- 576 papers

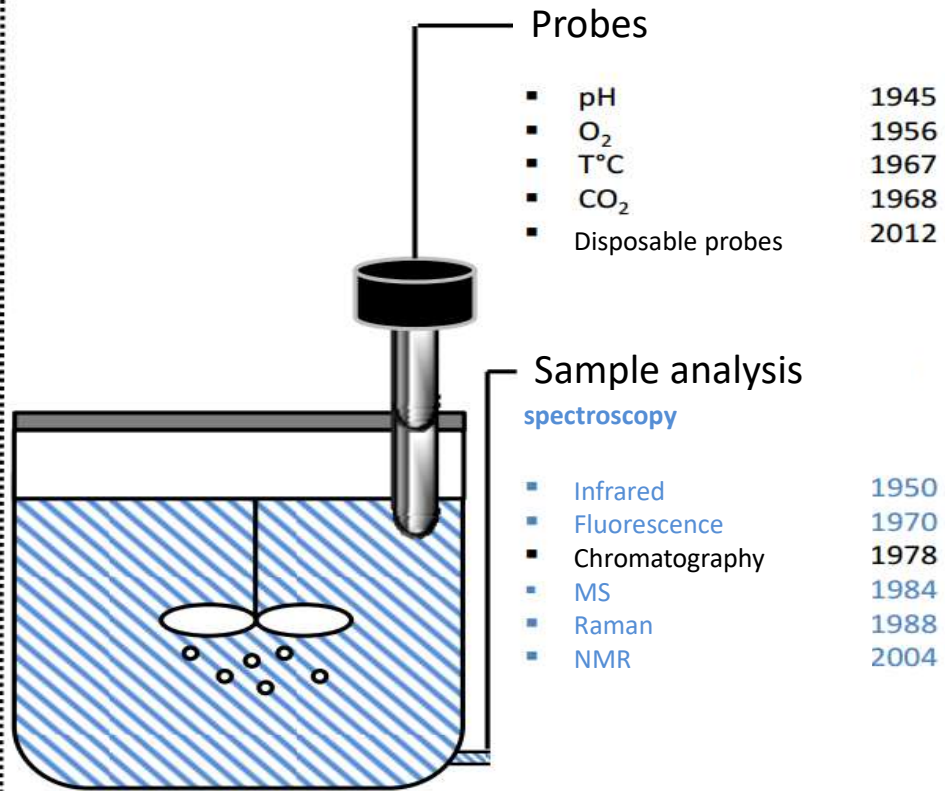
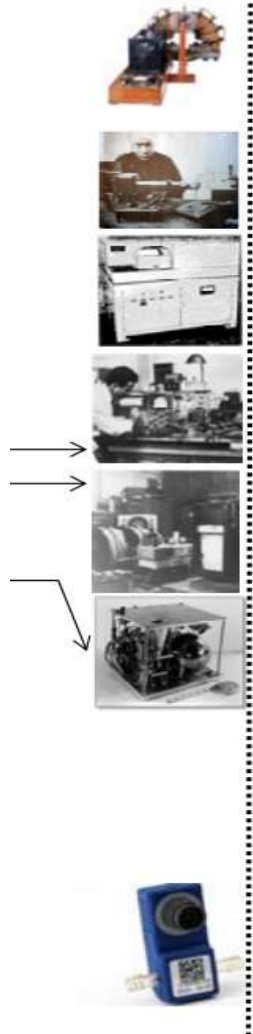
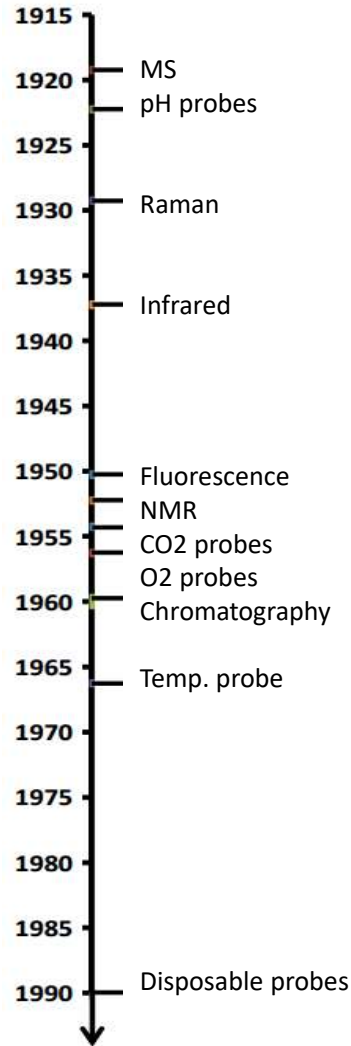
State of the art about bioprocesses monitoring



- pubmed from 1925 to 2020
- key words : “monitoring or control” and “bioprocess or fermentation”
- 576 papers

A matter of history

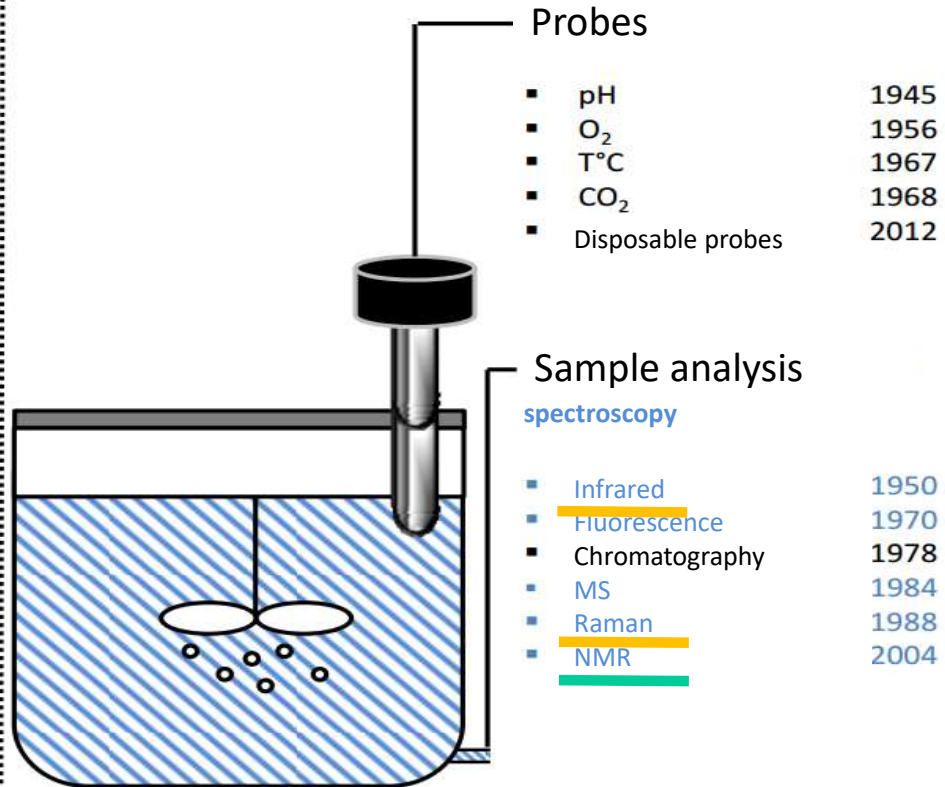
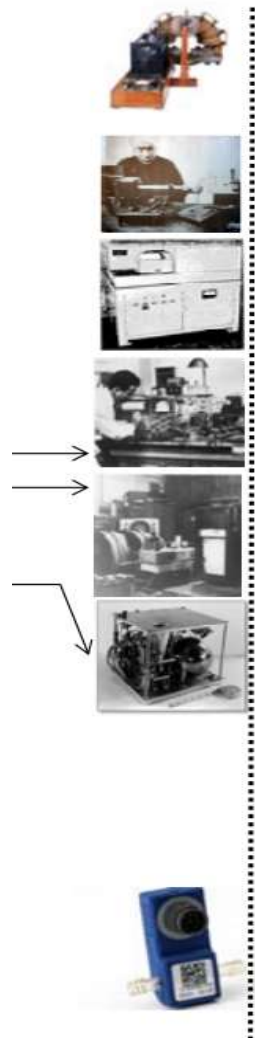
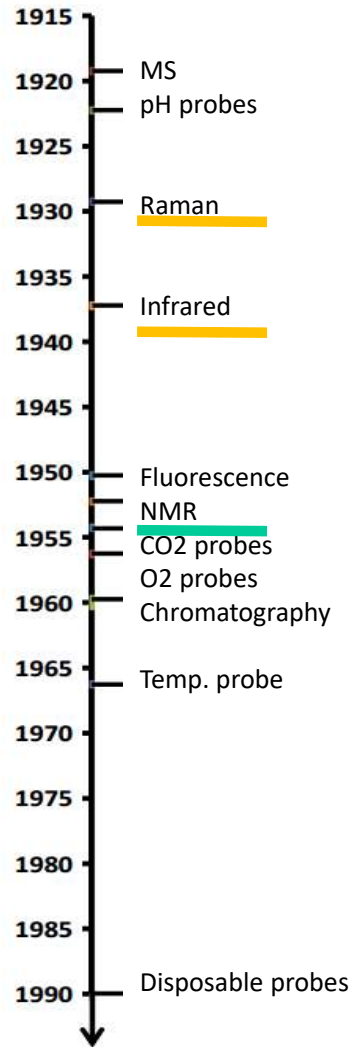
Conception



Application to Bioprocesses

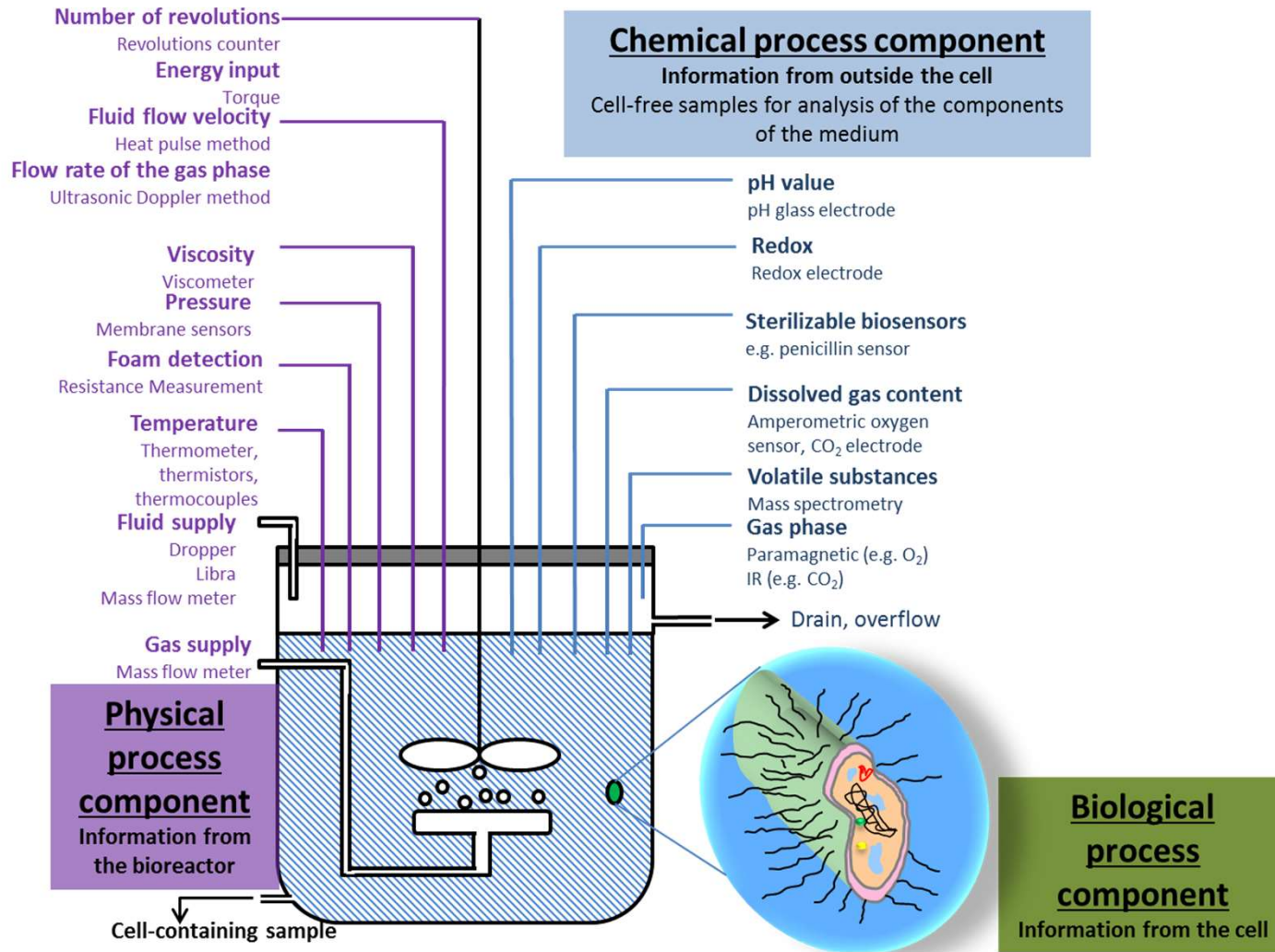
A matter of history with different time lags

Conception

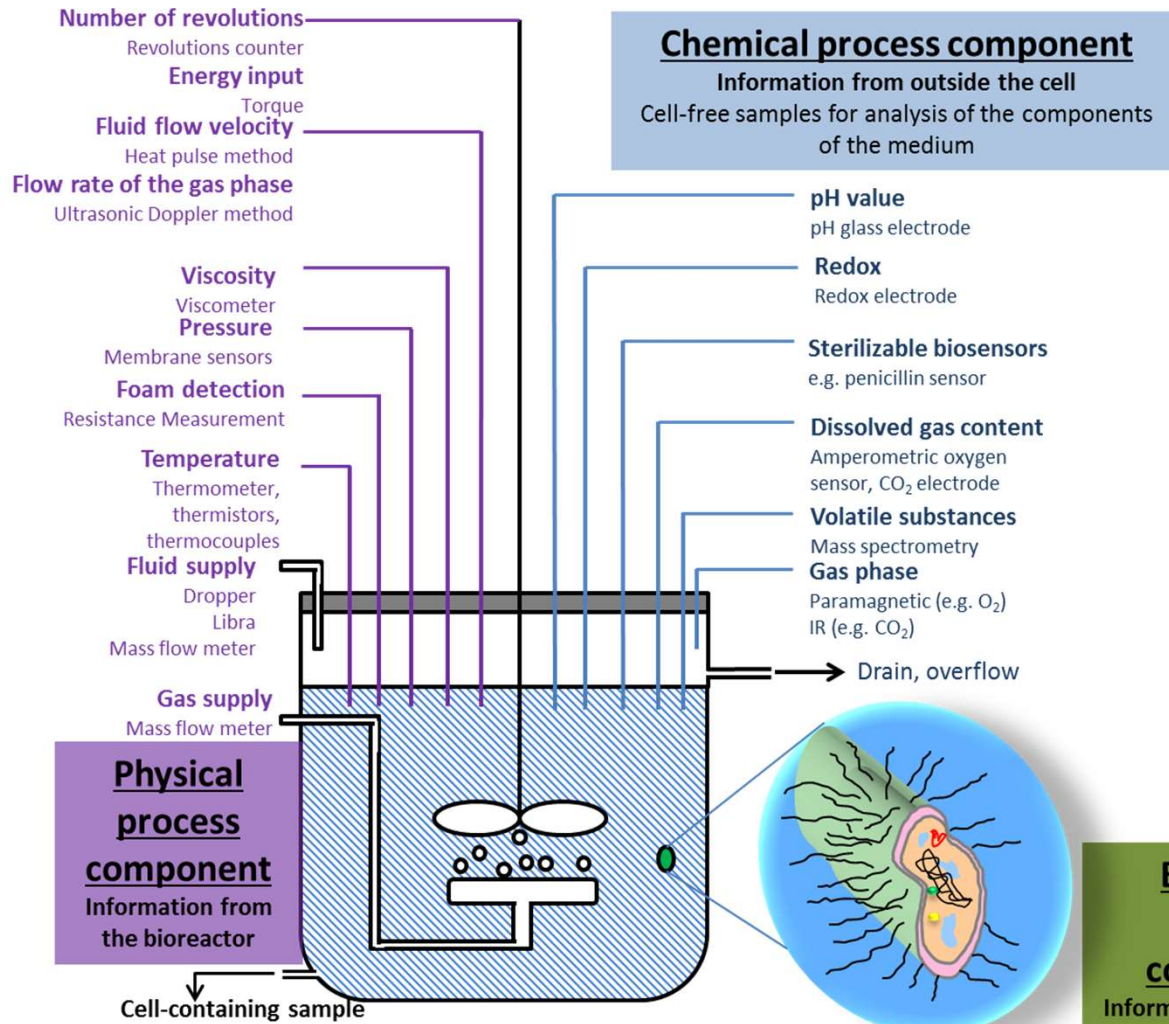


Application to Bioprocesses

Monitoring components of bioprocesses



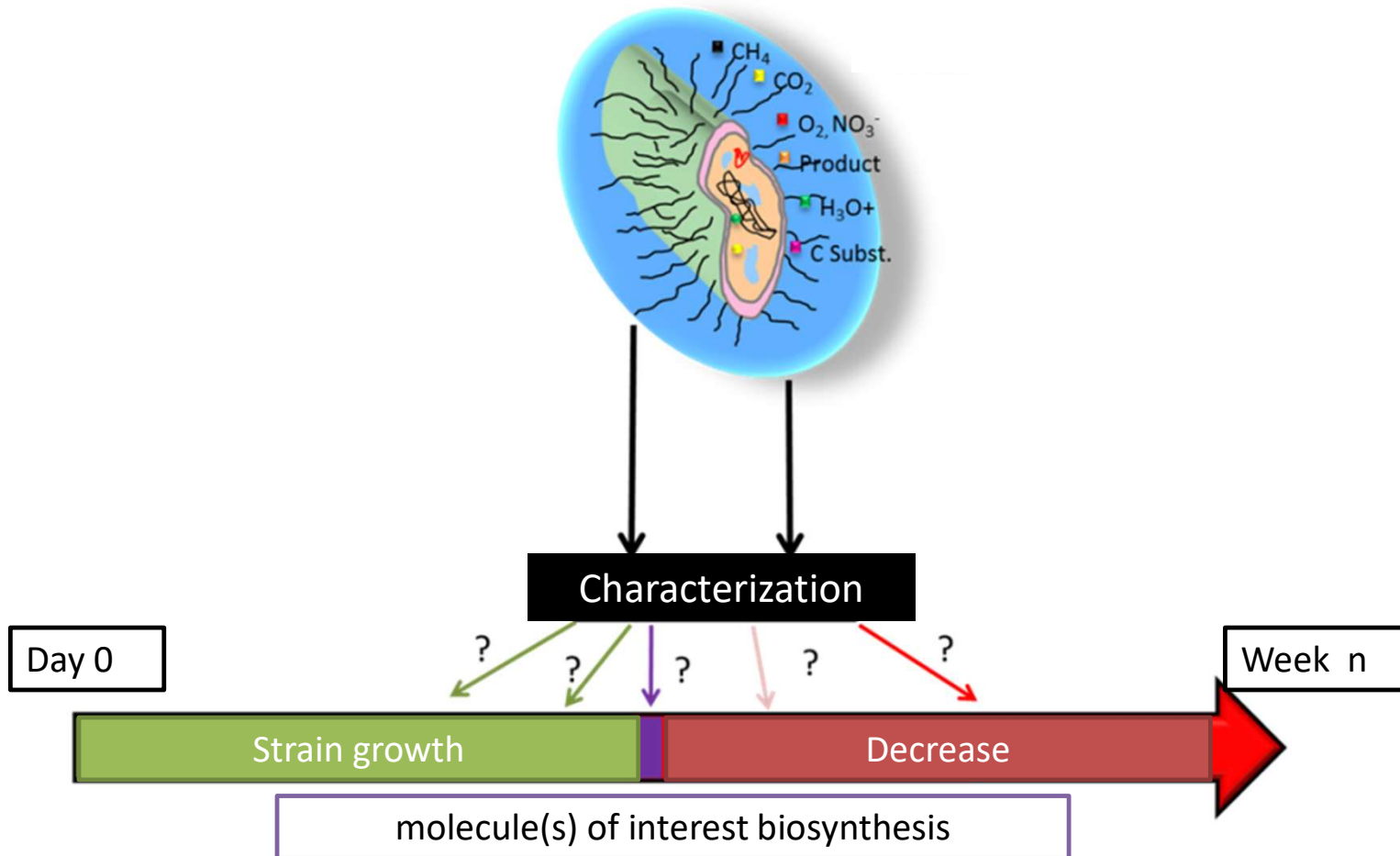
Monitoring components of bioprocesses



c.a. 20 physico-chemical probes

What about the cell?

Monitoring components of cells in reactors



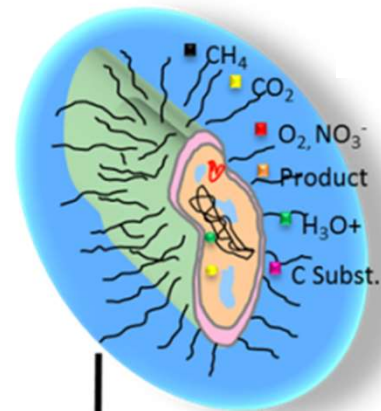
Monitoring components of cells in reactors

Actually

chromatography
turbidimetry
dry matter

cumulation of techniques
invasive, sampling, treatment

robust methods
time consuming methods



Characterization

Day 0



Week n

molecule(s) of interest biosynthesis

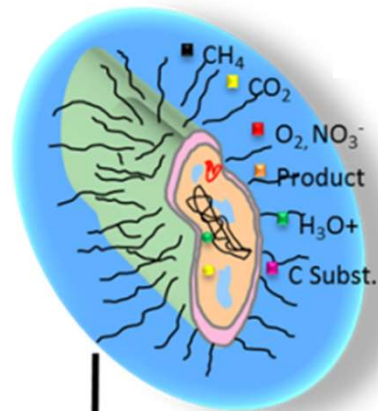
Monitoring components of cells in reactors

Actually

chromatography
turbidimetry
dry matter

cumulation of techniques
invasive, sampling, treatment

robust methods
time consuming methods



Minimal informations
1-cell physiology
2-cell biochemistry
3-high degree of expertise

Characterization

Day 0



Week n

molecule(s) of interest biosynthesis

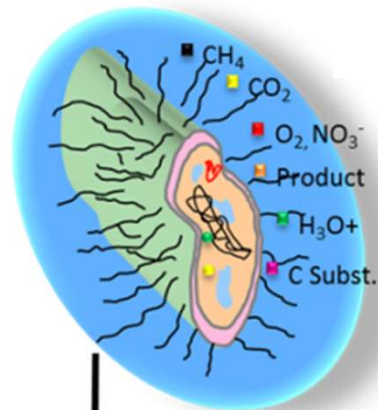
Monitoring components of cells in reactors

Actually

chromatography
turbidimetry
dry matter

cumulation of techniques
invasive, sampling, treatment

robust methods
time consuming methods



What if ? other approaches

alternative technics
spectroscopy

multiparametric
non invasive

robust methods?
time consuming methods?

Characterization

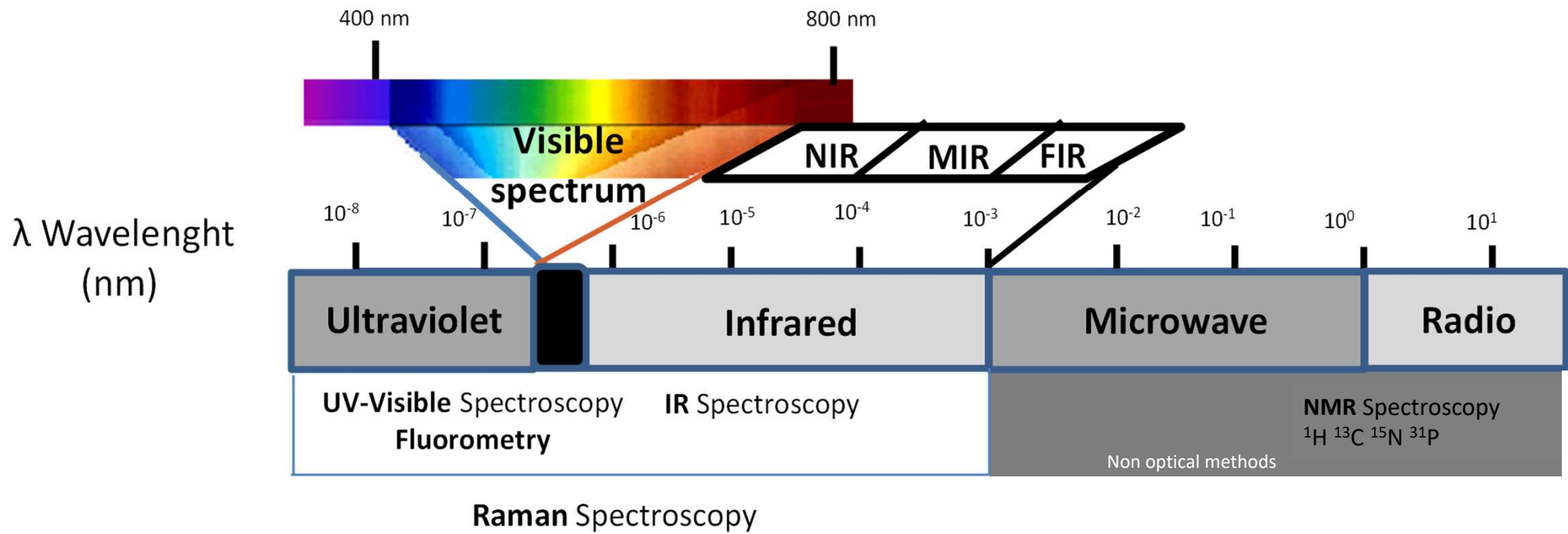
Day 0



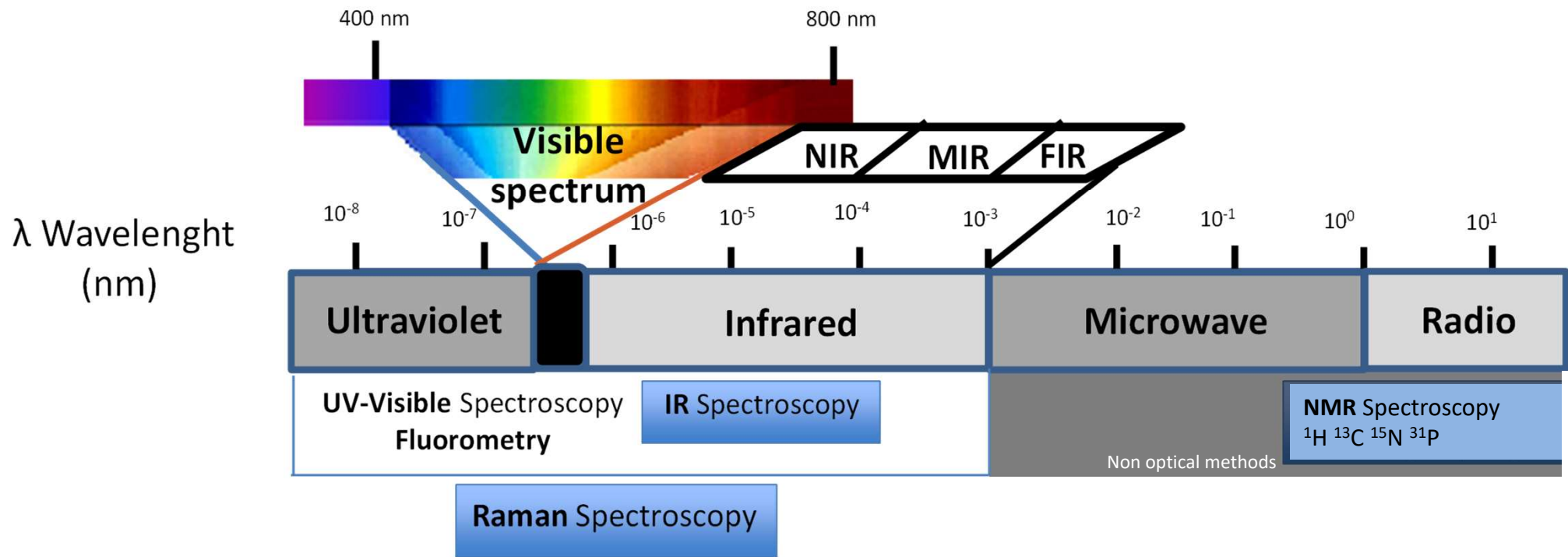
Week n

molecule(s) of interest biosynthesis

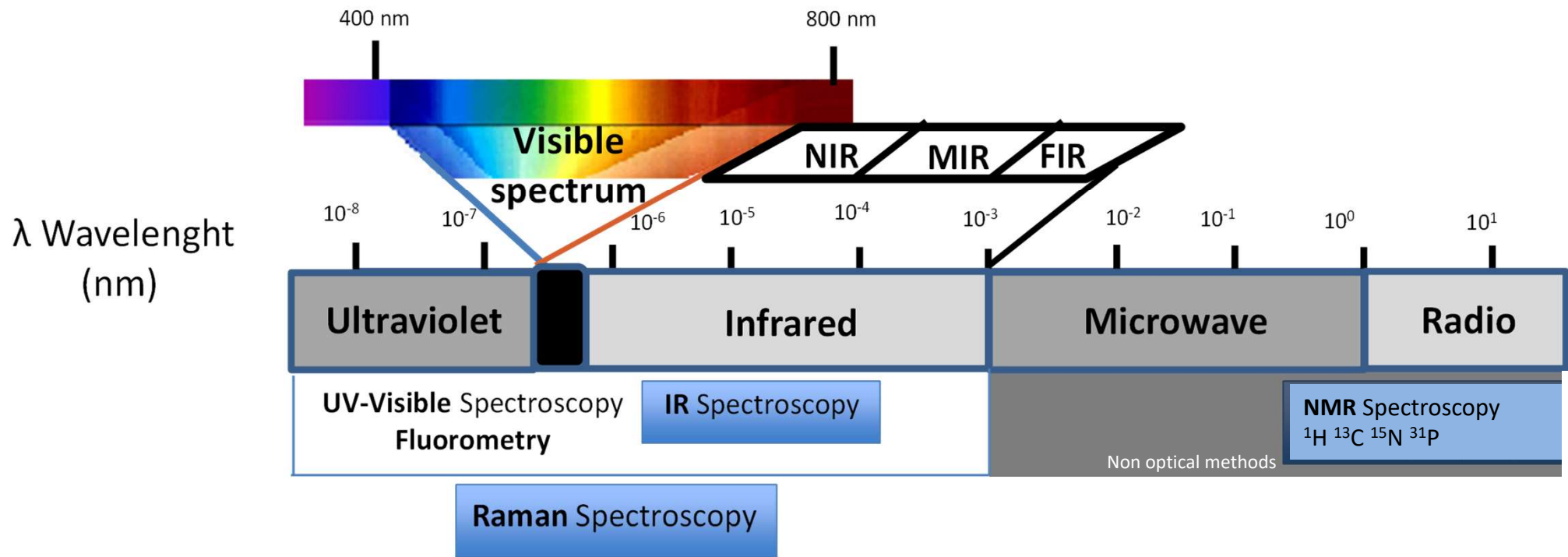
Non-invasive approaches ... spectroscopy



Non-invasive approaches ... spectroscopy

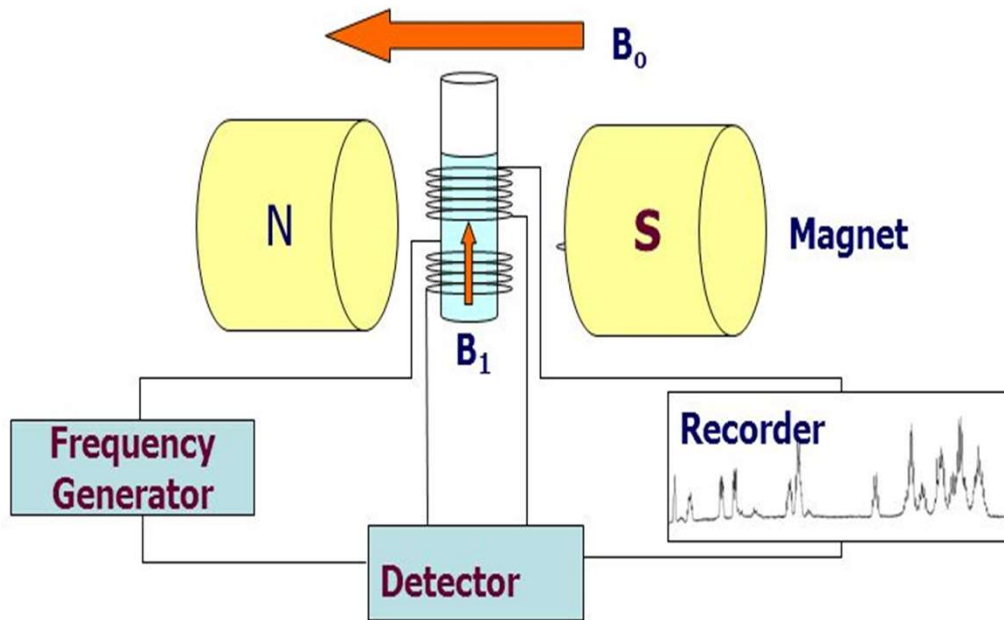


Non-invasive approaches ... spectroscopy



Radiowave spectroscopies to monitor lipid accumulation in oleaginous microalgae

NMR spectroscopy on entire microalgae



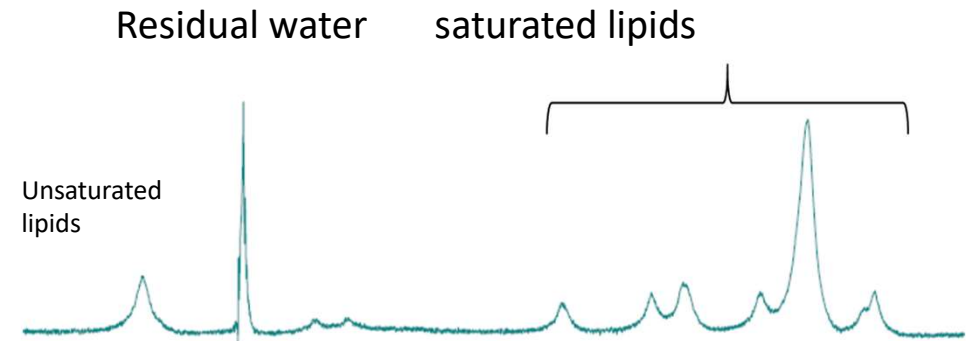
Magnet - Normally Superconducting.

Frequency generator Creates an alternating current that induces B_1 .

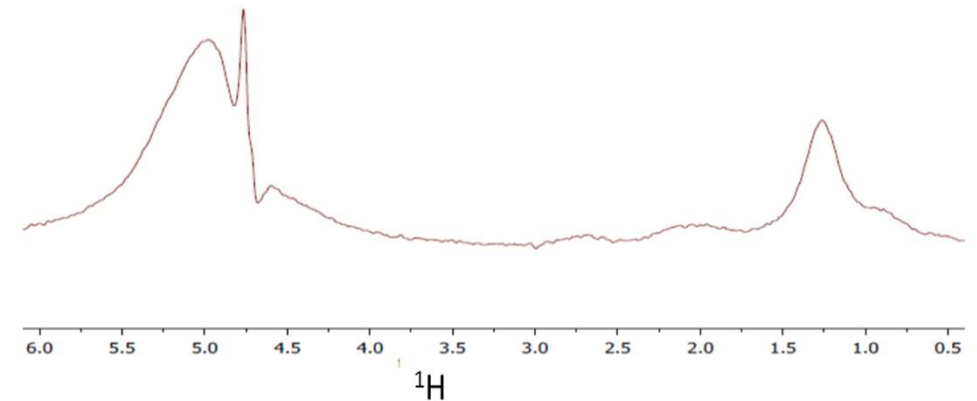
Detector

NMR recent technological breakthrough

High field spectrometer
700 MHz

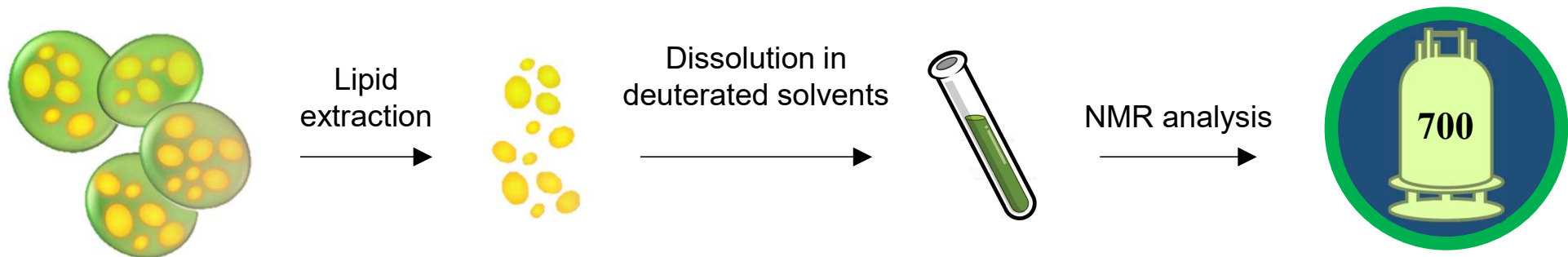


Low field spectrometer
43 MHz



^1H NMR spectra of *Parachlorella kesslerii* entire cells

NMR spectra informations interpretation

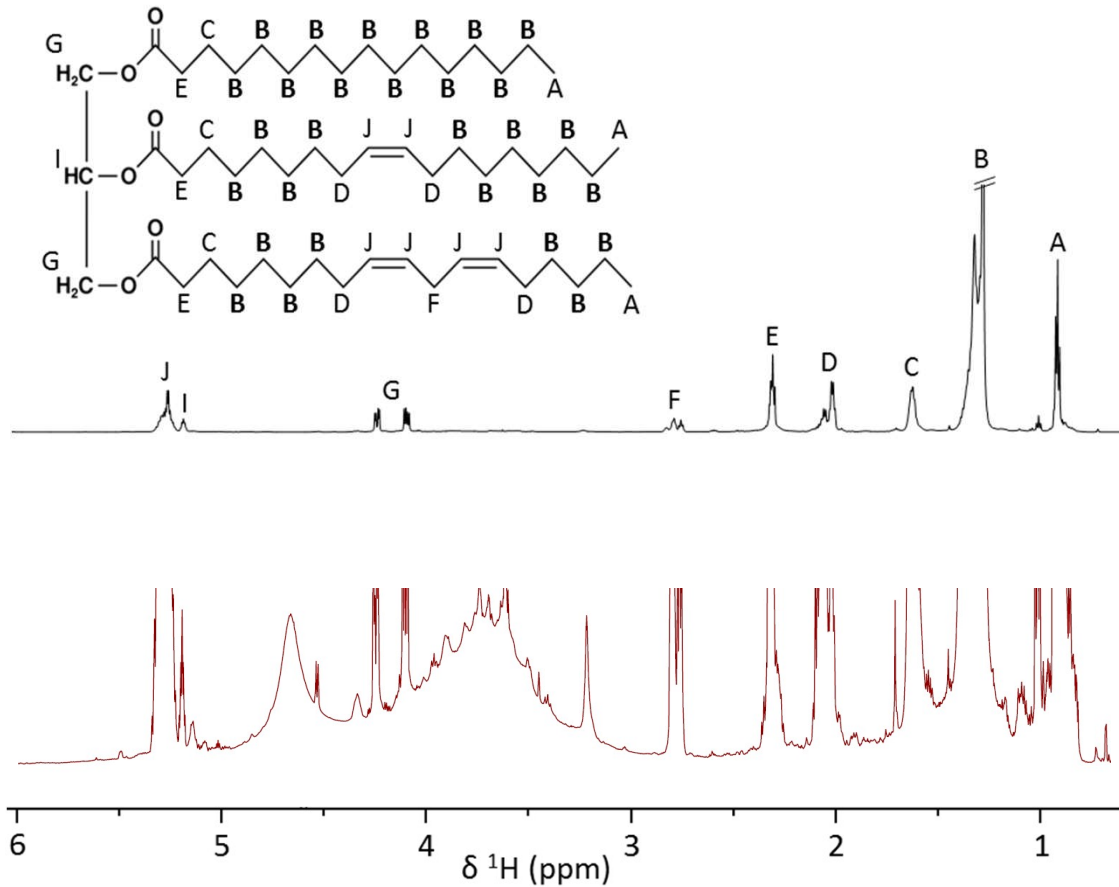


- Non-destructive, reproducible
- No lipid derivatization
- Only one internal standard (no lipid standards needed)

quantitative NMR approaches:

- 1D ^1H NMR
- 1D ^{13}C NMR
- Quantitative 2D NMR

^1H NMR



- Highly overlapped ^1H NMR

- Baseline distorted

Accessible information:

- concentration of total fatty chains

- concentration of unsaturated fatty chains

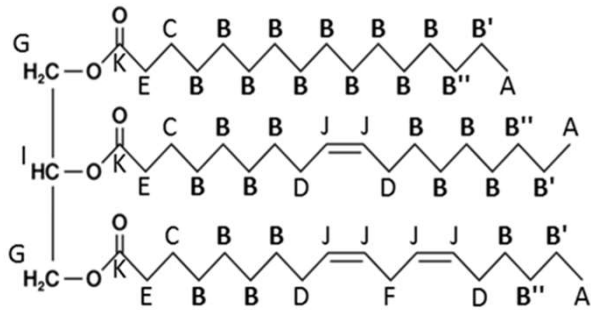
- triglyceride ratio

- ω -3 ratio

- **low accuracy**

- **fast**

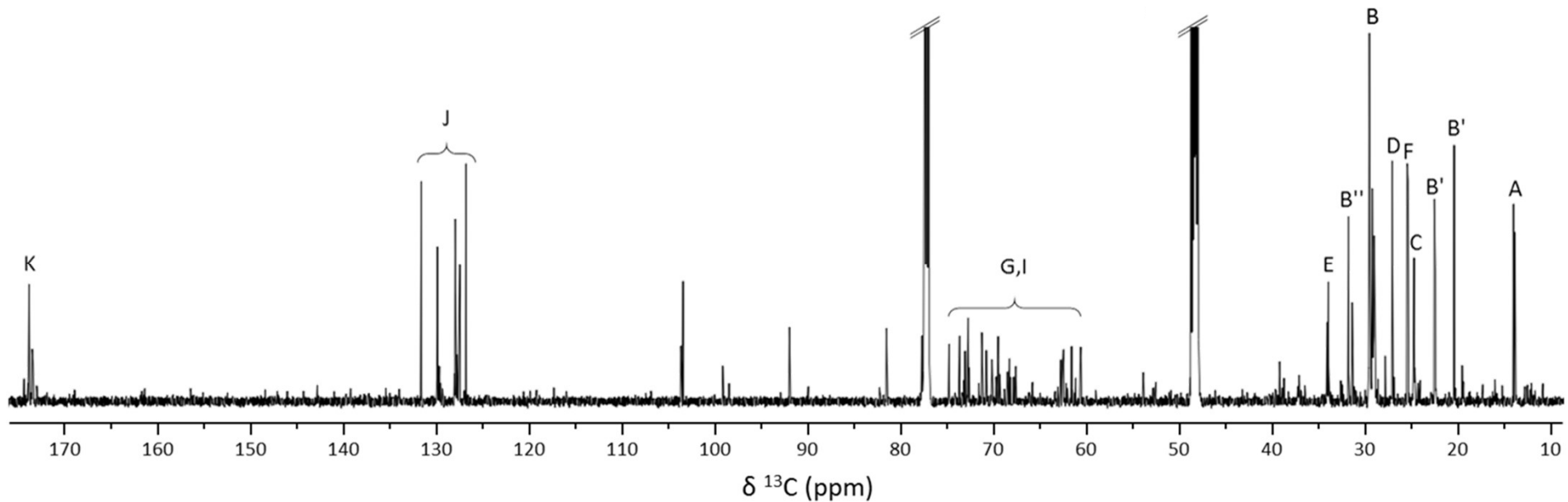
^{13}C NMR



- much more resolved spectrum
- more accurate
- more information
- less sensitive

Accessible information:

- concentration of total fatty chains
- concentration of unsaturated fatty chains
- triglyceride ratio
- ω -3, ω -6, ω -7, ω -9 ratios
- ...



Is NMR quantitative? Lipid extracts

^1H NMR quantification

Concentration (mmol.L ⁻¹)	Starved ?	Fatty chains	% Saturated	% Triglyceride
PK 61e-30	Yes	42	28	54
PK 61e-20	Yes	24	44	46
PK 62e	Yes	46	29	67
NGSCe	Yes	102	41	81
PK 59e-A	No	12	68	-
PK 59e-B	No	15	50	-
PK 50e-H	No	11	44	-
PK 33e	No	13	16	-

^{13}C NMR quantification

Concentration (mmol.L-1)	Fatty chains #1	Fatty chains #2	Fatty chains #3	Omega-3	Omega-6	Omega-7	Omega-9	Unsaturated
NGSCe	135	140	134	10	19,1	25	22	68,7
PK60e	126	163	131	51,7	21,6	-	0,5	73,6
PK61e-30	72	76,6	69,3	19	16,2	-	4,4	36,9

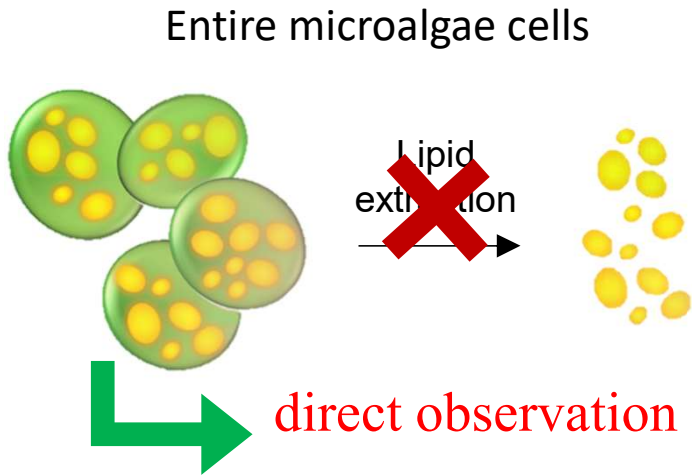
Benchtop NMR?

- ⊕ Improve NMR accessibility
 - reduced size
 - transportable
 - no cryogenic liquid
 - low cost
- ⊖ Lower sensitivity and resolution
- ⊕ Prototype : gradient coil
 - Advanced solvent suppression pulse sequences
 - UltraFast NMR
 - DOSY NMR

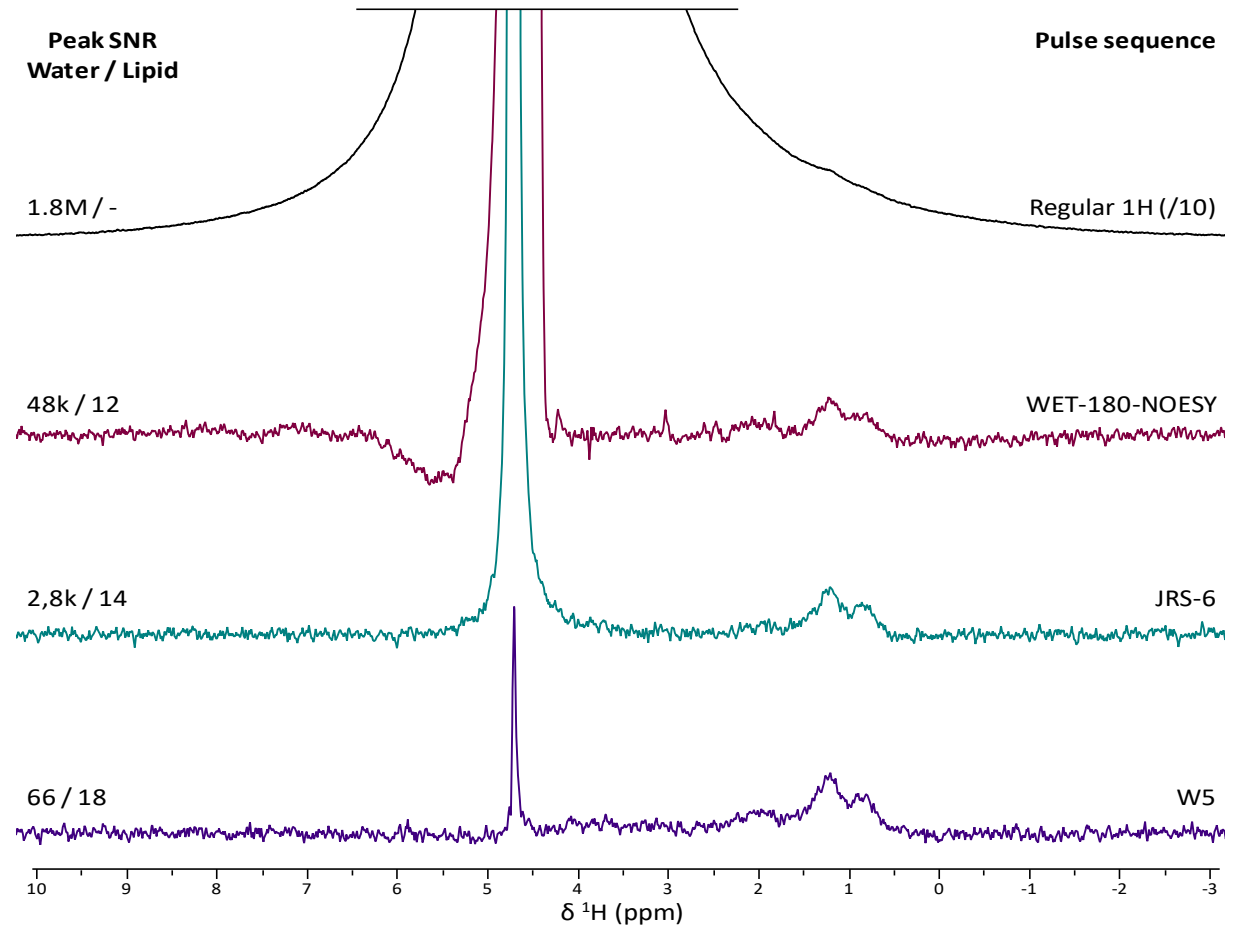


Benchtop NMR used for reaction monitoring

On line monitoring - water signal

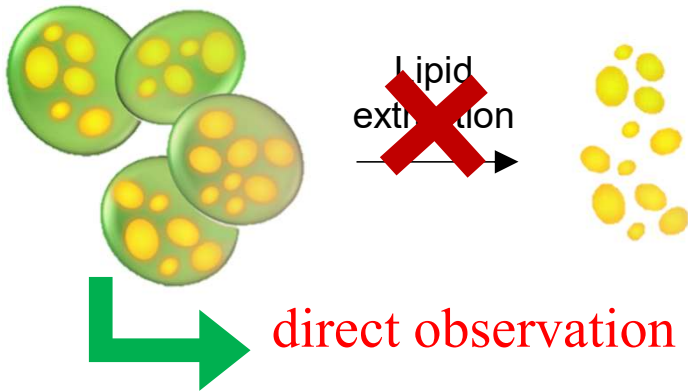


- Need to remove water peak
- W5 provides the best water peak reduction

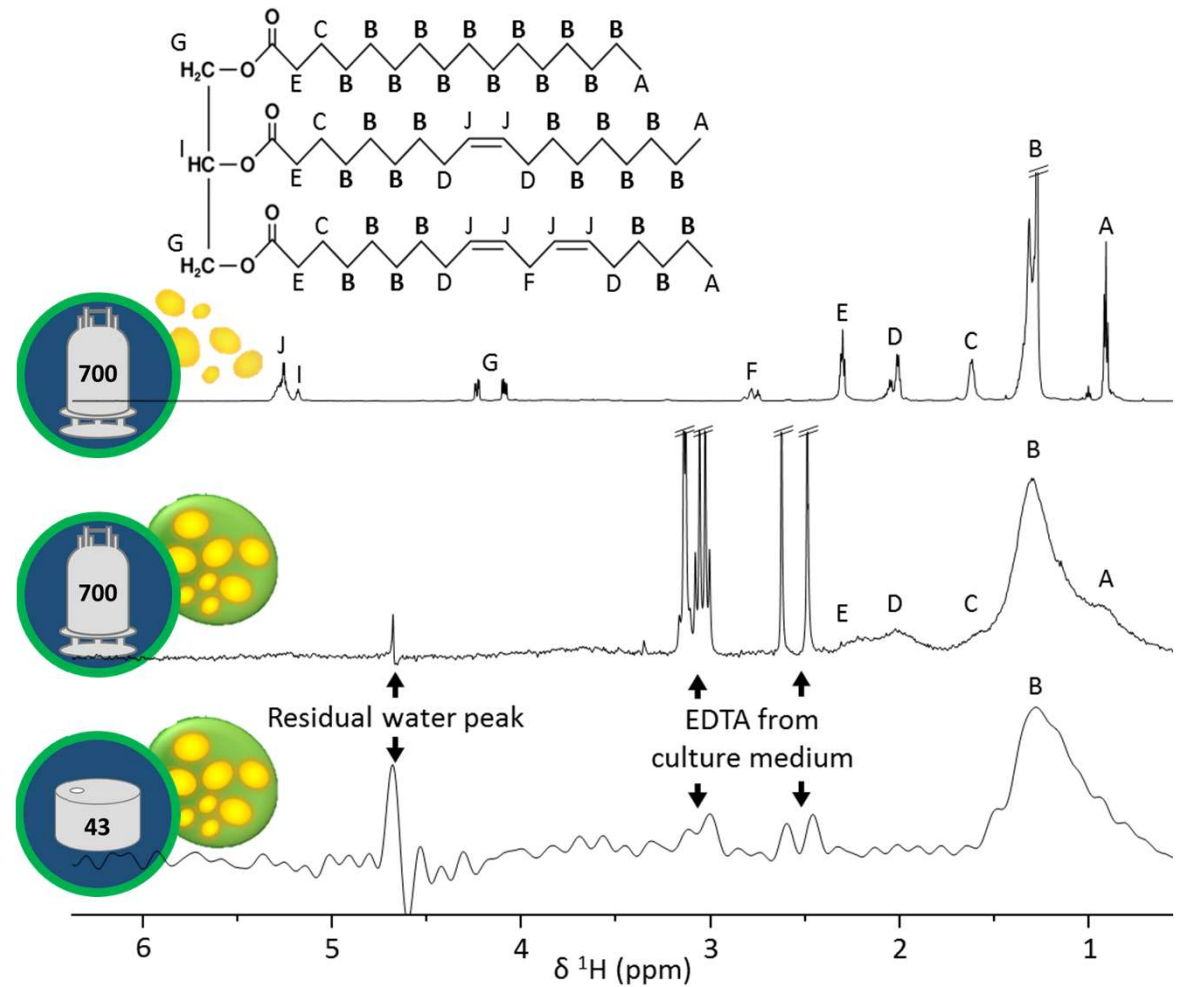


On line monitoring - matrix effect

Entire microalgae cells

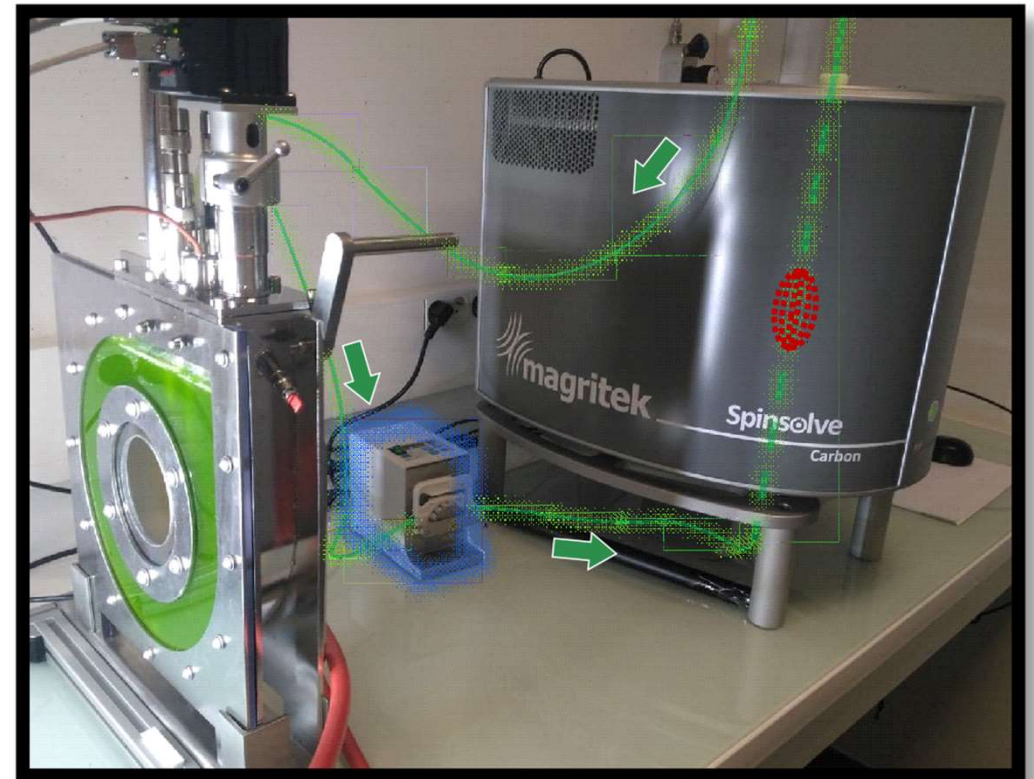


- Need of flow measurements
- Intracellular lipids give rise to large peaks
- the main one is still visible at 43 MHz, **used to monitor the lipid production**

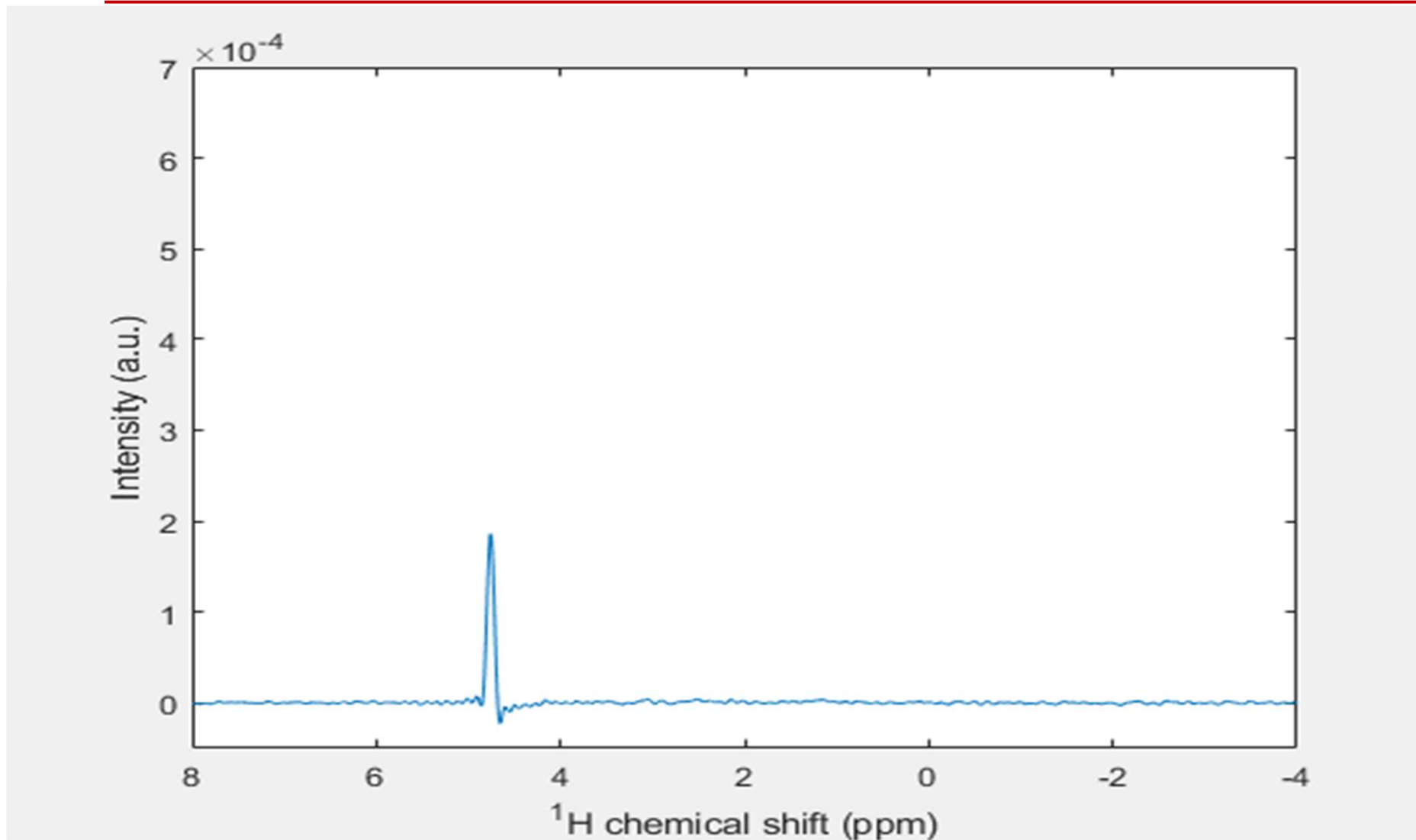


On line monitoring - coupling PBR/NMR

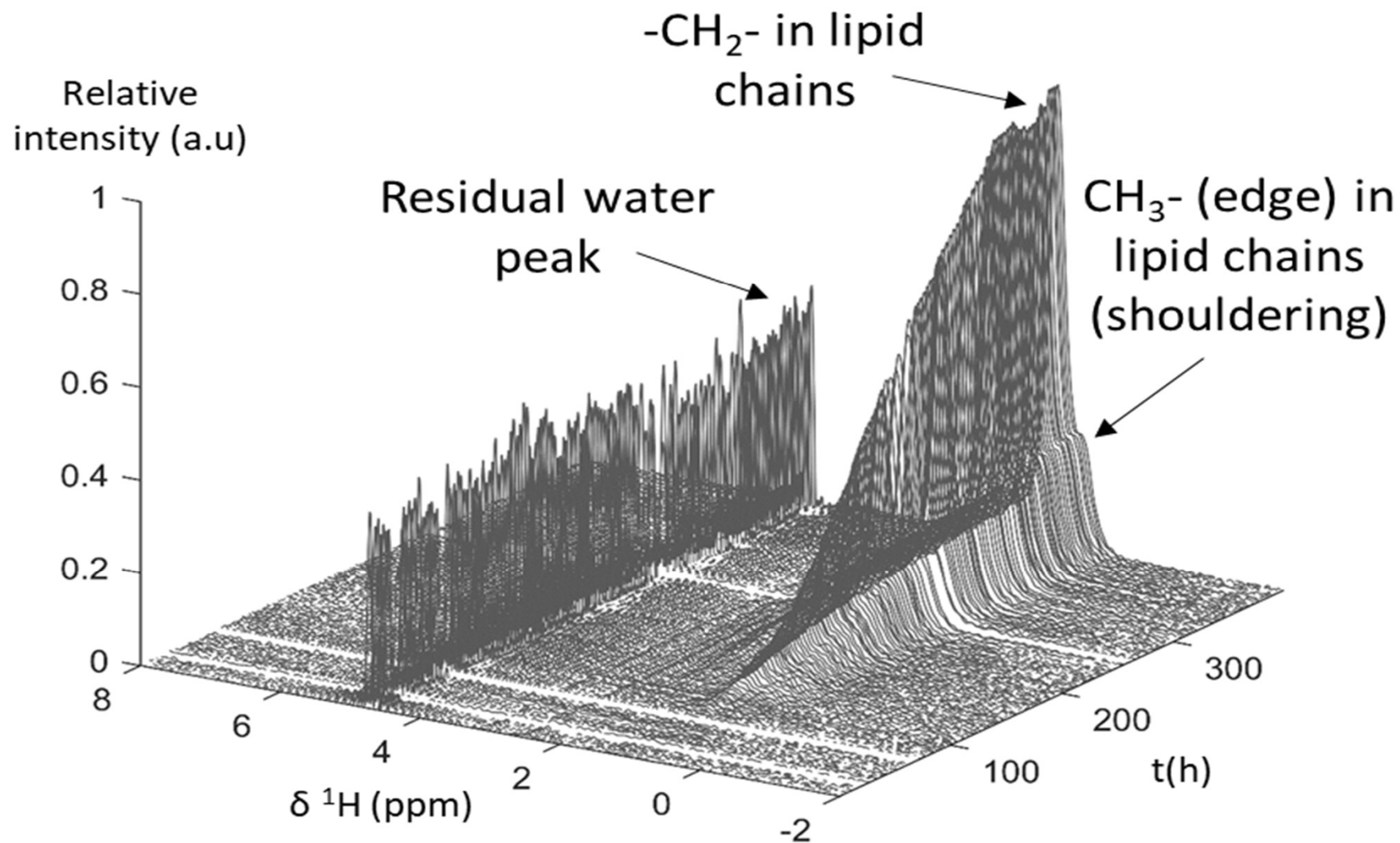
- Microalgae cultivation system
- Bypass loop (11 mL)
- Online analysis
- Flow rate 2 mL.min⁻¹
- One spectrum per hour



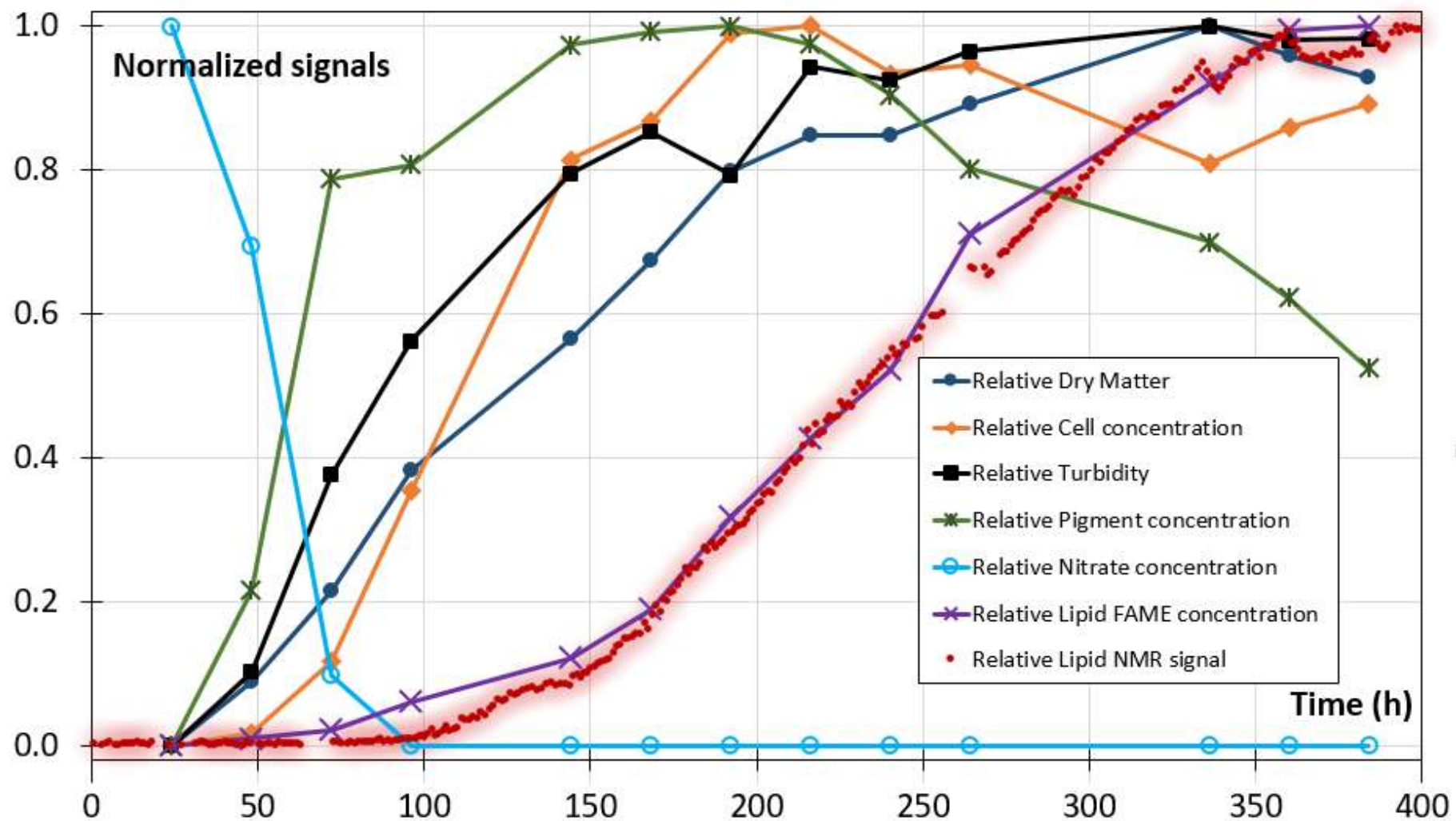
On line monitoring - the raw data in real time



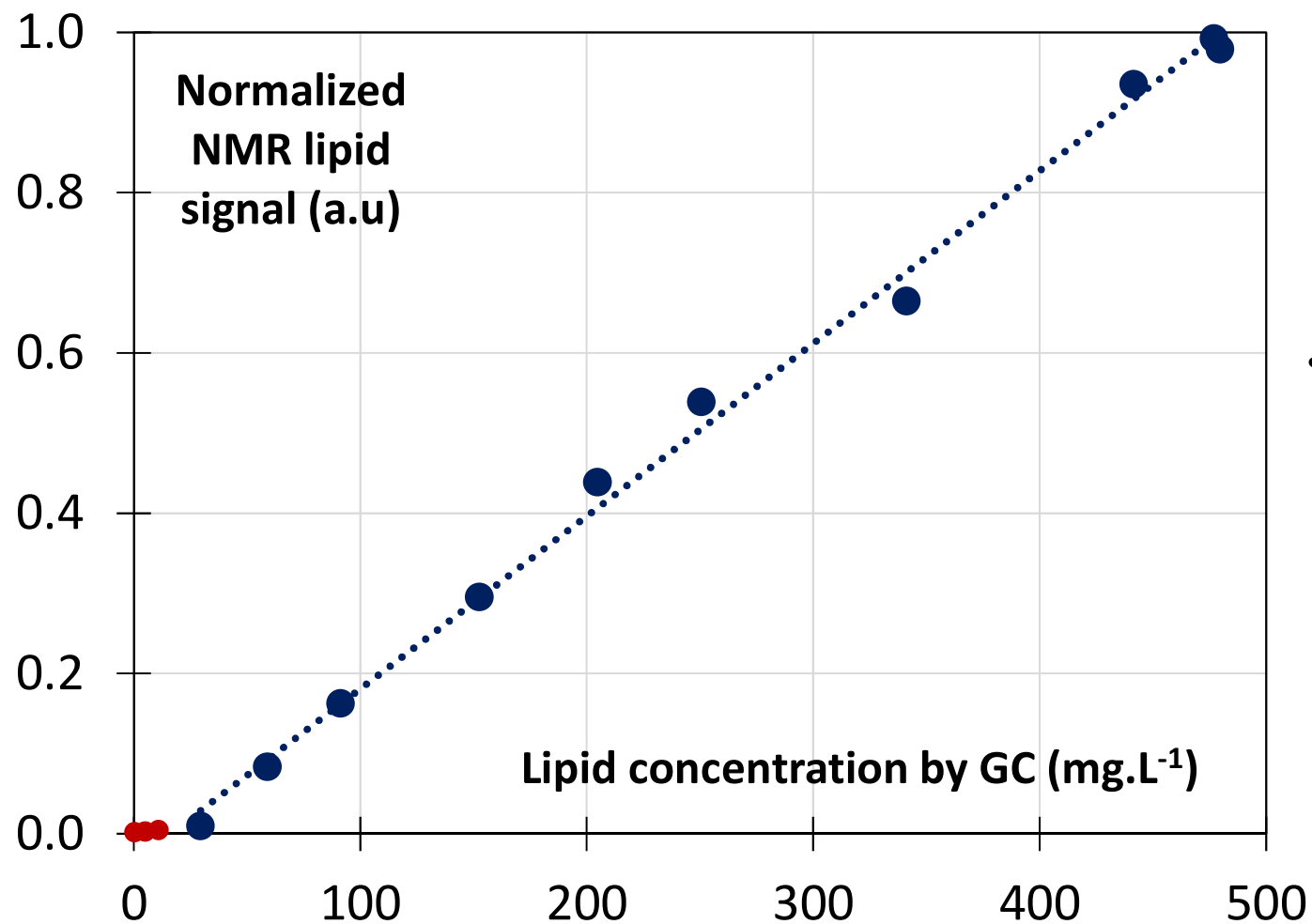
On line monitoring - the raw data



On line monitoring - results



On line monitoring - calibration



- ($R^2 > 0.99$ over 10 points)
- LOD : 9 mg.L⁻¹
- LOQ : 30 mg.L⁻¹




NMR spectroscopy

- More details in

Received: 26 October 2018 | Revised: 17 December 2018 | Accepted: 19 December 2018
DOI: 10.1002/mrc.4821

SPECIAL ISSUE MINI-REVIEW

Benchtop NMR for the monitoring of bioprocesses

Dylan Bouillaud^{1,2} | Jonathan Farjon¹  | Olivier Gonçalves²  | Patrick Giraudeau^{1,3} 



Benchtop flow NMR spectroscopy as an online device for the *in vivo* monitoring of lipid accumulation in microalgae

Dylan Bouillaud^{a,b}, Vladimir Heredia^b, Thomas Castaing-Cordier^a, Delphine Drouin^b, Olivier Gonçalves^b, Jonathan Farjon^{a,*}, Patrick Giraudeau^{a,c}

WILEY



DOI: 10.1002/cphc.201801116

CHEMPHYSCHEM
Articles

Highly Resolved Pure-Shift Spectra on a Compact NMR Spectrometer

Thomas Castaing-Cordier,^[a] Dylan Bouillaud,^[a] Paul Bowyer,^[b] Olivier Gonçalves,^[c] Patrick Giraudeau,^{*[a, d]} and Jonathan Farjon^{*[a]}

Process Biochemistry xxx (xxxx) xxx-xxx



Using benchtop NMR spectroscopy as an online non-invasive *in vivo* lipid sensor for microalgae cultivated in photobioreactors

Dylan Bouillaud^{a,b}, Delphine Drouin^b, Benoît Charrier^a, Corentin Jacquemmoz^a, Jonathan Farjon^a, Patrick Giraudeau^a, Olivier Gonçalves^{b,*}

^a Université de Nantes, CEISAM, UMR CNRS 6230, BP 92208, 2 rue de la Houssinière, 44322 Nantes Cedex 3, France
^b Université de Nantes, GEPEA, UMR CNRS 6144, 37 boulevard de l'Université, 44600 Saint-Nazaire Cedex, France

Very promising...

High-field spectrometer

- Assignment improvements
- Analytical specifications determination by comparison with reference techniques
- Approaches comparison

Benchtop spectrometer

- Relative quantitative data compared with reference techniques
- Biological interpretation
- Coupling with specific cultivations
 - ↳ biological information

However...

High-field spectrometer

- sensitivity improvement
- absolute quantification
- water signal suppression

Benchtop spectrometer

- sensitivity improvement
- sensitivity to temperature
- increase the force field

Conclusion - perspectives

- New spectrometric multiparametric sensors
- Very promising
- Still needing improvement
 - robustness
 - matrix effect
 - “growth stages” (physiology)
 - semi quantitative
 - RTO

Acknowledgements

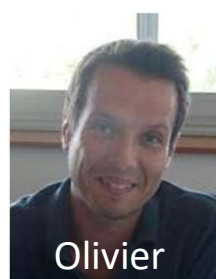
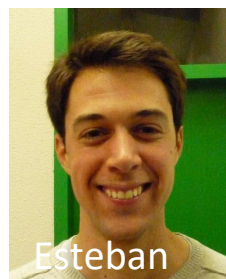


BAM + TEAM



EBSI

Acknowledgements



BAM + TEAM



MIMM

Atlantic ocean

La Baule

Saint-Nazaire

GEPEA lab. (CRTT)

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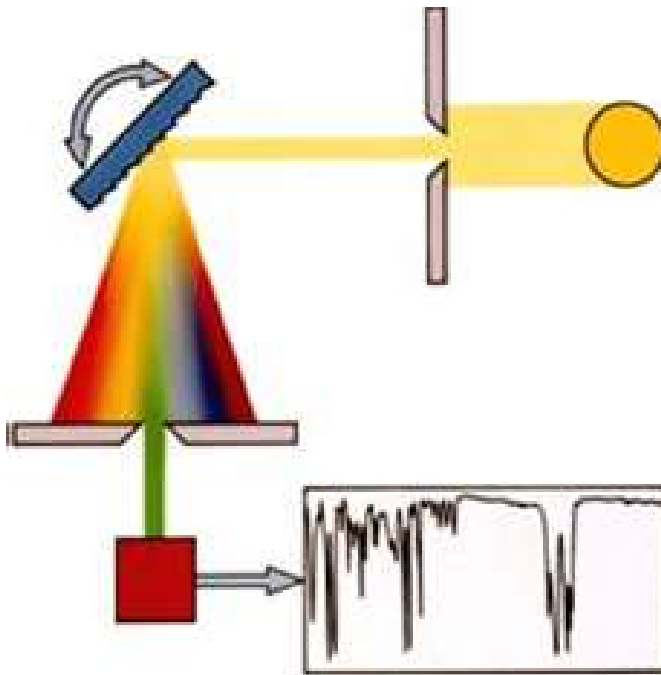
Thank you for your
attention

www.gepea.fr

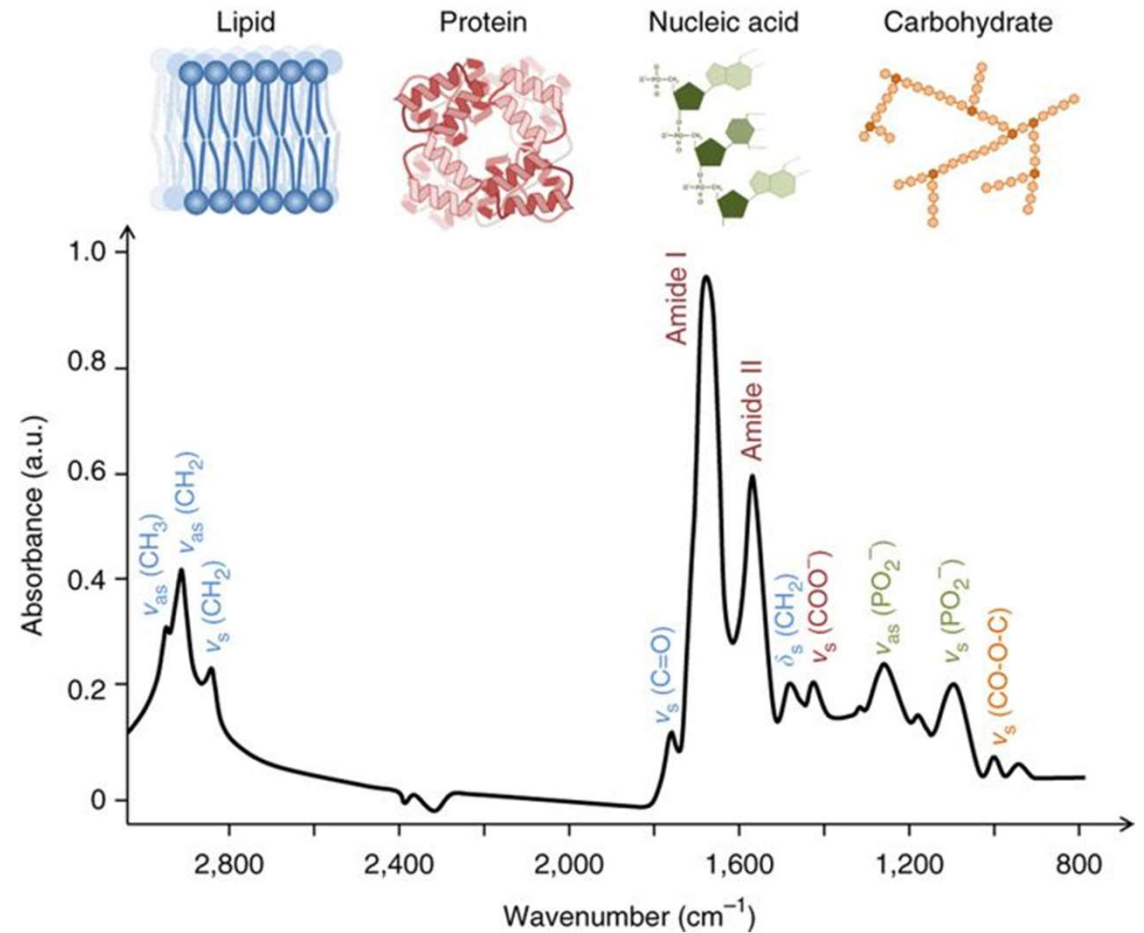
www.alaosolis.com

Olivier.Goncalves@univ-nantes.fr

Infrared spectroscopy



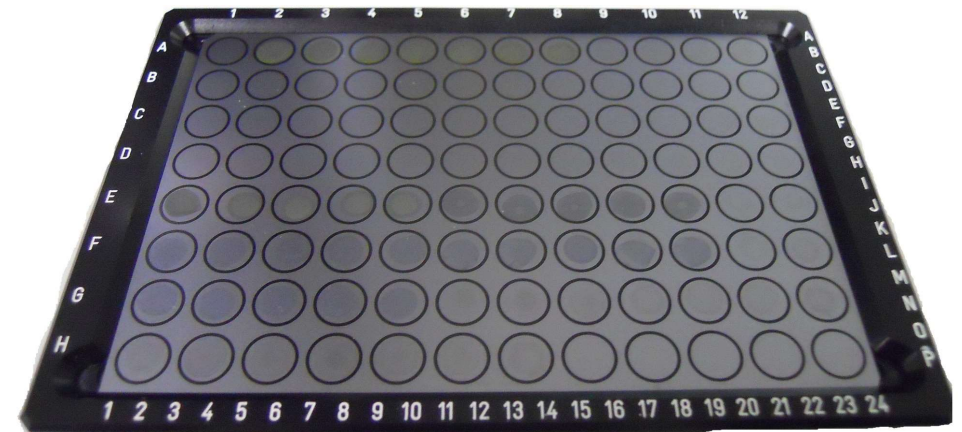
FTIR Principle



FTIR spectra of *Chlorella vulgaris*

Infrared spectroscopy for PBR monitoring

- Water sensitive : off-line and need to dry the sample



Influence of the sampling method

- Molecular probing approach - SCN⁻

Bioprocess Biosyst Eng (2014) 37:2371–2380
DOI 10.1007/s00449-014-1215-4

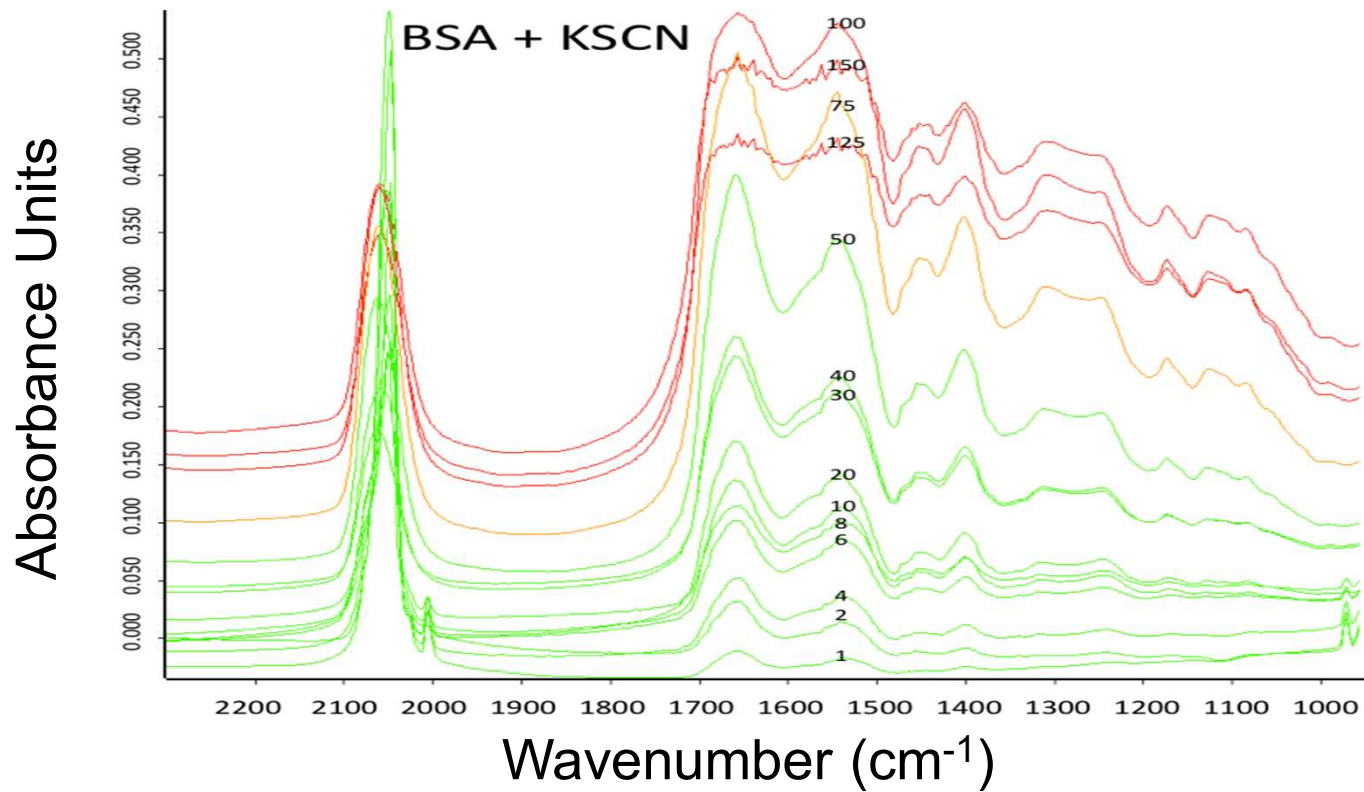
ORIGINAL PAPER

Influence of physical and chemical properties of HTSXT-FTIR samples on the quality of prediction models developed to determine absolute concentrations of total proteins, carbohydrates and triglycerides: a preliminary study on the determination of their absolute concentrations in fresh microalgal biomass

Esteban Serrano León · Rémy Coat ·
Benjamin Moutel · Jérémy Pruvost ·
Jack Legrand · Olivier Gonçalves

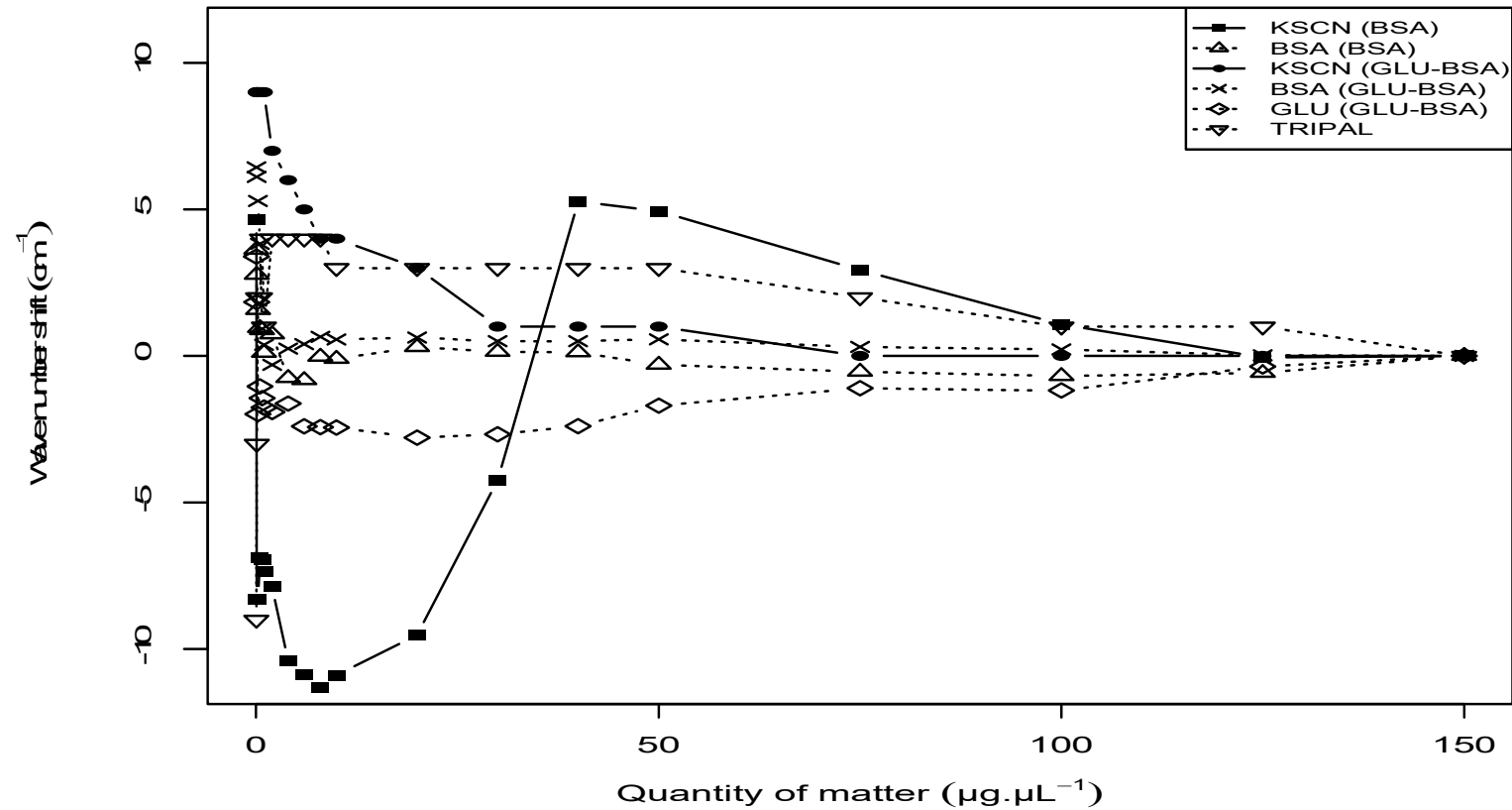
Influence of the sampling method

- Wave number shifts



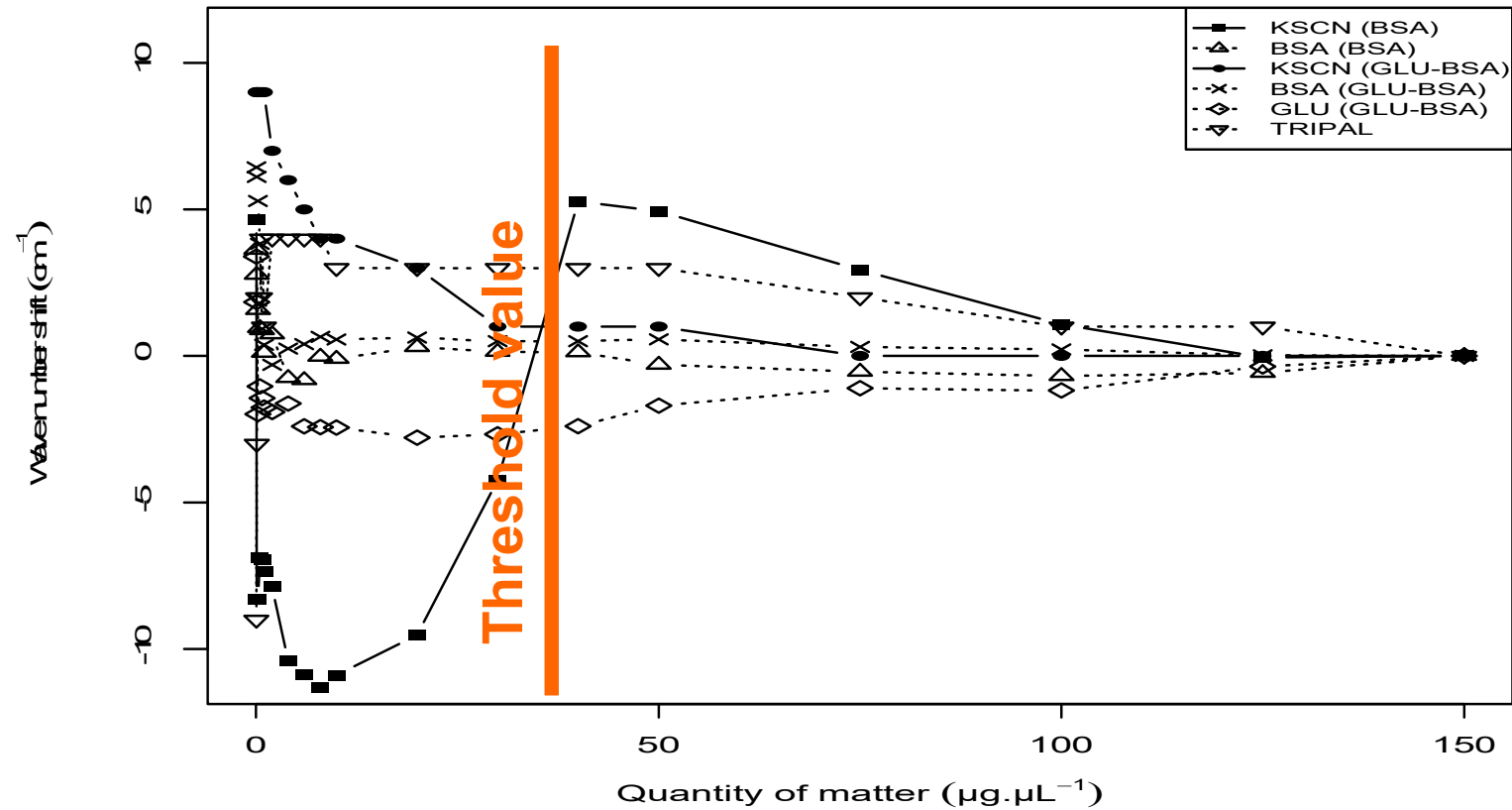
Influence of the sampling method

- Wave number shifts - hydrogen bonding network



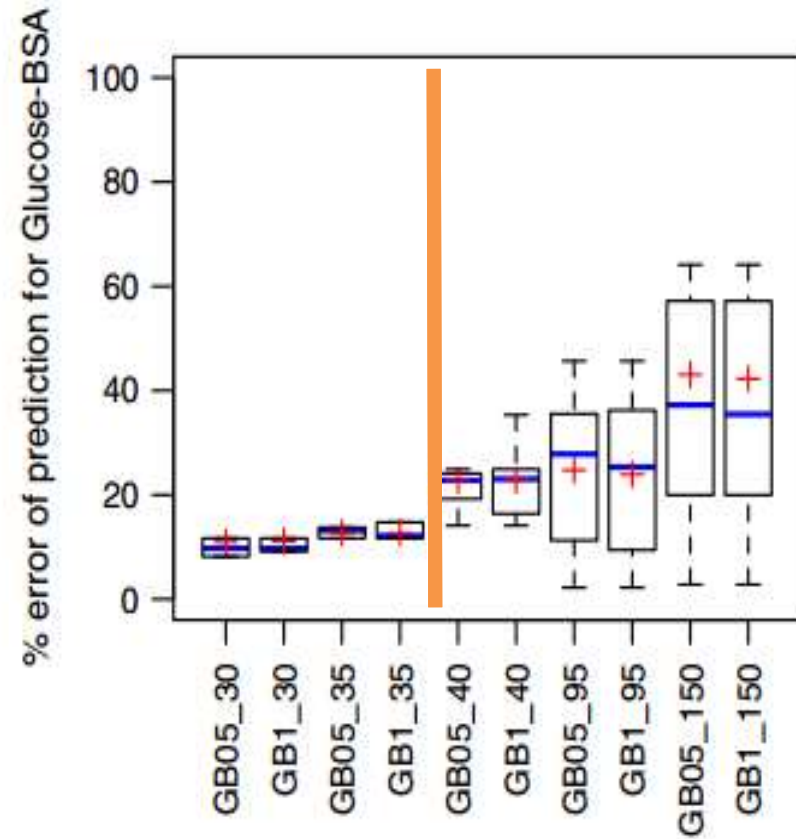
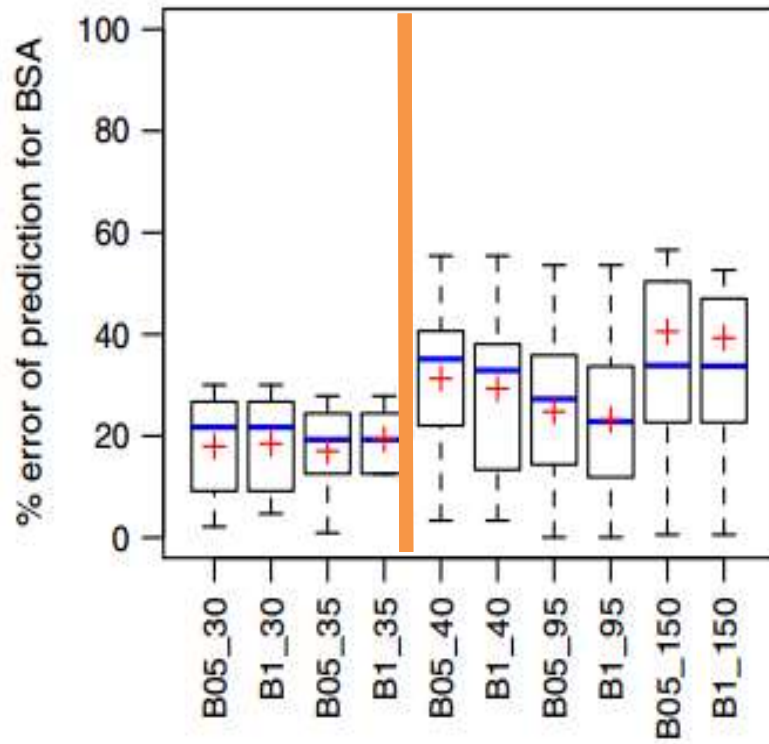
Influence of the sampling method

- Wave number shifts - hydrogen bonding network



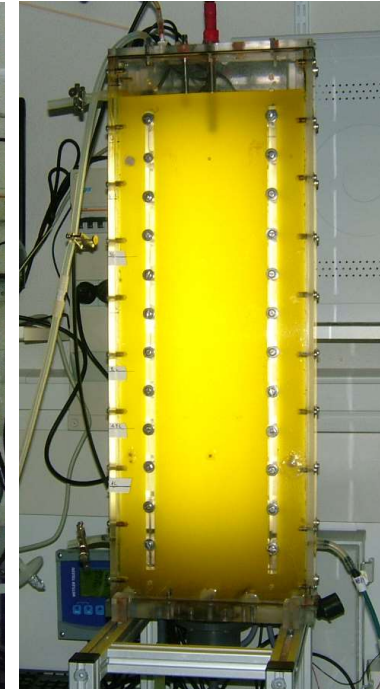
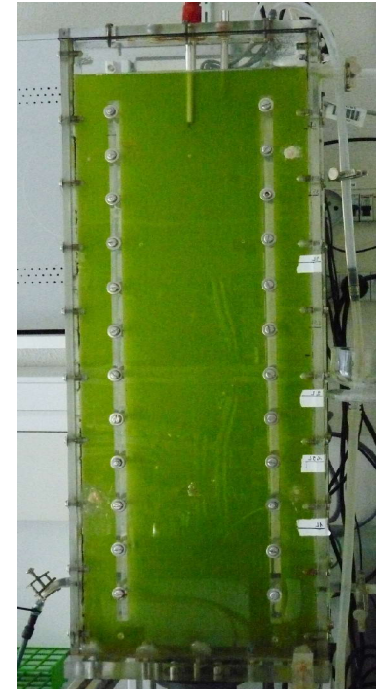
Influence on the error of prediction

- Strong influence of the sampling method thru molecular probing approach SCN⁻

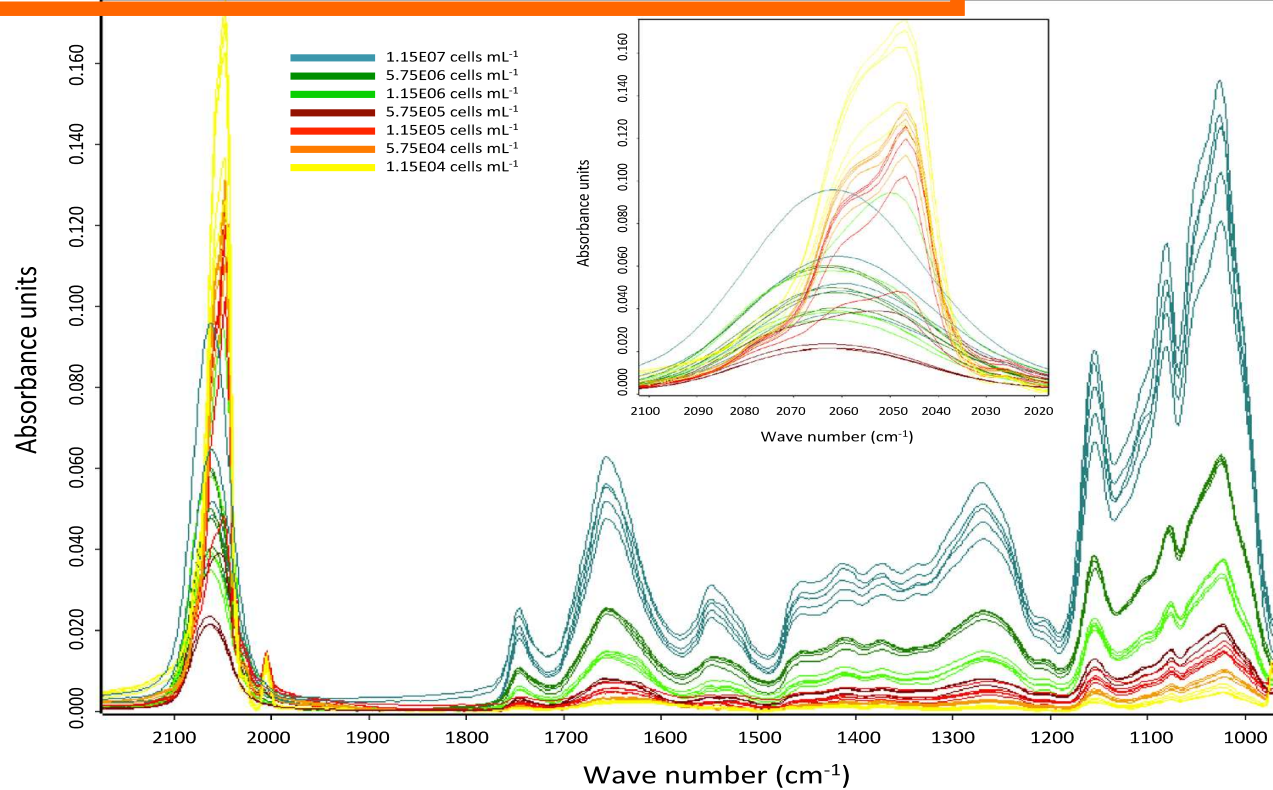
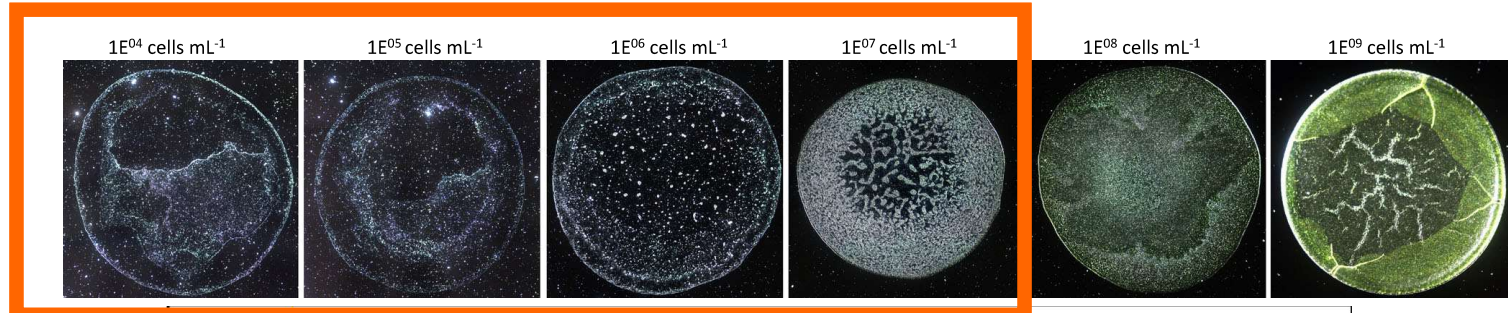


Infrared spectroscopy for PBR monitoring

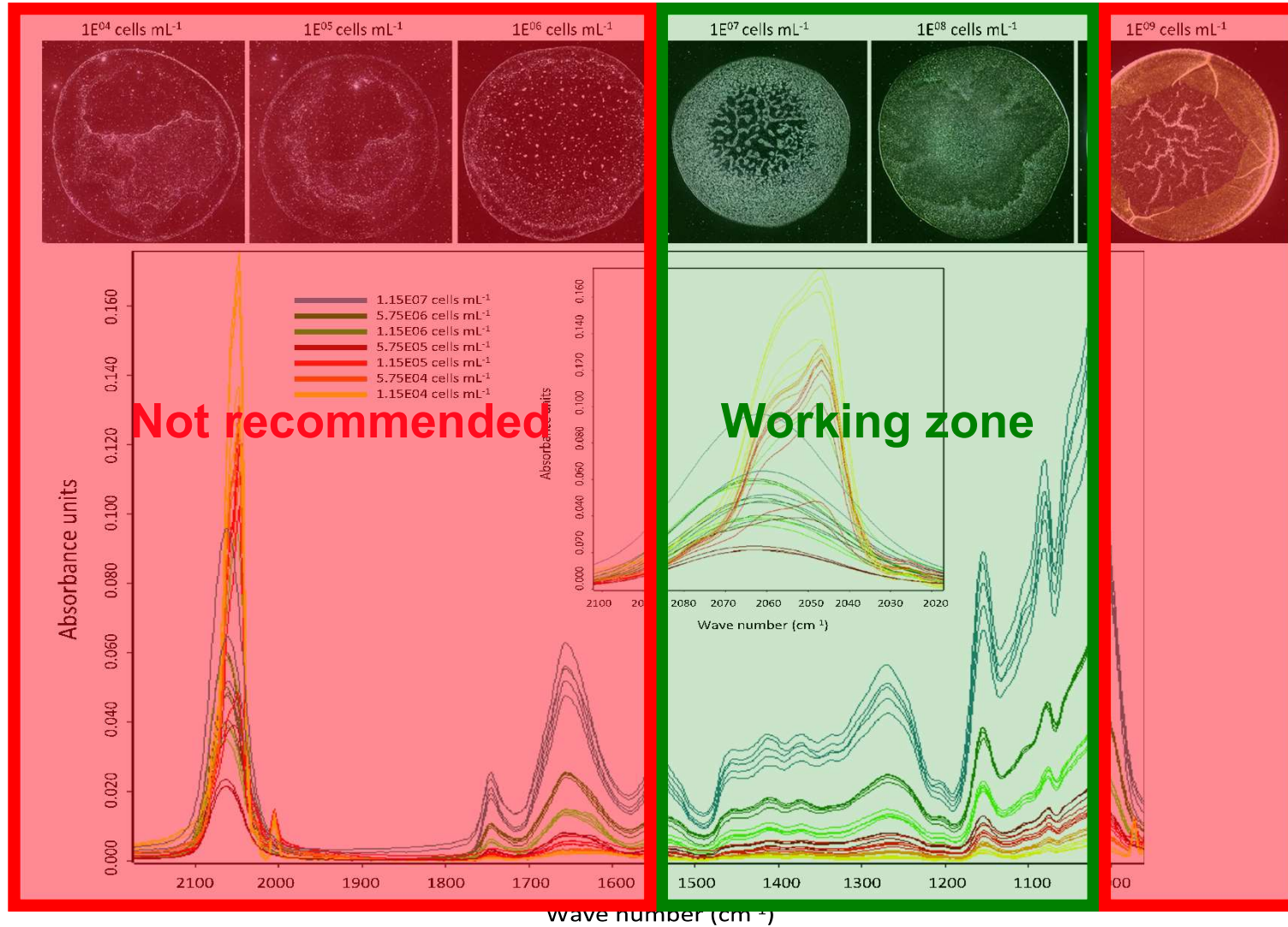
- Real conditions
 - *Nannochloropsis oculata* - Airlift PBR (5 L)
 - Filtered sea water (0.2 μm)
 - Conway 3N3P medium
 - Light: 110 $\mu\text{mol}/\text{m}^2/\text{s}$
- Model construction and test
 - Progressive starvation, optimize dynamical range of the intracellular TL (3N3P with 3P-N)
 - Calibration, max concentration : 0.4 g/L
 - Test, max concentration : 1.2 g/L
- Off-line monitoring FTIR/GCFID
 - 30 mL sampled (centri, washed, NB cells, DW, pigments)
 - GCFID (TL-FA, 5 replicates)
 - FTIR (1 μL deposit 384 wells plate , 5 replicates)



Infrared spectra on fresh cells

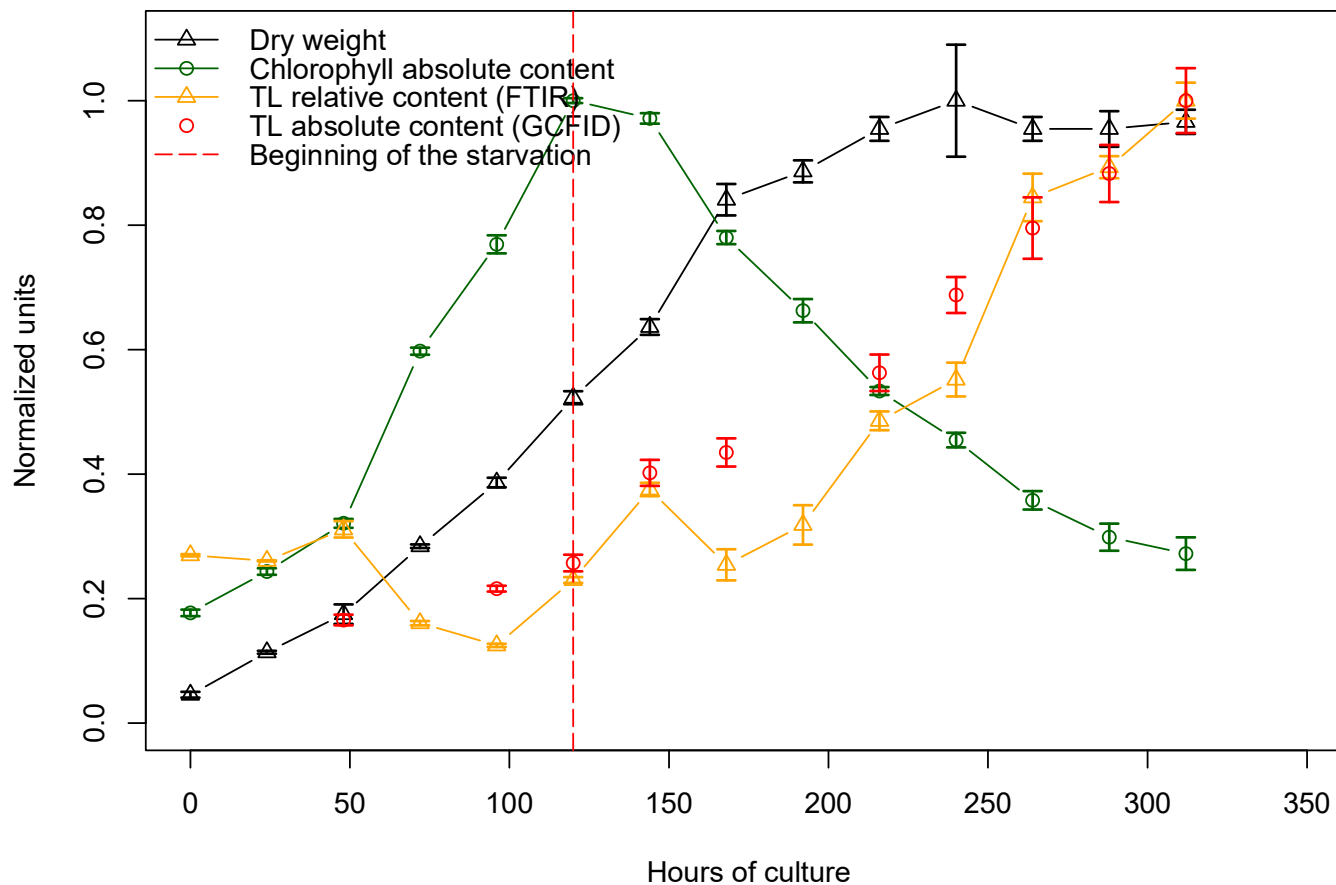


Infrared spectra on fresh cells : heterogeneity



Monitoring results

- From 10% to 60% lipids DW ; 50 data points with GCFID, 50 spectra with FTIR



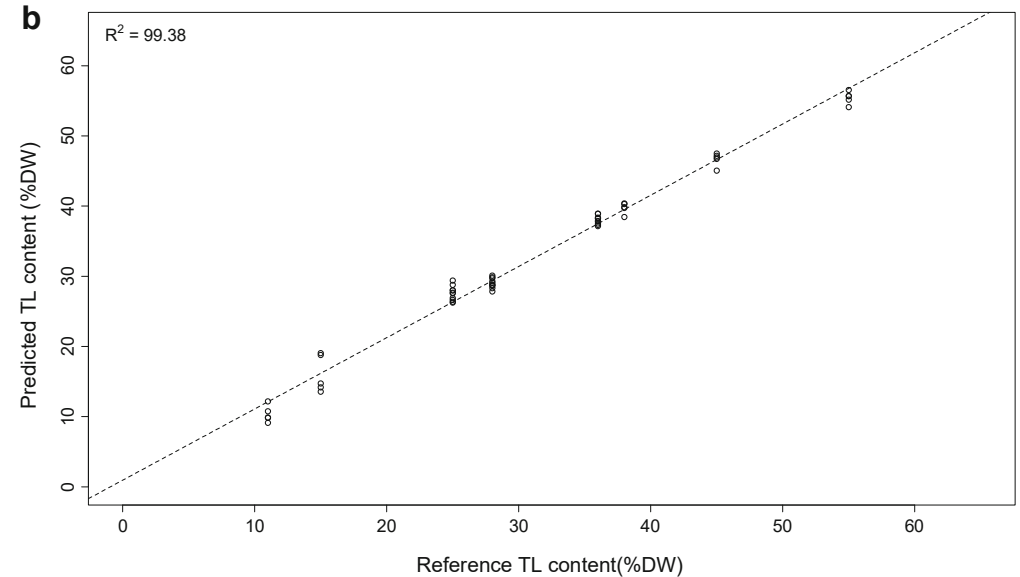
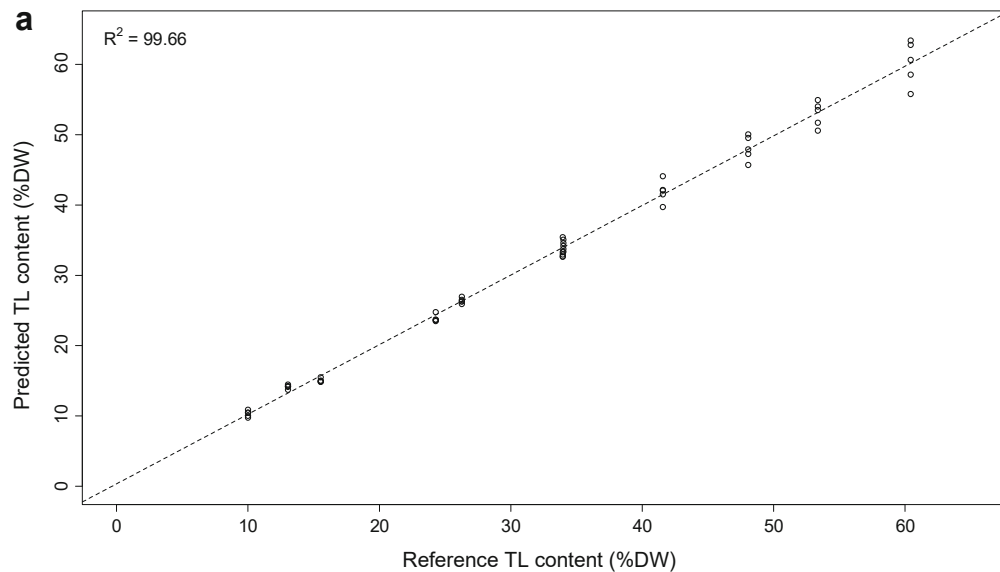
Performances of the multivariate model

Table 1 PLS-R quality parameters for cross and test set validation for the selected model

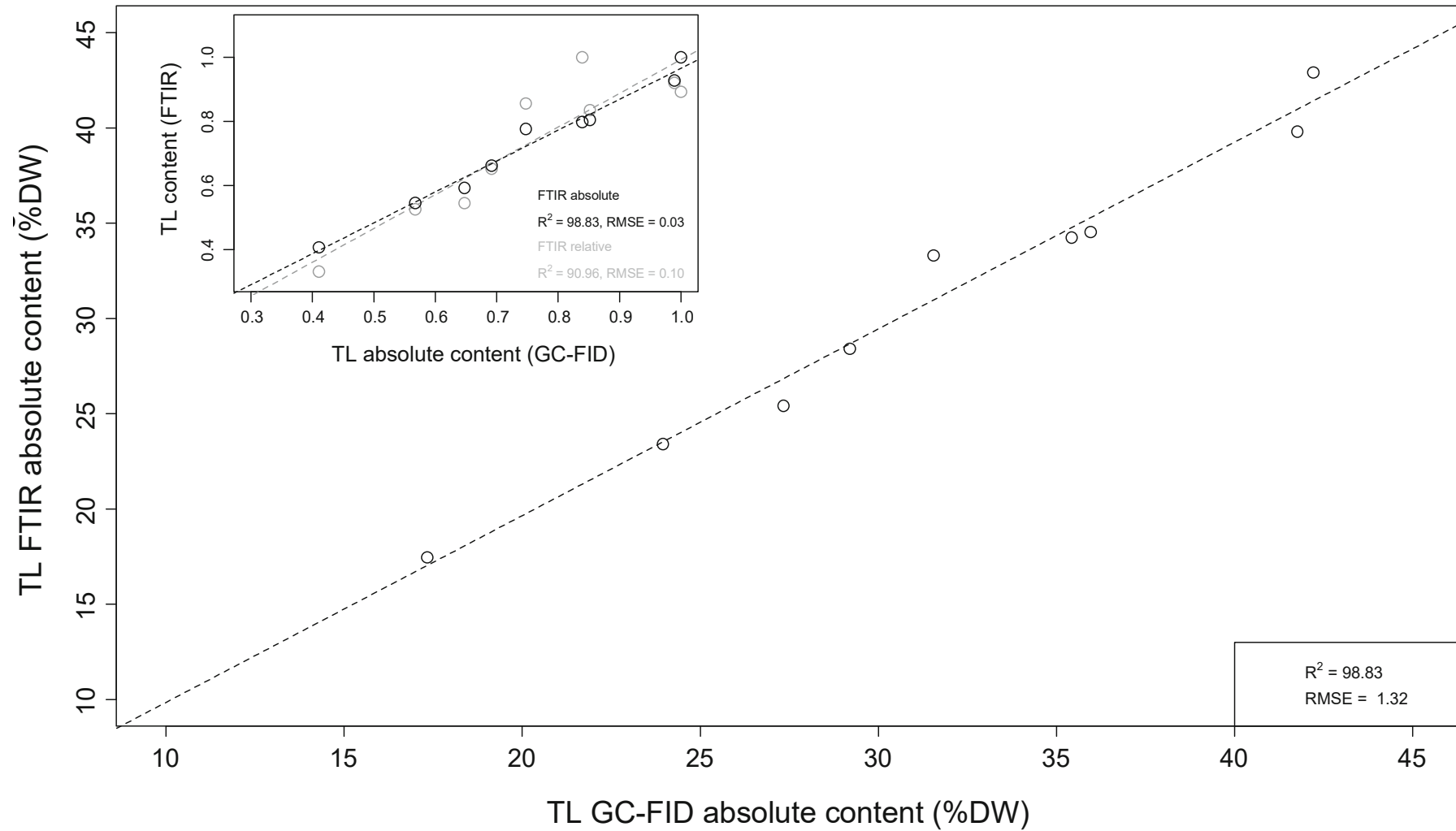
	Pre-processing	Spectral region (cm ⁻¹)	RMSE (CV and P)	Number of PLS components	R ² (%)
Cross validation	None	1,838–1,477 1,118–758	1.29	6	99.66
External validation	None	1,838–1,477 1,118–758	1.81	7	99.38

R² is the coefficient of determination

RMSECV root mean square error of cross validation, RMSEP root mean square error of prediction for the external validation



Robustness of the multivariate model



Interesting...

- Small volume sample (1 mL of a typical PBR culture)
- Fast (within 30 minutes)
- Accurate (as accurate as the reference method)
- Robust (physiological independant)
- High-throughput (up to 300 samples automatically)
- Can be inserted after your cells washing and counting protocol steps

Bioprocess Biosyst Eng (2014) 37:2175–2187
DOI 10.1007/s00449-014-1194-5

ORIGINAL PAPER

Unravelling the matrix effect of fresh sampled cells for in vivo unbiased FTIR determination of the absolute concentration of total lipid content of microalgae

Rémy Coat · Valeria Montalescot · Esteban Serrano León ·
Delphine Kucma · Candice Perrier · Sébastien Jubeau ·
Gérald Thouand · Jack Legrand · Jérémy Pruvost · Olivier Gonçalves

ANR
AGENCE
NATIONALE
DE LA
RECHERCHE

Diesalg
(2012 - 2015)

However...

- Microalgae dependent (error up to 150% for *C. reinhardtii*, *C. kessleri* and *N. oleoabundans*)
- Semi quantitative still interesting

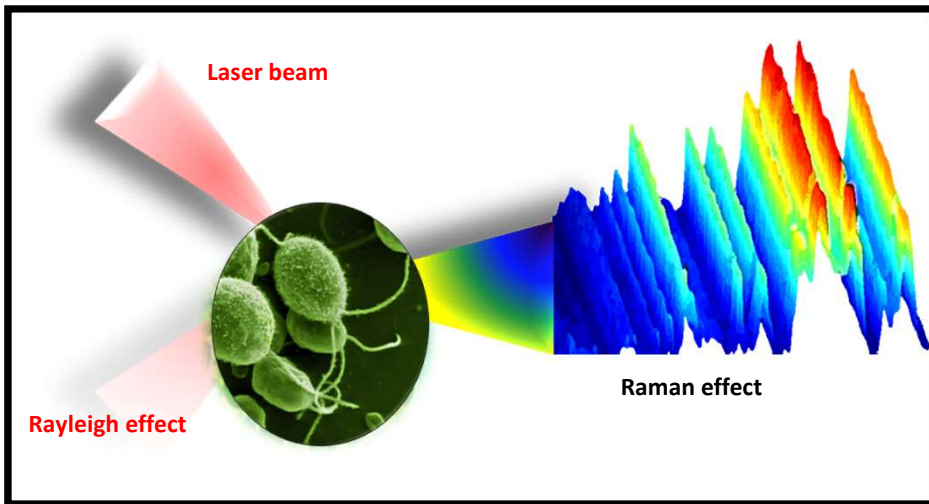
- **Remote IR probe (ATR) ?**
WATER contribution?
Automatized deposit and dry steps?
Bypass on the PBR?
Matrix effect?
EX SITU



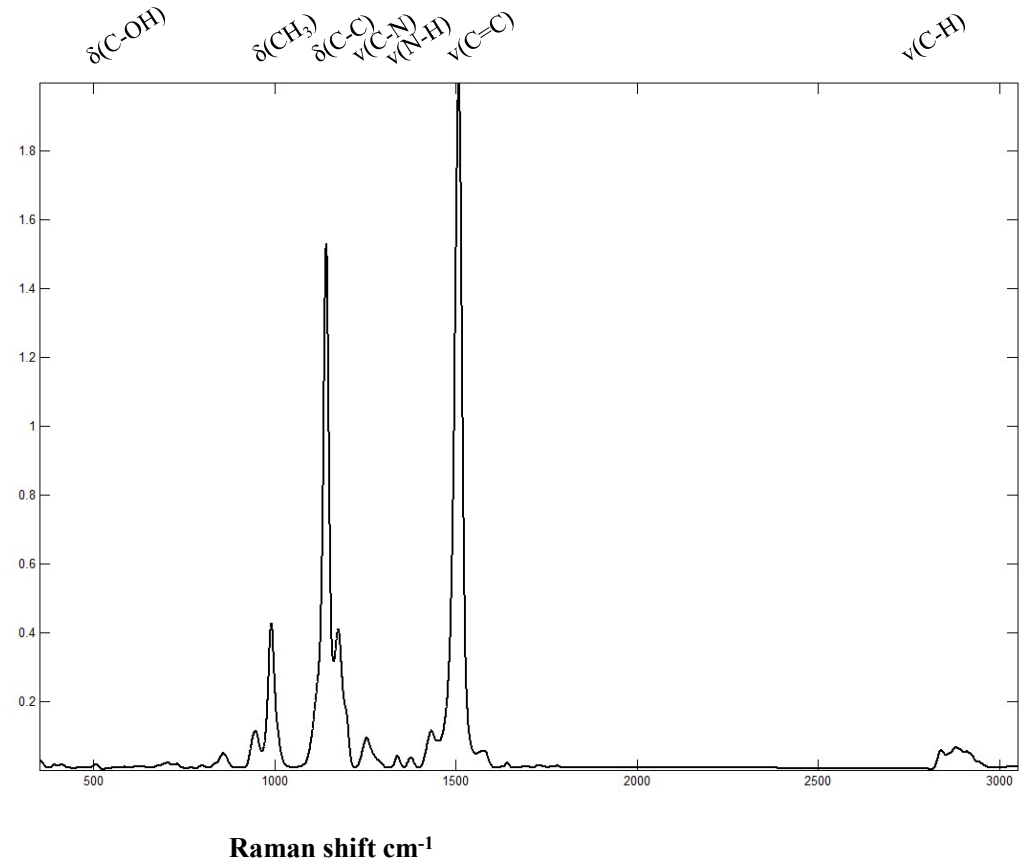
- **Raman for IN SITU?**



Raman spectroscopy



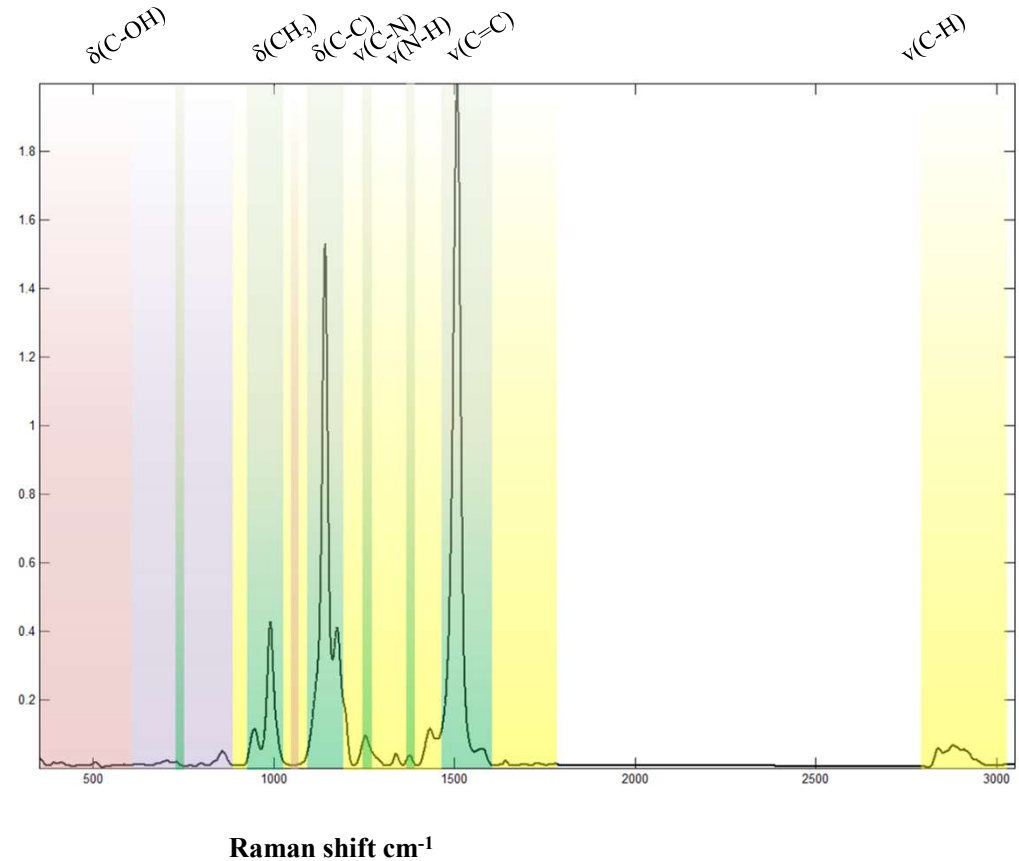
Raman effect



Raman spectra of *Chlorella vulgaris* (785 nm)

Raman spectroscopy

- Carbohydrates
- Nucleic acids
- Pigments
- Proteins and or Lipids



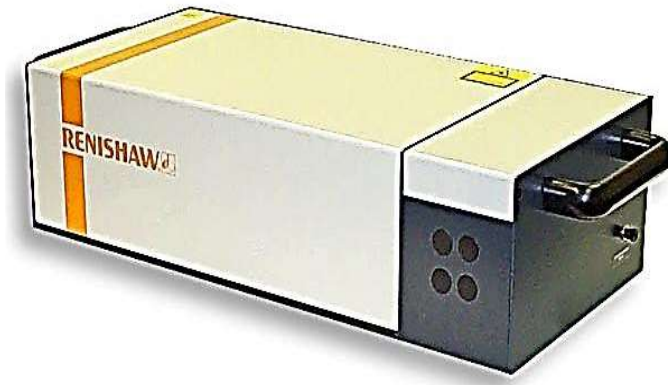
Raman spectra of *Chlorella vulgaris* (785 nm)

Raman spectroscopy for PBR monitoring

- Water non-sensitive : on-line possible but still need to dry the sample on gold surface to understand the effect of the laser beam power on the cells



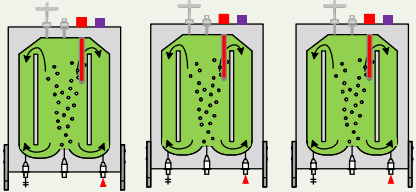
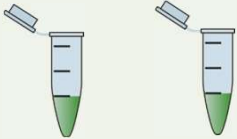
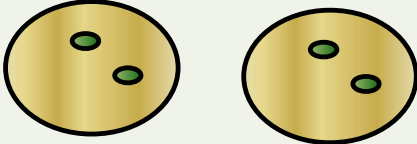
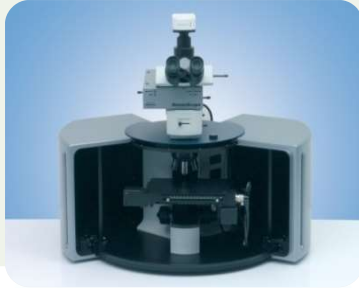
Microspectrometer
Bruker Senterra - 785 nm



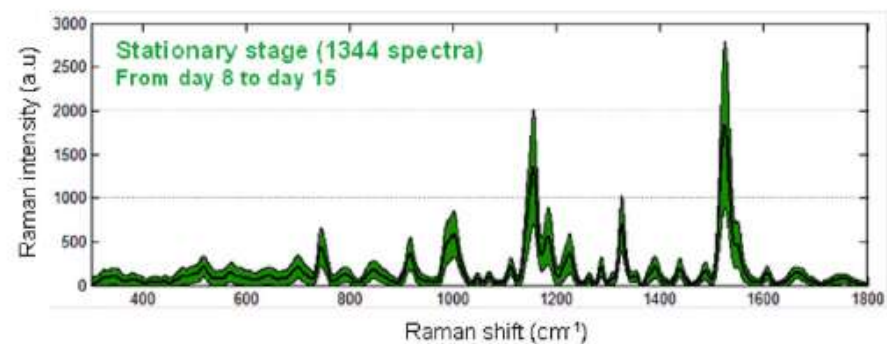
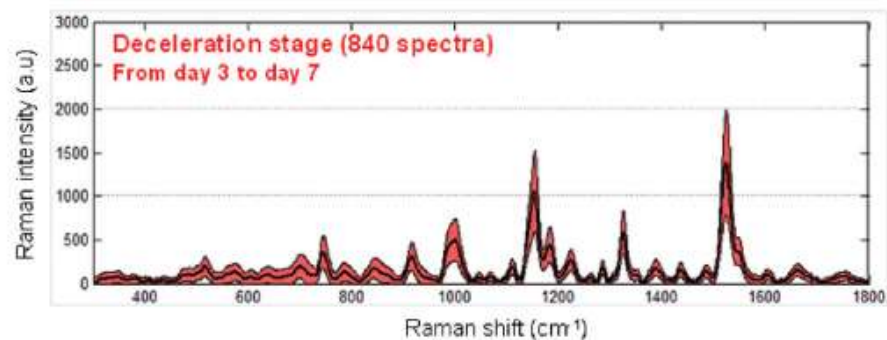
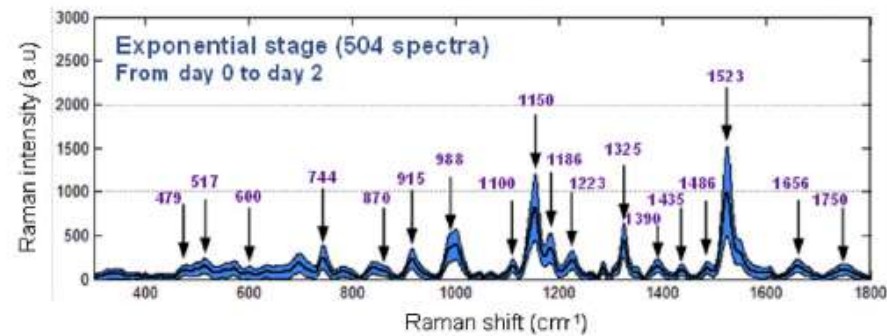
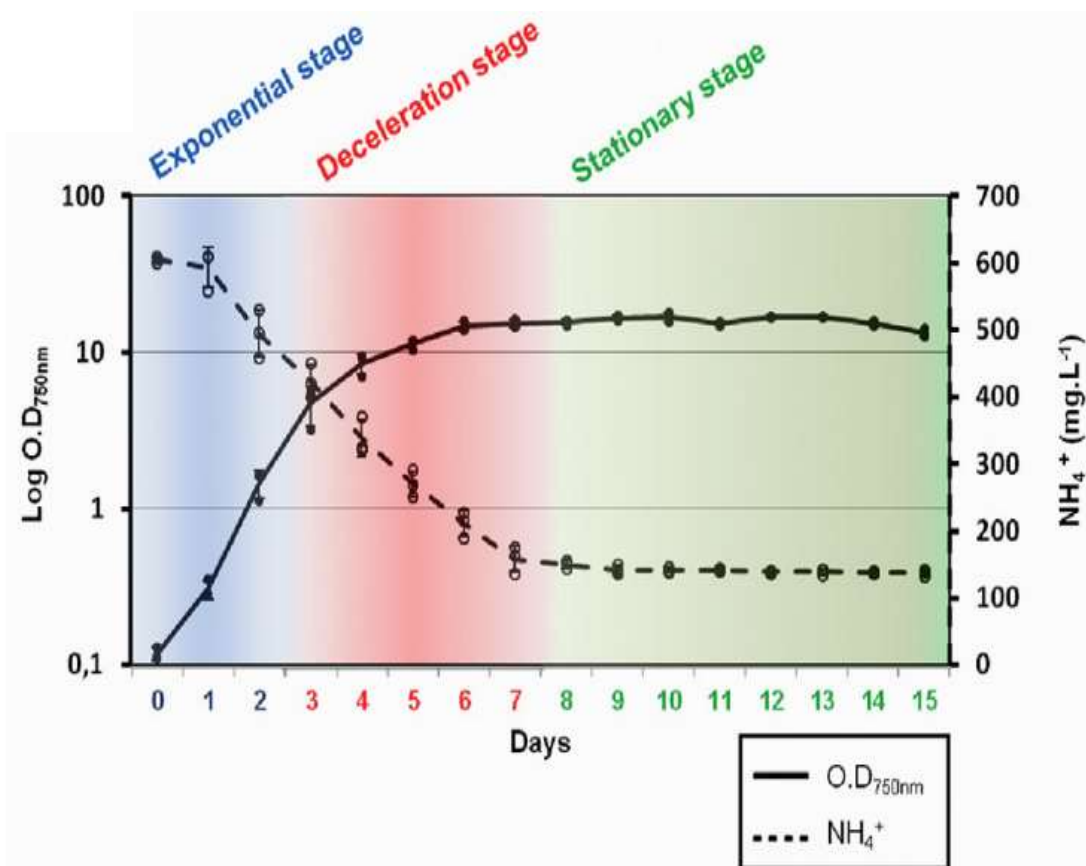
Spectrometer with optical fiber
Renishaw RA100 - 532 nm

Raman spectra on cells grown in PBR

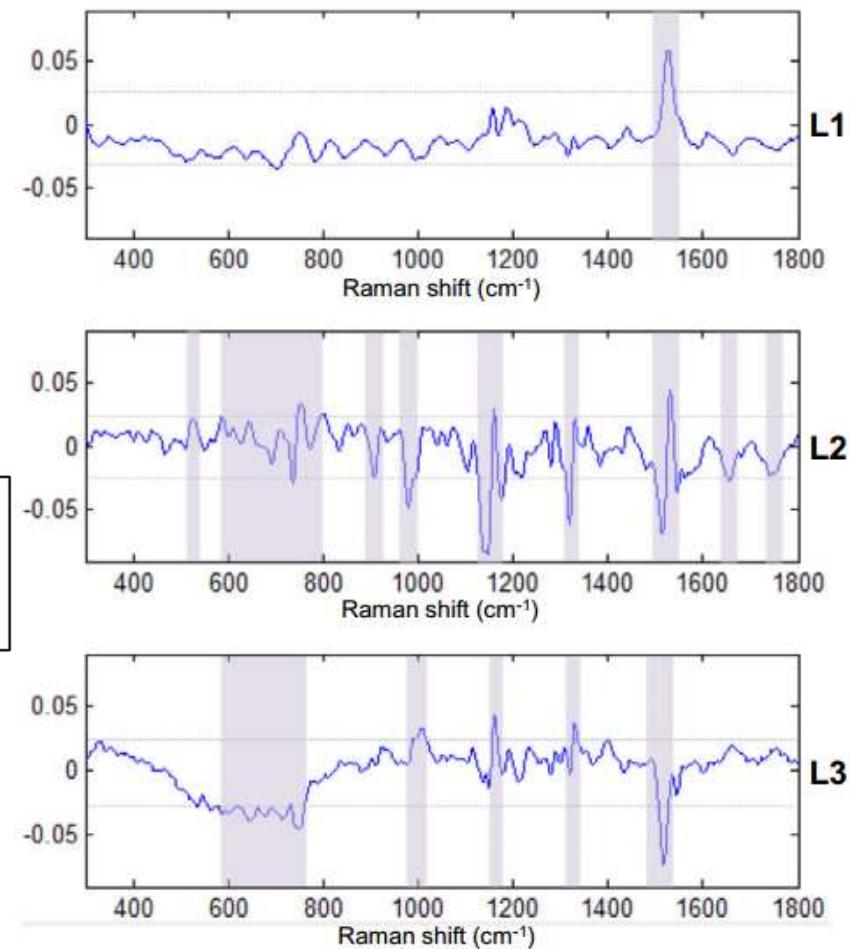
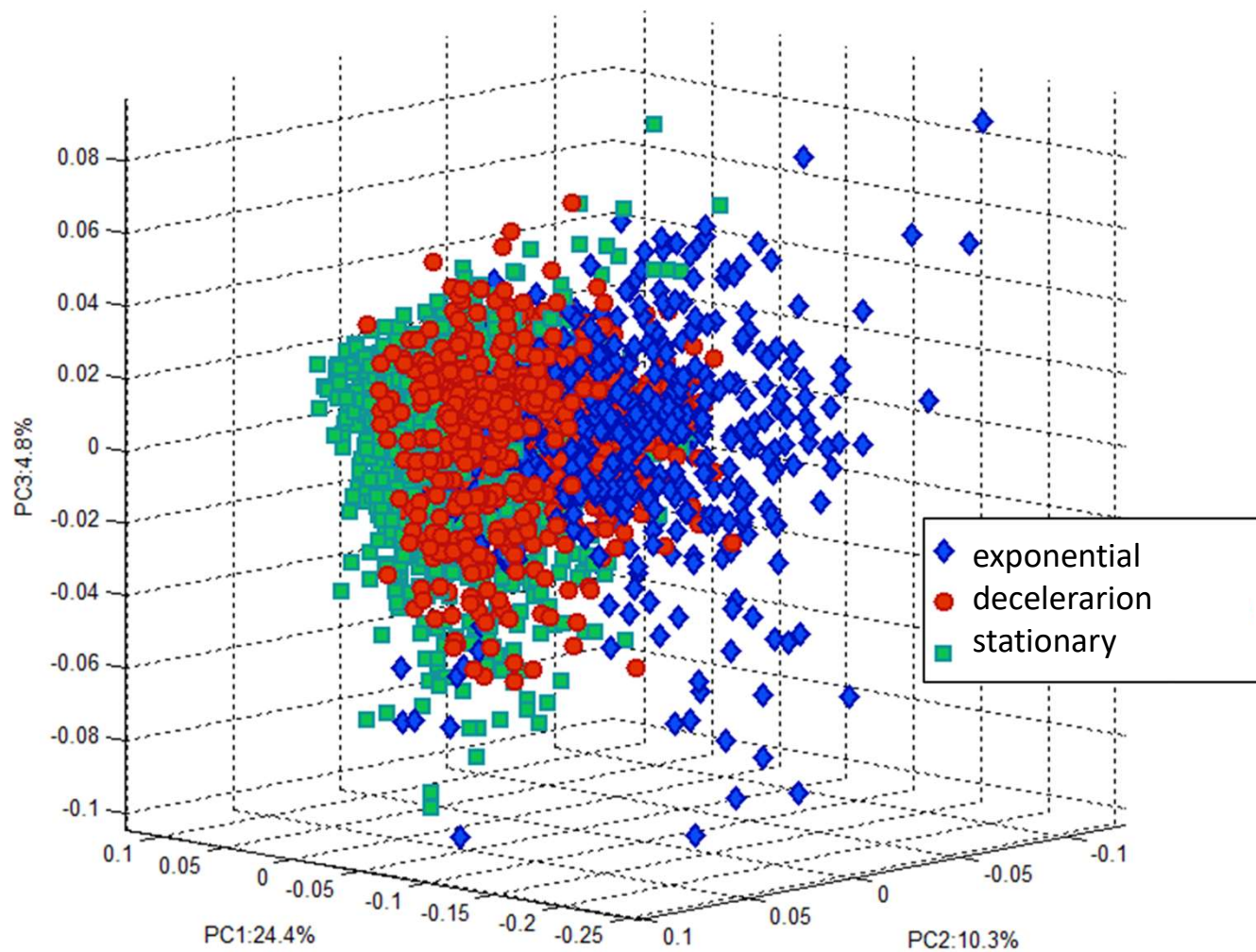
- Need to prepare a bank of spectra during the microalgae growth in PBR (off line)
- 3000 spectra and 16 days of growth in PBR

<u>Step 1</u> Robustness	<u>Step 2</u> Biological variability	<u>Step 3</u> Experimental variability	<u>Step 4</u> Data acquisition
Photobioreactors triplicates	Two samples by reactor	Two deposits by sample (<i>Chlamydomonas reinhardtii</i>)	14 spectra by deposit
			

Raman spectra are growth dependant

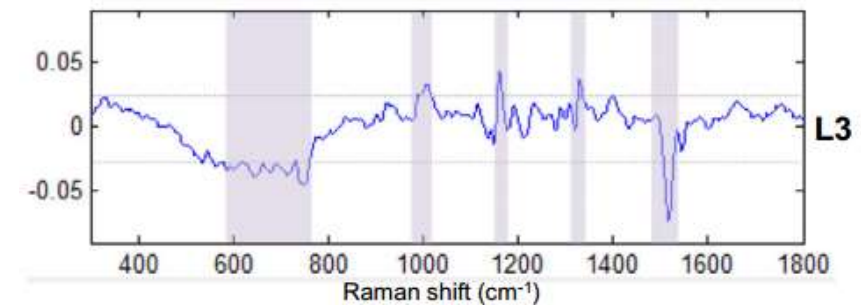
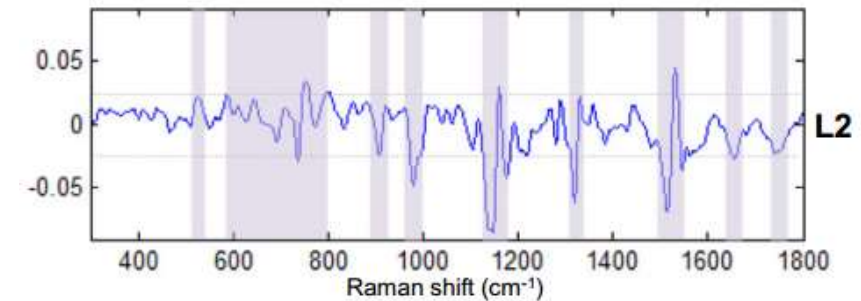
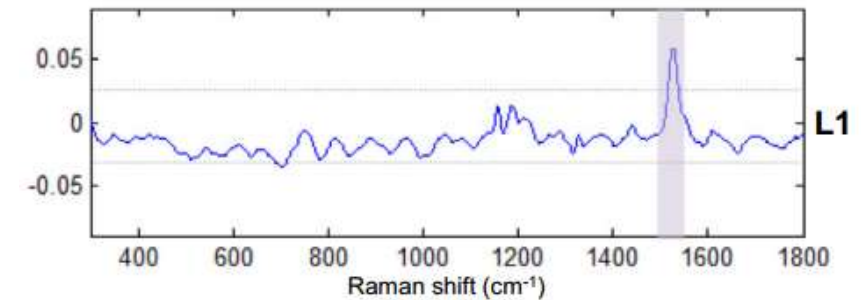


Raman spectra are growth dependant



Raman spectra are growth dependant

Raman shift (cm ⁻¹)	Main attribution
517	Polysaccharides $\delta(\text{C-H}_2)$, $\delta(\text{C-OH})$ [58]
600 - 800	DNA and RNA bases (ring breathing) [58]
744	Carbohydrates, chlorophyll a $\delta(\text{H-C-O})$, $\delta(\text{N-C-C})$ [28]
915	Chlorophyll a $\delta(\text{N-C-C})$, $\delta(\text{C-C-C})$ [22]
988	Chlorophyll a $\delta(\text{C-H}_3)$ [22]
1150	Carotenoid $\delta(\text{C-C})$, $\delta(\text{C-H})$ [22,57,58]
1186	Amino acids leucine, phenylalanine, chlorophyll a $\delta(\text{C-H})$, $\nu(\text{N-C})$ [22]
1325	Chlorophyll a $\nu(\text{C-N})$, $\delta(\text{C-H})$ [22]
1523	Carotenoid $\nu(\text{C=C})$ [22,57,60]
1656	Lipid, amide I $\nu(\text{C=C})$ cis [28,31,57]
1750	Lipid $\nu(\text{C=O})$ [26,31]



Interresting...

- Small volume sample (0,5 mL of a typical PBR culture)
- Fast (within few minutes)
- Accurate
- More details in

Algal Research 42 (2019) 101595



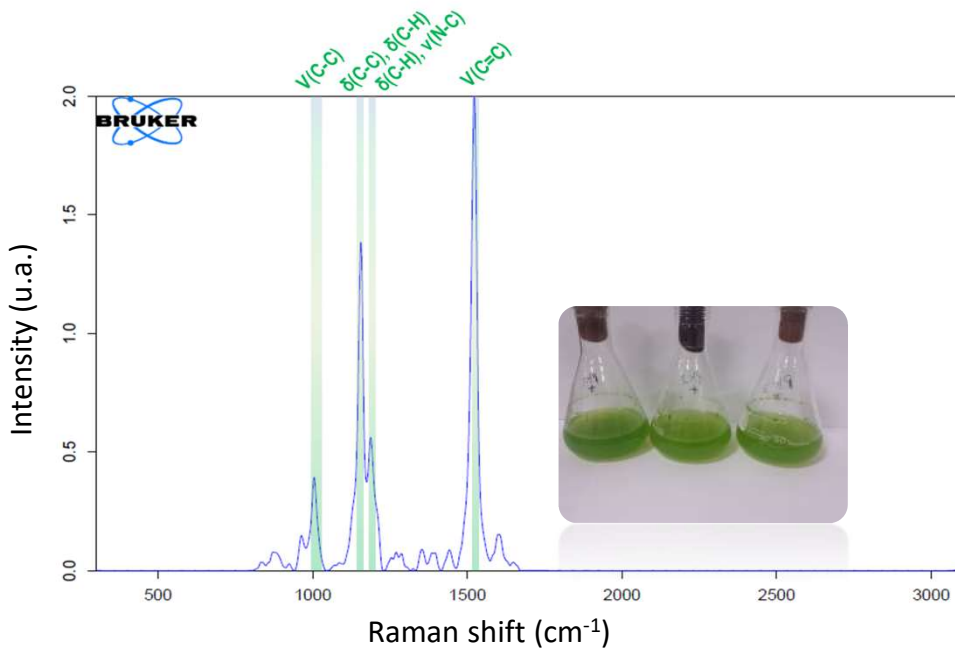
Fast non-invasive monitoring of microalgal physiological stage in photobioreactors through Raman spectroscopy

Christopher Lieutaud^a, Ali Assaf^a, Olivier Gonçalves^b, Gaëtane Wielgosz-Collin^c,
Gérald Thouand^{a,*}

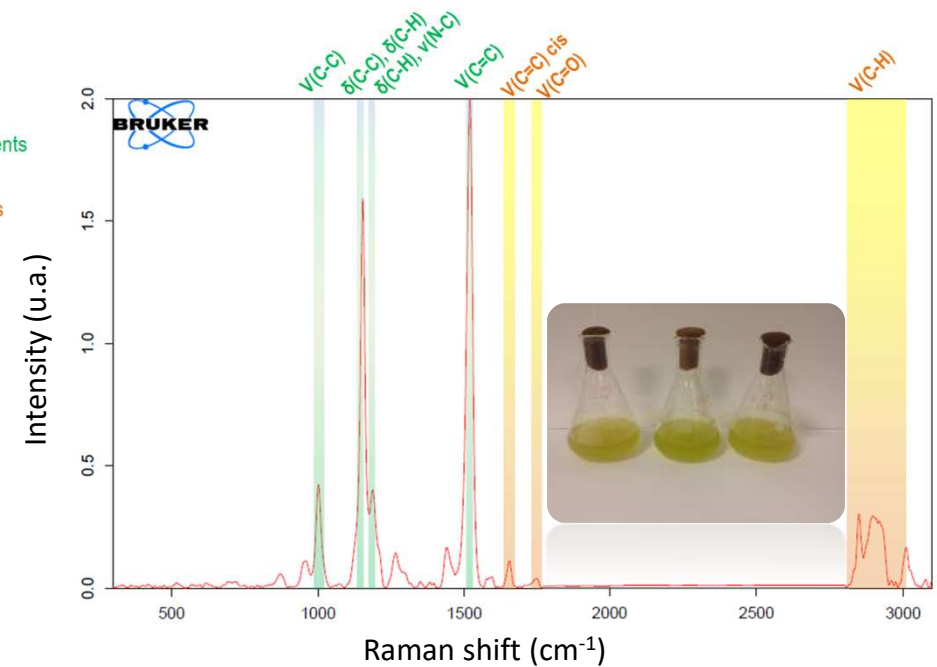


And what about the monitoring?

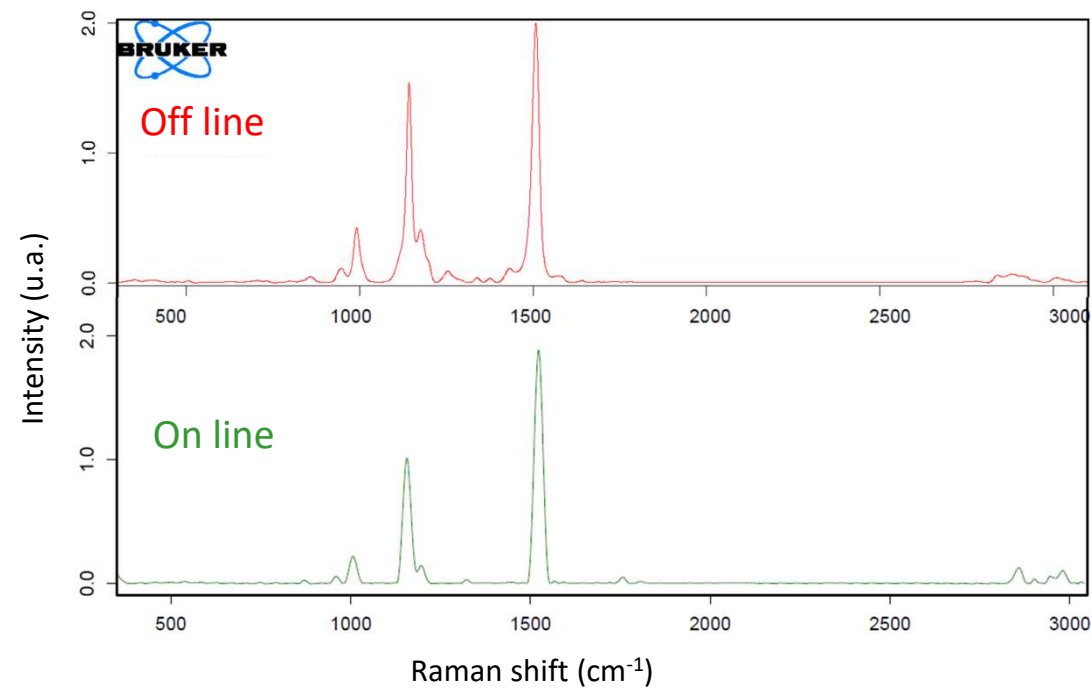
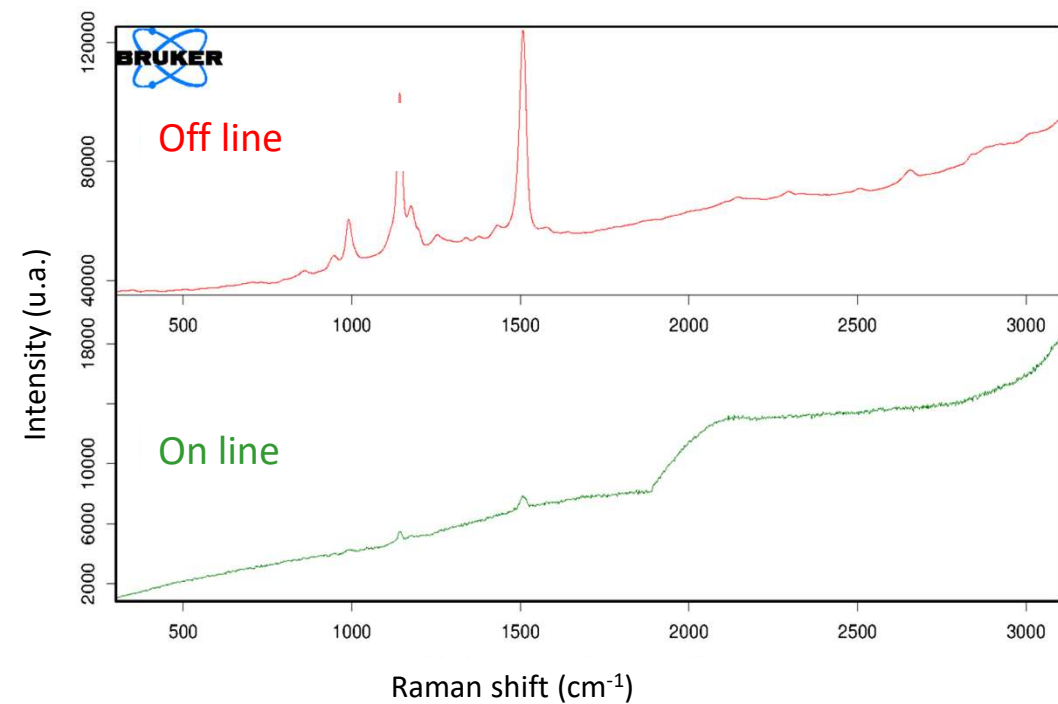
- Possible to measure lipids for *P. kesslerii* at 532 nm, 5 mW, 3 sec acquisition time, on gold surface



■ Bandes pigments
■ Bandes lipides

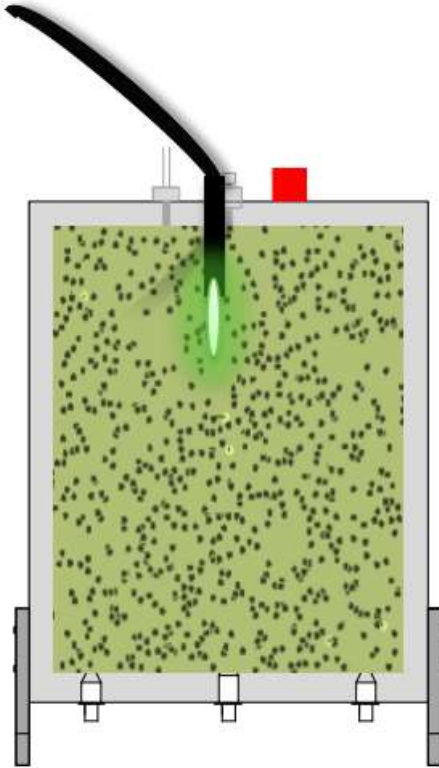


Signal loss when working on line



Signal loss needs parameters optimisation

- Optimisation of time and laser power
- Focal point of the laser and the cells in the PBRs



Laser power

40 mW
80 mW



Acquisition time:

1 sec
10 sec
100 sec



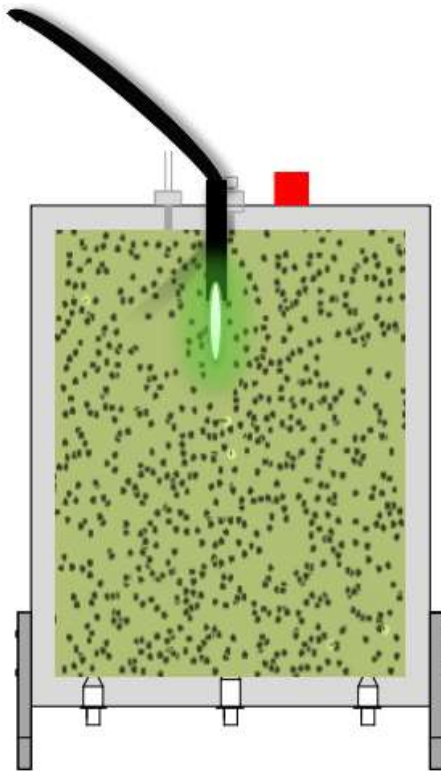
Turbidity

1 log
2 log
3 log



Signal loss needs parameters optimisation

- Optimisation is growth stage dependant



Laser power

40 mW

80 mW

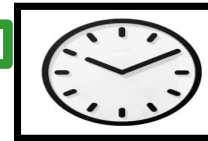


Acquisition time:

1 sec

10 sec

100 sec

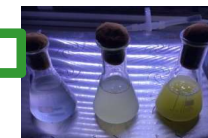


Turbidity

1 log

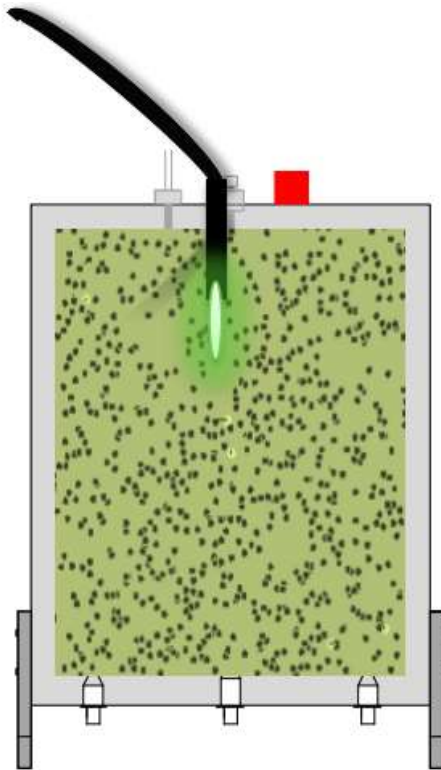
2 log

3 log



Signal loss needs parameters optimisation

- Optimisation is growth stage dependant



Laser power

40 mW

80 mW



Acquisition time:

1 sec

10 sec

100 sec

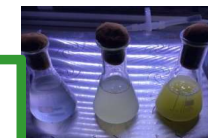


Turbidity

1 log

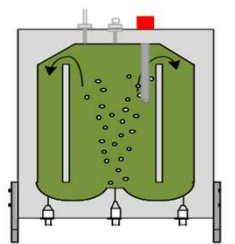
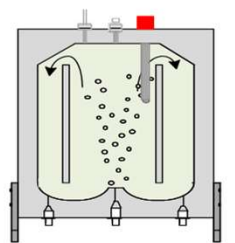
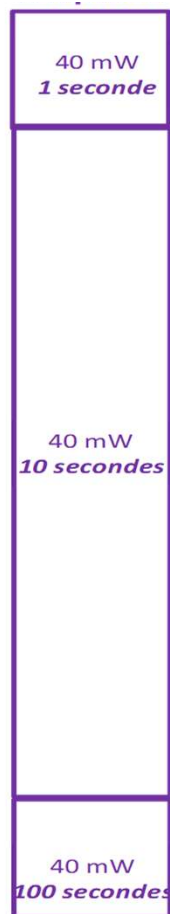
2 log

3 log

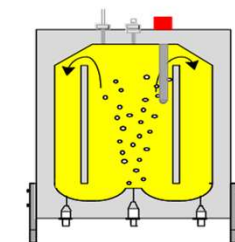


First proof of concept

Test
adaptative
parameters

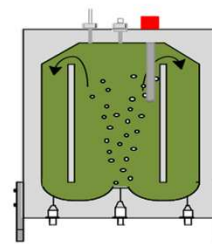
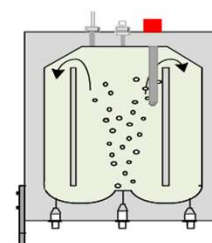
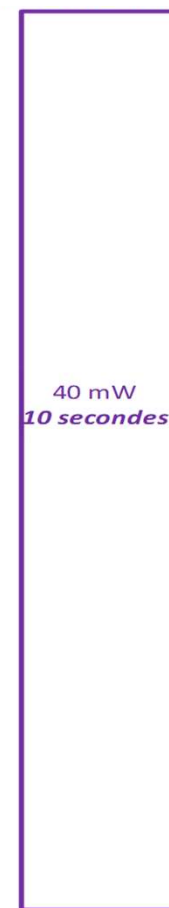


Nitrogen
starvation

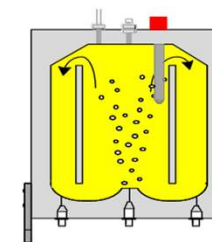


Day 30

Control
median
parameters

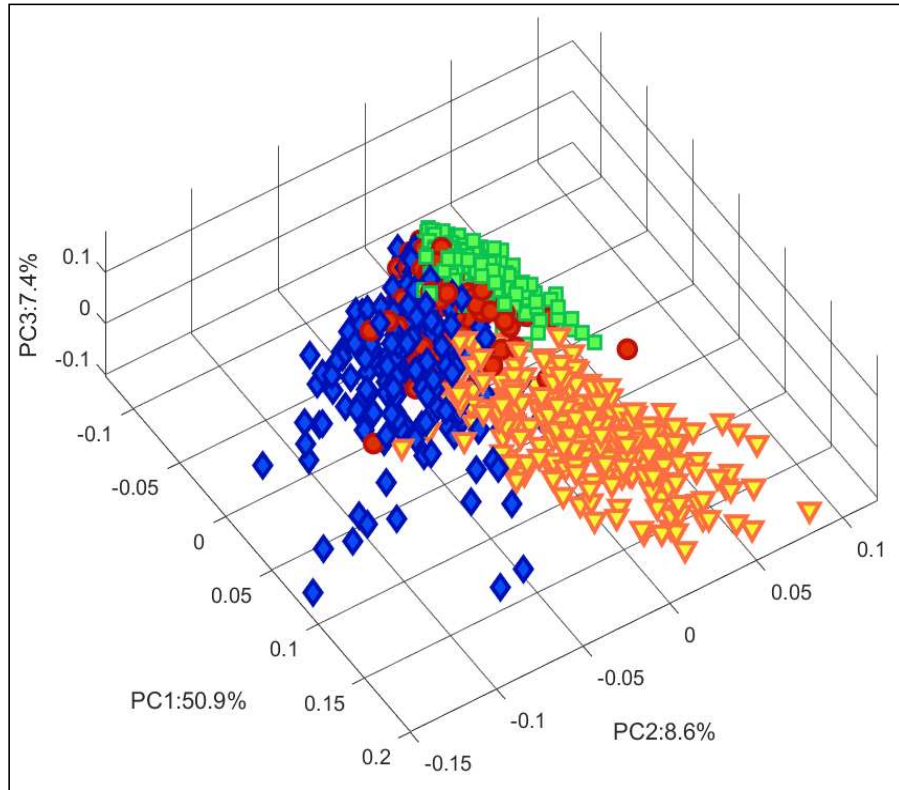


Nitrogen
starvation

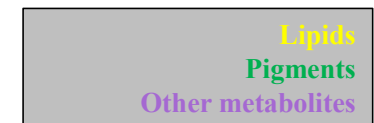
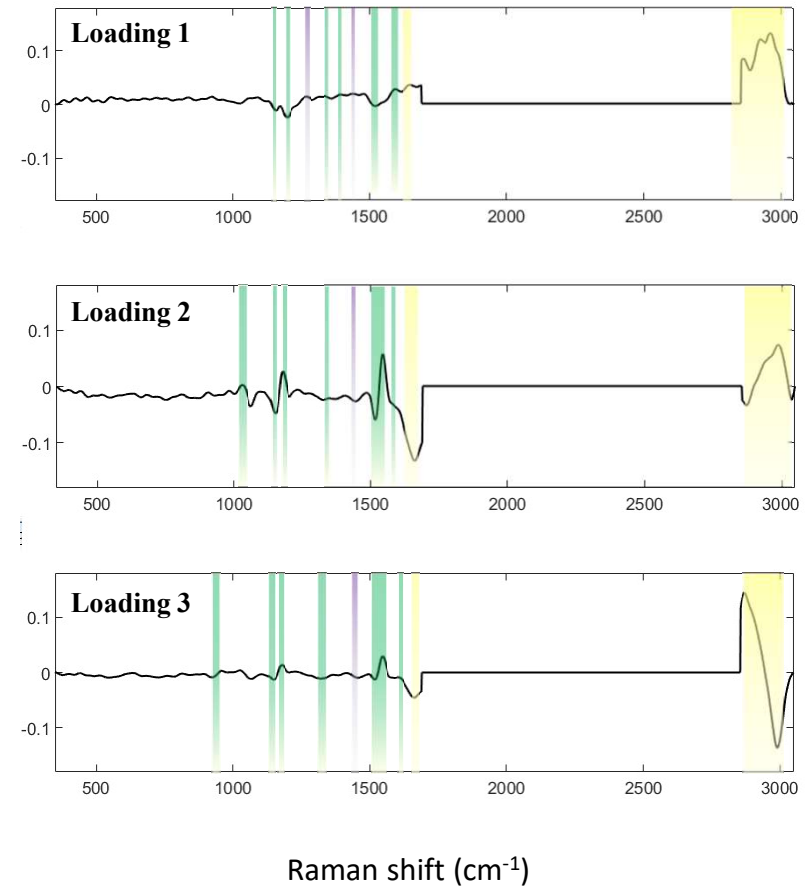


Day 30

Online Raman spectra were phases dependant

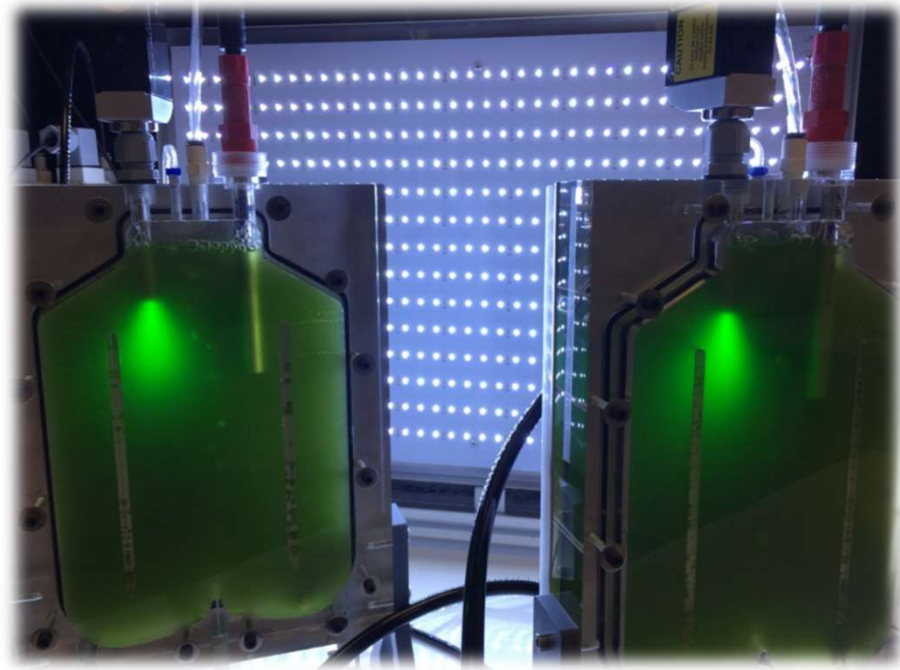


- Exponential (J0-J3)
- Deceleration (J5-J8)
- Stationnary (J10-J17)
- Lipid production (J28-J33)



Very promising...

- On line measurements are possible
- Lipids, pigments are easily detectable
- Semiquantitative



However...

- Very dependent on the bank of spectra
- Developments of new probes, with enhanced features



- **Matrix effect?**
- **What about control strategies? RTO?**
- **And what if NMR new technological rupture could bring something ?**